

**Drug Discovery & Therapy
World Congress**

2015



July 22 - 25, 2015, Boston, MA, USA

**Global Biotechnology
Congress**

2015



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Abstracts

John B. Hynes Veterans
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Drug Discovery and Therapy World Congress – 2015

Welcome Note



Atta-ur-Rahman, *FRS*
Co-President

The “**Drug Discovery & Therapy World Congress 2015**”, scheduled to be held from 22nd - 25th July, 2015 will bring together the world's leading scientists in the field of drug discovery and therapy to discuss their latest researches in the exciting setting of Boston.



Ferid Murad, *NL*
Co-President

The conference should provide an occasion for the participating scientists not only to present their researches and interact with eminent colleagues but also to enjoy the intellectually stimulating environment of Boston.

We would like to welcome the participants to DDTWC 2015. We hope that this would be an exciting event since the leading authorities in their respective fields will be presenting their latest researches during the conference. A large number of students will also be participating in it which should be very educational for them.

Drug Discovery and Therapy World Congress 2015

CONTENTS

PLENARY LECTURES

PL-293	CURRENT STATUS OF OBESITY AND THE NEED FOR A PARADIGM SHIFT <u>Richard L. Atkinson</u>	1
PL-122	APPLICATION OF NITRIC OXIDE RESEARCH TO DRUG DEVELOPMENT <u>Ferid Murad</u>	2
PL-123	EVOLUTIONARY DYNAMICS AND TREATMENT OF CANCER <u>Martin Nowak</u>	3
PL-224	EMERGING PHARMACO-MPE (MOLECULAR PATHOLOGICAL EPIDEMIOLOGY) PARADIGM FOR GLOBAL PRECISION MEDICINE <u>Shuji Ogino</u>	3
PL-222	RECONSTRUCTION, MODELING AND USE OF GENOME-SCALE NETWORKS IN BIOLOGY <u>Bernhard Palsson</u>	4
PL-223	NOVEL PRO-RESOLVING LIPID MEDIATORS IN INFLAMMATION & INFECTION LEADS FOR RESOLUTION PHYSIOLOGY AND PHARMACOLOGY <u>Charles N. Serhan</u>	4
PL-2	MICRORNAs, SMALL INTERFERING RNAs AND MODULAR THERAPEUTICS <u>Phillip A. Sharp</u>	5
PL-1	DESIGNING BIOLOGY FOR A HEALTHY WORLD <u>Pamela A. Silver</u>	5
PL-291	ADVANCED THERAPEUTICS WHILE PRESERVING THE MICROBIOME <u>Ada Yonath</u>	6
PL-292	SPECIFIC ANTI-TUBERCULIN (IgG) HIGH LEVELS AND LYMPHOCYTE <i>IN VITRO</i> PROLIFERATION WITH TUBERCULIN ARE BETTER INDICATORS OF LTBI THAN TST Edmond J. Feris, Joaquin Zuñiga, Liliana Encinales, Joel N.H. Stern, Julio Granados, Marcelo Fernandez-Vina and <u>Edmond J. Yunis</u>	6

KEYNOTE LECTURES

KNL-250	SCIENTIFIC MISCONDUCT IN BIOMEDICAL SCIENCES <u>Rathnam Chaguturu</u>	8
----------------	--	---

KNL-53	ATP SYNTHESIS BY CANCER CELLS: BEYOND WARBURG Gautam Dhar, Suvajit Sen and <u>Gautam Chaudhuri</u>	8
KNL-82	NATURE-BASED DRUG DISCOVERY – <i>REEMERGING PARADIGM</i> <u>M. Iqbal Choudhary</u> and Atta-ur-Rahman	9
KNL-5	DIFFERENT STRATEGIES TO TARGET RESISTANT CANCER CELLS OVEREXPRESSING MULTIDRUG ABC TRANSPORTERS: INHIBITION AND COLLATERAL SENSITIVITY <u>Attilio Di Pietro</u>	10
KNL-12	THERAPEUTIC MONOCLONAL ANTIBODIES AGAINST CANCER AND VIRUSES <u>Dimitar S. Dimitrov</u>	11
KNL-167	VBP15 FOR DMD: A COLLABORATIVE PUBLIC/PRIVATE PARTNERSHIP ENABLED BY VENTURE PHILANTHROPY SUPPORT FROM THE INTERNATIONAL STAKE-HOLDER COMMUNITY <u>Eric Hoffman</u>	11
KNL-321	ENGINEERED HYDROGELS FOR REGENERATIVE MEDICINE APPLICATIONS <u>A. Khademhosseini</u>	12
KNL-60	TARGETING MTA1 MASTER CHROMATIN REMODELER IN CANCER AND STEM CELL BIOLOGY <u>Rakesh Kumar</u>	12
KNL-226	SEEING A ROAD FROM THE CAR: COULD EFFECTIVE MICRORNA “BRAKES” SLOW DOWN THE TRIP TO ALZHEIMER'S DISEASE? <u>Debmoy K. Lahiri</u> , Justin M. Long, Nipun Chopra, Xudong Huang, Nigel H. Greig, Kumar Sambamurti, Jack T. Rogers, Weihong Song and Bryan Maloney	13
KNL-93	SYNTHETIC AND BIOENGINEERED HEPARIN-BASED THERAPEUTICS <u>Robert J. Linhardt</u>	14
KNL-225	EQUISETIN, REUTERICYCLIN AND STREPTOLODYGIN: NATURAL PRODUCT LEAD STRUCTURES FOR NOVEL ANTIBIOTICS <u>Mark G. Moloney</u>	14
KNL-127	ON THE ORIGIN AND COMPLETENESS OF LIGAND BINDING POCKETS WITH APPLICATIONS TO DRUG DISCOVERY <u>Jeffrey Skolnick</u>	15
KNL-40	NEW TECHNOLOGIES FOR IMPROVED VACCINES AGAINST INFECTIOUS DISEASES AND CANCER <u>Jeffrey B. Ulmer</u>	16
KNL-158	PREVENTION OF EGFR-TYROSINE KINASE INHIBITOR COMPLICATIONS BY TREATMENT WITH NK-1 RECEPTOR BLOCKADE <u>William B. Weglicki</u> , J.H. Kramer, J.J. Chmielinska, C.F. Spurney and I.T. Mak	16

INVITED LECTURES

IL-148	NOVEL BIOMEDICAL AGENTS FROM ABORIGINAL MEDICINALLY IMPORTANT PLANTS OF CANADA <u>Athar Ata</u>	17
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IL-231	PRESENILINS ARE TRANSCRIPTIONAL TARGETS OF PARKIN Frédéric Checler, Eric Duplan, Jean Sévalle, Julien Viotti, Thomas Goiran, Charlotte Bauer, Paul Renbaum, Ephrat Levy-Lahad, Clément A. Gautier, Olga Corti, Nathalie Leroudier and Cristine Alves da Costa	17
IL-124	TISSUE SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs): A PATH TO A CLINICAL CANDIDATE <u>Eugene L. Piatnitski Chekler</u>	18
IL-173	“OPEN INNOVATION” IN DRUG DISCOVERY & TRANSLATIONAL RESEARCH IN ACADEMIA <u>Thomas D.Y. Chung</u>	19
IL-236	BUTYRYLCHOLINESTERASE AS A DIAGNOSTIC AND THERAPEUTIC TARGET FOR ALZHEIMER’S DISEASE <u>Sultan Darvesh</u>	19
IL-295	NANOSCIENCE APPROACHES TO DIAGNOSIS AND THERAPY OF HUMAN DISEASES <u>Silvia De Paoli</u>	20
IL-4	DISCOVERING ANTAGONISTS OF GLYCOSAMINOGLYCAN-BINDING PROTEINS FOR TREATING THROMBOSIS, CANCER, AND OTHER DISEASES <u>Umesh R. Desai</u>	20
IL-241	NEW PHARMACOLOGICAL TOOLS TO UNDERSTAND AND EXPERIMENTAL DRUGS TO TREAT MILD TRAUMATIC BRAIN INJURY AND RELATED DISORDERS <u>Nigel H. Greig, David Tweedie, Yazhou Li, Ian Tamargo, Barry J. Hoffer, Kumar Sambamurti, Debomoy K. Lahiri and Chaim G. Pick</u>	21
IL-196	SYNTHESIS OF HYDROLYSABLE NANOGELS FOR CONTROLLED TEMPORAL DELIVERY OF VASCULOGENIC FACTORS IN PATTERNED CELLULAR CONSTRUCTS <u>Esmail Jabbari</u>	22
IL-62	CO-SUPPRESSION OF INTRACELLULAR CALCIUM AND OXIDATIVE STRESS, A KEY STRATEGY FOR DEVELOPMENT OF NEUROPROTECTIVE DRUGS <u>Zhi-Gang Jiang</u>	23
IL-6	CHILDHOOD OBESITY AND NUTRITIONAL INTERVENTIONS <u>Emilio Jirillo</u>	24
IL-125	ROLE OF MICROVASCULAR ENDOTHELIAL TYPE B ENDOTHELIN RECEPTOR DURING PREGNANCY. IMPLICATIONS IN HYPERTENSIVE PREGNANCY AND PREECLAMPSIA <u>Raouf A. Khalil</u>	24
IL-251	IMAGING AGENTS FOR DIAGNOSIS OF NEURODEGENERATIVE DISEASES <u>Jeff Kuret</u>	25
IL-126	GENOMICS WITH GRAPHENE NANOTECHNOLOGY <u>Jean-Pierre Leburton</u>	25
IL-301	A COMPREHENSIVE APPROACH TO UNDERSTAND GLYCOSYLATION OF MONOCLONAL ANTIBODIES Jun Qian, David Mahon, Babita Parekh, Qinwei Zhou and <u>Tun Liu</u>	26

IL-7	MECHANICALLY TUNABLE AND DEGRADABLE HYDROGELS FOR REGENERATIVE MEDICINE <u>Tao L. Lowe</u>	26
IL-227	FRAGMENT BASED DESIGN OF NON-ATP COMPETITIVE KINASE INHIBITORS THROUGH REPLACE <u>Campbell McInnes</u>	27
IL-20	THE USE OF BARIATRIC SURGERY IN PATIENTS WITH DIABETES AND OBESITY <u>Jeffrey I. Mechanick</u>	27
IL-170	SMALL-MOLECULE “DRUG-LIKE” TSH RECEPTOR AGONISTS AND ANTAGONISTS <u>Susanne Neumann</u>	28
IL-67	PROSTATE CANCER-SPECIFIC DESTRUCTION OF ANDROGEN RECEPTOR WITH DEUBIQUITINASE INHIBITORS Teresita Reiner, Alicia de las Pozas, Ricardo Parrondo, Deanna Palenzuela and Carlos Perez-Stable	28
IL-165	NITROGEN AND OXYGEN INTERMEDIATES AND ANTIBODY MEDIATED PLATELET CYTOTOXICITY AS PROGNOSTIC MARKERS FOR DENGUE SEVERITY <u>Sunil Premawansa</u> , M.S. Mapalagamage, M.N.S. Bandara, Premawansa G. Mark Loeb, A.D. De Silva and S.M. Handunnetti	29
IL-298	PHOTOTRIGGERABLE LIPID ASSEMBLIES AND THEIR APPLICATIONS FOR DUAL DRUG DELIVERY <u>Anu Puri</u>	30
IL-13	ROLE OF HIF1 α /NF κ B PATHWAY IN OBESITY <u>Matteo A. Russo</u>	31
IL-266	DIETARY RESTRICTION TO PREVENT AD PATHOGENESIS <u>Kumar Sambamurti</u> , Pannerselvam Chinnakannu, Miguel A. Pappolla, Narayan R. Bhat, Debomoy K. Lahiri, Nigel H. Greig and Vasudeva Padmaraju	32
IL-133	USEFUL DESCRIPTORS IN BEYOND RULE-OF-FIVE DRUG DESIGN SPACE? <u>Marina Shalaeva</u>	33
IL-262	DEVELOPING STRATEGIES TO BUILD COMBINED DIRUTHENIUM-PHARMACEUTICAL METALLODRUGS TARGETING CANCER THERAPY <u>Denise de Oliveira Silva</u>	33
IL-271	PRENATAL VITAMIN A DEFICIENCY FACILITATES ALZHEIMER'S DISEASE PATHOGENESIS <u>Weihong Song</u>	34
IL-97	LARGE-SCALE CONTINUOUS MANUFACTURE OF NANO-COCRYSTAL DRUGS AND THERAPEUTICS OR HOW DEFENCE RESEARCH MAY CONTRIBUTE TO PROTECT HEALTH <u>Denis Spitzer</u> and Marc Comet	34
IL-164	EXPANDING CHEMICAL SPACE BEYOND DRUGABILITY-RULES: THE SEARCH FOR MECHANISTICALLY DISTINCT PROTEASOME INHIBITORS <u>Jetze J. Tepe</u>	35

IL-130	TARGET BASED VS PHENOTYPIC SCREENING APPROACHES IN DRUG DISCOVERY <u>Tove Tuntland</u>	36
IL-38	CHEMICAL, PHARMACOLOGICAL AND QUALITY CONTROL STUDIES OF TRADITIONAL CHINESE MEDICINE (TCM) AND ETHNIC MEDICINE <u>Wei Wang</u>	36
IL-123	ADVANCES IN NANOTECHNOLOGY FOR DRUG DELIVERY: A SUMMARY OF 15 YEARS OF RESEARCH AND COMMERCIALIZATION <u>Thomas J. Webster</u>	37
IL-294	SPECIFIC DELIVERY OF DRUGS TO MITOCHONDRIAL TARGETS: MANIPULATING MITOCHONDRIAL FUNCTIONS <u>Volkmar Weissig</u>	37
IL-128	CONTROLLED RELEASED SCAFFOLDING BIOMATERIAL FOR BONE TISSUE ENGINEERING: A NOVEL THERAPEUTIC STRATEGY FOR CLEFTS PATIENTS <u>Adam Qingsong Ye</u>	38
IL-8	QUANTITATIVE MULTIMODAL MULTI-PARAMETRIC IMAGING: ITS INTEGRATION AND APPLICATIONS <u>Yun Zhou</u>	38

SESSION LECTURES

SL-31	MODULATORY EFFECT OF CELASTROL ON TH1/TH2 CYTOKINES PROFILE, TLR2 AND CD3+ T-LYMPHOCYTE EXPRESSION IN A RELAPSING-REMITTING MODEL OF MULTIPLE SCLEROSIS IN RATS <u>Amany A. Abdin</u>	40
SL-61	NOVEL PLATINUM COMPLEXES [Pt(N-N)CL ₄], AS SUGGESTED ANTICANCER DRUGS <u>Anita Abedi, Nasser Safari, Vahid Amani and Amir-Hassan Zarnani</u>	40
SL-172	DEVELOPMENT OF NOVEL CLASS OF M1 ERAP AMINOPEPTIDASE INHIBITORS FOR TREATING AUTOIMMUNE DISEASE <u>Nitish Agrawal</u>	41
SL-17	FROM BASIC RESEARCH TO DRUG DEVELOPMENT - THE STORY OF COPAXONE IN THE TREATMENT OF MULTIPLE SCLEROSIS, MECHANISM OF ACTION, AND NEW APPLICATIONS <u>Rina Aharoni</u>	42
SL-265	BOTTOM-UP APPROACHES FOR POLYMERIC VASCULATURE TISSUE CONSTRUCTS AND CELLULAR MICROENVIRONMENT <u>Shamsher Ali and Alireza Khademhosseini</u>	43
SL-208	VDACs DEFICIENT MICE: AN ORIGINAL MODEL TO TARGET SELECTIVELY METASTASIS <u>Keltoum Anflous-Pharayra</u>	43
SL-68	COMBINATORIAL MULTIVALENT DISPLAY USING A SYNTHETIC, PNA-BASED SCAFFOLD TO CHARACTERIZE LIGAND-RECEPTOR INTERACTIONS <u>Daniel Appella</u>	44

SL-200	TARGETING HIV NUCLEOCAPSID PROTEIN <i>VIA</i> A SMALL MOLECULE PLATFORM AND RELATED PRECLINICAL DEVELOPMENT <u>Daniel Appella</u>	44
SL-213	BIOACTIVE PROPERTIES OF TURKISH ENDEMIC <i>ASTRAGALUS CHRYSOCHLORUS</i> <u>Süle Arı</u> , Semra Hasançebi, Neslihan Turgut Kara and Özgür Çakır	45
SL-155	SYNTHETIC HEPARAN SULFATE MIMETICS AS NOVEL ANTICOAGULANTS: <i>IN VITRO</i> EVALUATION FOR XENOTRANSPLANTATION P.K. Benipal, H.Iwase, R.A. Al-Horani, A.Y. Mehta, R. Karuturi, L. Burdorf, X.F. Cheng, D.G. Harris, R.N. Pierson III, S.C. Robson, D.K.C. Cooper, U.R. Desai and <u>A.M. Azimzadeh</u>	46
SL-183	TARGETING IMMUNE COSTIMULATION USING A NOVEL aCD28 PEGGYLATED FAB AND CD154 BLOCKADE: EFFICACY IN A PRE-CLINICAL TRANSPLANT MODEL <u>Agnes M. Azimzadeh</u> , Tianshu Zhang, Braileanu Gheorghe, Wenji Sun, Anthony Kronfli, Joshua Miller, Siamak Dahi, Natalia Kubicki, Jhade Whoddal, Mehmet C Uluer, Evelyn Sievert, Lars Burdorf, Xiangfei Cheng, Bernard Vanhove and Richard N. Pierson III	46
SL-179	NEW FRAMEWORKS FOR TRANSLATIONAL RESEARCH AT THE MAYO CLINIC: CENTER FOR CLINICAL & TRANSLATIONAL SCIENCES (CCATS) <u>Andrew D. Badley</u>	47
SL-117	BISVANILLIN AND BIS ARYL ETHER SCAFFOLDS FOR ELABORATION OF ANTIATHEROGENIC AGENTS: SYNTHESIS AND BIOLOGICAL ACTIVITIES <u>Michel Baltas</u> , Florence Bedos-Belval, Corinne Bacqué, Christiane André-Barrès and Chantal Carayon	48
SL-132	NANOPORE-BASED DETECTION OF BIOMARKERS FOR CANCER DIAGNOSTICS Jiwook Shim, Ann M. Nardulli, Farhad Kosari, George Vasmatzis, David A. Ahlquist and <u>Rashid Bashir</u>	49
SL-95	MULTIFACETED APPROACH TO CANCER BIOMARKER DISCOVERY IN A CLINICAL SETTING <u>Darrell R. Borger</u>	50
SL-39	THE MOLECULAR BASIS OF PURINERGIC SIGNAL METABOLISM REGULATING HEMOSTASIS AND ECTOPIC CALCIFICATION <u>Demetrios T. Braddock</u>	50
SL-214	3D ANALYSIS OF THE BINDING SITES FOR PREDICTING BINDING AFFINITIES IN DRUG DESIGN Ali Osman Atac, Ozlem Erdas, Ferda Nur Alpaslan and <u>Mehmet Erdem Buyukbingol</u>	51
SL-45	A NATURALLY-DERIVED IMMUNOSUPPRESSIVE DRUG, RAPAMYCIN, DECREASES CIRCULATING TUMOR CELLS IN PATIENTS WITH LYMPHANGIOLEIOMYOMATOSIS <u>Xiong Cai</u> , Gustavo Pacheco-Rodriguez, Mary Haughey, Suowen Xu, Hai-Ping Wu, Thomas N. Darling and Joel Moss	52
SL-10	ACAT1/SOAT1 AS A THERAPEUTIC TARGET FOR ALZHEIMER'S AND OTHER RELATED NEURODEGENERATIVE DISEASES <u>Ta Yuan Chang</u>	52

SL-87	EFFECTS OF ELECTROACUPUNCTURE ON THE ULTRASTRUCTURE OF ICC AND EXPRESSION OF PROTEIN CX43 IN FD RATS Mi Liu, Guoshan Zhang, Wei Hu, Shen Xie, Yuer Liu, Mailan Liu and <u>Xiao-Rong Chang</u>	53
SL-94	INFLUENCE OF ELECTROACUPUNCTURE PRETREATMENT ON SERUM NO AND NOS AND MYOCARDIAL ULTRASTRUCTURES IN MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY Liu Zhao, <u>Wang Chao</u> , Zhang Jiali, Yan Jie, Liu Mi and Chang Xiaorong	53
SL-22	MULTIPARAMETRIC PET VALUES IN THE PREDICTION OF ALZHEIMER'S DISEASE PROGRESS <u>Xueqi Chen</u> , Rongfu Wang, Rui Gao, Haoyin Cao, Dean F. Wong, Yun Zhou	54
SL-209	DEVELOPMENT OF A CLOUD-BASED HISTOLOGY DATABASE FOR COLLABORATIVE CANCER RESEARCH <u>Ke Cheng</u>	54
SL-306	CO-DELIVERY OF ETOPOSIDE AND VORINOSTAT USING POLY(ETHYLENE GLYCOL) BASED BIODEGRADABLE NANOGELS <u>Aruna Chhikara</u> , Parveen Kumar, Madhu Chopra	55
SL-189	SYNERGISTIC EFFECT OF HDAC INHIBITOR (PANOBINOSTAT) AND TOPOISOMERASE INHIBITORS ON CERVICAL CANCER CELLS <u>Madhu Chopra</u> and Lubna Wasim	56
SL-320	ROLE OF NEPRILYSIN IN ALZHEIMER'S DISEASE <u>Nipun Chopra</u> , Kwangsik Nho, Apoorva Bharthur, Justin Long, Kumar Sambamurti , Nigel H. Greig, Andrew J Saykin and Debomoy K Lahiri	56
SL-88	OVERVIEW OF THE CURRENT AND FUTURE ROLE OF IMMUNE CHECKPOINT BLOCKADE IN ONCOLOGY <u>James Cleary</u>	57
SL-115	SYNTHESIS OF COVALENT BIOCHEMOTHERAPEUTICS WITH PROPERTIES OF SELECTIVELY "TARGETED" ANTI-NEOPLASTIC CYTOTOXICITY <u>Cody P. Coyne</u>	58
SL-156	NANOPARTICLE BASED SENSORS WITH BIOLOGICAL APPLICATIONS <u>Georgeta Crivat</u>	58
SL-303	IMMUNOLOGICAL PROPERTIES OF ENGINEERED NANOMATERIALS AND CHALLENGES WITH THEIR PRECLINICAL CHARACTERIZATION <u>Marina A. Dobrovolskaia</u>	59
SL-302	THREE DIMENSIONAL CELL CULTURE MODELS OF THE SOLID TUMOR MICROENVIRONMENT FOR THE TESTING OF PHARMACEUTICAL NANOCARRIERS <u>Gerard G.M. D'Souza</u>	59
SL-240	COMPARISON OF <i>IN VIVO</i> AND <i>IN VITRO</i> TESTS OF RESISTANCE IN <i>PLASMODIUM FALCIPARUM</i> POSITIVE PATIENTS TREATED WITH ARTEMISININ BASED COMBINATION THERAPY IN NORTHWESTERN NIGERIA <u>Mukhtar Dauda</u>	60
SL-319	RELEASE-ACTIVE ANTIBODIES ARE INNOVATIVE ANTIBODIES-BASED DRUGS: NEW WAY TO QUALITY ASSESSMENT <u>Elena S. Don</u> , Vyacheslav V. Grechenko, Suzanna E. Pokhil, Alena A. Borshcheva, Darya V. Barykina, Marina V. Nikiforova, Evgeny A. Gorbunov and Sergey A. Tarasov	60

SL-138	BIOMOLECULE ANALYSIS AND DNA SEQUENCING WITH SOLID-STATE NANOPORES <u>Marija Drndic</u>	61
SL-178	IDENTIFYING NOVEL DIFFERENTIATION AGENTS FOR NEUROBLASTOMA THERAPY <u>Liqin Du, Zhenze Zhao, Xiuye Ma, Yidong Chen, Alexander Pertsemlidis, Susan Mooberry, Matthew Hart, Tzu-Hung Hsiao and Gregory Lin</u>	61
SL-212	DISCOVERY, OPTIMIZATION, AND CHARACTERIZATION OF NOVEL CHLORCYCLIZINE DERIVATIVES FOR THE TREATMENT OF HEPATITIS C VIRUS INFECTION <u>Shanshan He, Jingbo Xiao, Andrés E. Dulcey, Adam Rolt, Zongyi Hu, Xin Hu, Amy Q. Wang, Xin Xu, Noel Southall, Marc Ferrer, Wei Zheng, T. Jake Liang and Juan J. Marugan</u>	62
SL-232	TARGETING ARGININE METHYLTRANSFERASES: IDENTIFICATION AND OPTIMIZATION OF FIRST-IN-CLASS PRMT5 INHIBITOR EPZ015666 (GSK3235025) <u>Kenneth W. Duncan</u>	62
SL-136	DISCOVERY OF FIRST-IN-CLASS DRUGS: TARGET- AND SYSTEMS-BASED APPROACHES <u>Joerg Eder</u>	63
SL-55	BETAINE SUPPLEMENTATION IMPROVES SYSTEMIC METABOLISM AND GLYCEMIA IN HFD-FED MICE <u>Asma Ejaz, Elizabeth Li, Allison Goldfine, Carles Lerin, Miranda Liang, Robert Gerszten and Mary-Elizabeth Patti</u>	63
SL-325	NANO-LAYERED MATERIALS FOR DRUG DELIVERY AND TRAPPING HARMFUL ANIONS IN TUMOR <u>Amin A. El-Meligi</u>	64
SL-219	KRUPPEL LIKE FACTORS AND HEAT SHOCK PROTEIN 27: NEW POTENTIAL MARKERS OF LARYNX AND LUNG CARCINOMAS <u>Tannous Rita, Fadous Khalifé Marie-Claire, Aftimos Georges, Paris Francois and Hadchity Elie</u>	64
SL-197	EXPLORING NEW IDEAS FOR THE ACADEMIC INDUSTRY INTERFACE <u>Stephen Freedman</u>	65
SL-139	INTEGRATION OF <i>IN SILICO</i> PREDICTION IN DRUG DISCOVERY PROCESS <u>Hua Gao</u>	65
SL-29	ASSOCIATIONS BETWEEN THE ALPHA-SYNUCLEIN SPLICED VARIANTS AND MONOAMINERGIC DEGENERATION IN PARKINSON'S DISEASE <u>Rui Gao, Guangjian Zhang, Xueqi Chen, Aimin Yang, Ming Zhang and Yun Zhou</u>	66
SL-185	FRIENDS WITH BENEFITS: WHY PARTNERING WITH AN ACADEMIC CRO MAKES ULTIMATE SENSE <u>Ralph J. Garippa</u>	66
SL-161	NOVEL DRUG CANDIDATES FOR TYPE-2 DIABETES MELLITUS (T2DM): FROM BENCH TO BEDSIDE <u>Mustafa Guzel</u>	67

SL-33	VASCULAR TARGETING SYSTEM - A PROPRIETARY GENE THERAPY PLATFORM TECHNOLOGY <u>Dror Harats, Yael Cohen, Erez Feige and Eyal Breitbart</u>	67
SL-280	EVALUATION OF SECONDARY METABOLITES, MICRO-MACRO ELEMENTS AND ANTIMICROBIAL ACTIVITY OF <i>CINNAMOMUM TAMALA</i> LEAVES <u>Waseem Hassan and Syeda Nida Zainab Kazmi</u>	68
SL-46	INTACT STABLE ISOTOPE LABELED PLASMA PROTEINS FOR MULTIPLEXED LC-MS/MS QUANTIFICATION <u>Adam M. Hawkrige</u>	68
SL-109	INTRATUMORAL MODULATION THERAPY FOR GLIOBLASTOMA <u>Hu Xu, Mitchell Cooper, Cleusa de Oliveira, Eugene Wong, Susanne Schmid and Matthew O. Hebb</u>	69
SL-195	A FULL-360-TRANSLATIONAL PHARMACOLOGY CONCEPT – FROM PRE-CLINICAL EXPERIMENTS TO CLINICAL DATA AND BACK <u>Thomas Herz</u>	69
SL-154	RATIONAL IDENTIFICATION OF ENOXACIN AS A NOVEL V-ATPASE (AND/OR MICRORNA?)-DIRECTED OSTEOCLAST INHIBITOR <u>Lexie Shannon Holliday</u>	70
SL-194	DEVELOPMENT OF A β -ARRESTIN BIASED D2R ANTAGONIST FOR THE TREATMENT OF SCHIZOPHRENIA <u>Edward Holson</u>	71
SL-118	POTENTIAL THERAPEUTIC EFFECT OF HEMATOPOIETIC STEM CELLS ON CEREBELLAR ATAXIA IN ADULT FEMALE RATS SUBJECTED TO CEREBELLAR DAMAGE BY MONOSODIUM GLUTAMATE <u>Horeya E. Korayem, Mohamed Abdo, Magda M. Naim, Soha E. Yones and Somaya Hosny</u>	71
SL-246	A BRAIN-PERMEABLE ANTIOXIDANT TARGETING AB AMYLOID PATHOLOGY <u>Xudong Huang, Ying Shi, Mohammed Abid, Charles R. Vanderburg, Nigel H. Greig, Debomoy K. Lahiri, Alpaslan Dedeoglu and Jack T. Rogers</u>	72
SL-59	TOXICOLOGICAL FINDING IN BEAGLE DOGS AFTER A SINGLE OR 4 WEEKS ORAL GAVAGE OF TRIPTOLIDE <u>Jinqian Liang, Yuwen Qiu, Xinlu Fu and Zhiying Huang</u>	73
SL-230	NEW STRATEGIES FOR FIGHTING MALARIA <u>Hidayat Hussain</u>	74
SL-90	LOGICAL REFINEMENT OF THERAPEUTIC TARGETS FOR GLIOBLASTOMA BASED ON MULTIDISCIPLINARY PROTEOMIC APPROACHES <u>Yasuo Iwadata</u>	75
SL-177	<i>IN VIVO</i> AND <i>IN VITRO</i> IMMUNOSTIMULATORY ACTIVITY OF SRI LANKAN WILD TYPE CARICA PAPAYA L. MATURE LEAF CONCENTRATE IN RATS <u>Chanika D. Jayasinghe and Preethi Udagama</u>	75
SL-328	LIGHT TRIGGERED NANOPARTICLE THERANOSTICS FOR CANCER <u>Amit Joshi</u>	76

SL-160	NANOENCAOSULATION OF COENZYME Q10 BY A NEW EMULSIFICATION PROCESS USED IN COSMETIC <u>Messaouda Kaci, Elmira Arab-Tehrany, Emilie Velot, Gabriel Dostert, Guillaume Gillet, Isabelle Lavissee and Stéphane Desobry</u>	77
SL-111	ENZYMATIC SYNTHESIS OF PROTEIN-MIMIC POLYSACCHARIDES AS CANDIDATE FOR NEW BIOMEDICAL MATERIALS <u>Jun-ichi Kadokawa</u>	77
SL-191	WHEN OPEN INNOVATION MEETS REPURPOSING <u>Edward Kahn</u>	78
SL-24	NOVEL IMMUNOSPECIFIC THERAPY WITH SYNTHETIC MULTI-EPIOTOPE TARGETING AGENT MODULATE MS –LIKE DISEASE BY INDUCTION OF PERIPHERAL REGULATORY MECHANISMS <u>N. Kaushansky, R. Zilkha-Falb, L. Cohen and A. Ben-Nun</u>	79
SL-235	ANTIBACTERIAL POLYKETIDE FROM THE MARINE ALGA-DERIVED ENDOPHITIC <i>STREPTOMYCES SUNDARBANSENSIS</i> : A STUDY ON HYDROXYPYRONE TAUTOMERISM <u>Mouloud Kecha, Ibtissem Djinni, Andrea Defant and Ines Mancini</u>	79
SL-314	MITOCHONDRIAL TARGETING OF THERAPEUTICS <u>Shana Kelley</u>	80
SL-247	INHIBITING EGFR DIMERIZATION USING TRIAZOLYL-BRIDGED DIMERIZATION ARM MIMICS <u>Eileen J. Kennedy</u>	80
SL-276	SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TACRINE DERIVATIVES AND TACRINE-COUMARIN HYBRIDS AS CHOLINESTERASE INHIBITORS <u>Kamil Kuca, S. Hamulakova, L. Janovec, M. Hrabínova, K. Spilovska, J. Korabecny, P. Kristian, E. Nepovimova, P. Mareosva and J. Imrich</u>	81
SL-275	UNTANGLING THE OPPORTUNITIES FROM SOUTH AFRICAN PLANTS <u>Namrita Lall</u>	81
SL-97	NEGATIVE FEEDBACK REGULATION OF INFLAMMATION & NOVEL ANTI-INFLAMMATORY STRATEGY <u>Jian-Dong Li</u>	82
SL-104	DIRECT ARYLATION AND ITS APPLICATION ON SCALE TO THE SYNTHESIS OF PF-06463922, A MACROCYCLIC INHIBITOR OF <i>ALK/ROS1</i> <u>Phuong T. Le</u>	83
SL-256	ALZHEIMER’S DISEASE VACCINES: BREAKTHROUGHS AND SETBACKS <u>Cynthia A. Lemere</u>	83
SL-66	THE METABOLIC AND PHARMACOKINETIC ANALYSIS OF CURCUMINOIDS BASED ON NANOPARTICLE FORMULATIONS BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY <u>Rui Li and Min Ye</u>	84
SL-80	3D QUALITY CONTROL OF ENTIRE MOLECULE COMPLEX OF COMPOUND DANSHEN DRIPPING PILLS <u>Wei Li</u>	85

SL-52	EFFECT OF MOXIBUSTION ON T LYMPHOCYTE SUBSETS OF <i>HELICOBACTER PYLORI</i> -ASSOCIATED GASTRITIS RATS Jing Shen, Yan Peng, Shou-Xiang Yi, Ying-Shuai Feng and <u>Ya-Ping Lin</u>	86
SL-166	MORPHOLOGICAL AND BIOPHYSICAL PROPERTIES OF NATURAL PHOSPHOLIPIDS-BASED BIOMEMBRANES Elmira Arab-Tehrany, A. Jacquot, G. Francius, A. Razafitianamaharavo and <u>M. Linder</u>	86
SL-18	RE-INTRODUCTION OF BOVINE HEPARIN: REGULATORY AND COMPENDIAL ISSUES <u>Robert J. Linhardt</u>	87
SL-101	EFFECT OF SHENYUAN ON ANTI-INFLAMMATORY AND HEMODYNAMIC FACTORS IN A PORCINE MODEL OF ACUTE MYOCARDIAL INFARCTION Wen-yi Liang, Zi-long Zhang, Xin-yuan Zhu, Xue-ru Feng and <u>Mei-lin Liu</u>	87
SL-108	IDENTIFICATION OF ASPIRIN RESPONSE RELATED GENE PROFILES IN THE ELDERLY POPULATION WITH CORONARY ARTERY DISEASE <u>Tengfei Liu</u> , Jingwei Zhang, Yahong Chen, Xueru Feng, Lu wang and Meilin Liu	88
SL-315	DEVELOPMENT OF PROTEIN AGENT TARGETING INTEGRINS $\alpha_v\beta_3$ AT A NOVEL SITE BY RATIONAL PROTEIN DESIGN Ravi Chakra Turaga and <u>Zhi-Ren Liu</u>	89
SL-149	CHEMOENZYMATIC SYNTHESIS OF LOW MOLECULAR WEIGHT HEPARINS <u>Jian Liu</u>	89
SL-159	TOTAL SYNTHESIS OF 6-DEOXYPLADIENOLIDE D AND ASSESSMENT OF SPLICING INHIBITORY ACTIVITY IN A MUTANT SF3B1 CANCER CELL LINE Kenzo Arai, Silvia Buonamici, Betty Chan, Laura Corson, Atsushi Endo, Baudouin Gerard, Ming-Hong Hao, Craig Karr, Kazunobu Kira, Linda Lee, <u>Xiang Liu</u> , Jason T. Lowe, Tuoping Luo, Lisa A. Marcaurelle, Yoshiharu Mizui, Marta Nevalainen, Morgan Welzel O'Shea, Eun Sun Park, Samantha A. Perino, Sudeep Prajapati, Mingde Shan, Peter G. Smith, Parcharee Tivitmahaisoon, John Yuan Wang, Markus Warmuth, Kuo-Ming Wu, Lihua Yu, Huiming Zhang, Guo Zhu Zheng and Gregg F. Keaney	90
SL-145	COMPUTATIONAL APPROACHES TO PK-FRIENDLY (DRUG-LIKE) MOLECULES <u>Franco Lombardo</u>	91
SL-32	A NOVEL, ACTIVATED PROTEIN C-INDEPENDENT ROLE OF PROTEIN S IN REGULATING THROMBOSIS <u>Rinku Majumder</u>	91
SL-116	UNDERSTANDING BIOLOGICAL ACTIVITY AND SELF-ASSEMBLY OF ANTIMICROBIAL CYCLIC LIPODEPSIPEPTIDE USING A MOLECULAR APPROACH <u>Jose C. Martins</u> , Matthias De Vleeschouwer, Niels Geudens, Annemieke Madder, Davy Sinnaeve and Tim Van Kersavond	92
SL-81	NOVEL LIPOSOMES INCLUDING TREHALOSE SURFACTANT INHIBIT THE GROWTH OF TUMOR CELLS ALONG WITH APOPTOSIS <u>Yoko Matsumoto</u>	92
SL-74	SYNTHETIC LETHAL TARGETING OF COLORECTAL CANCER CELLS THROUGH SOD1 INHIBITION Babu V. Sajesh, Zelda Lichtensztejn and <u>Kirk J. McManus</u>	93

SL-47	ADVANCING MOLECULAR DIAGNOSTICS AND PRECISION MEDICINE USING NON-CODING RNA VARIANTS <u>Prasun J. Mishra</u>	94
SL-112	NOVEL THERAPEUTIC STRATEGIES FOR DELIVERING THERAPEUTICS TO BRAIN - CIRCUMVENTING THE EFFLUX AND PERMEABILITY BARRIERS <u>Ashim K. Mitra</u>	94
SL-207	EFFECT OF <i>ALTHAEA OFFICINALIS L.</i> ROOT EXTRACT ON CONCENTRATION OF BLOOD PROTEINS IN MICE <u>Mehrdad Modaresi</u>	95
SL-310	TREATMENT STRATEGIES THAT ENHANCE THE EFFICACY AND SELECTIVITY OF MITOCHONDRIA-TARGETED ANTICANCER AGENTS <u>Josephine S. Modica-Napolitano</u>	95
SL-54	NOVEL HIGH DENSITY PEPTIDE ARRAYS WITH COMBINATORIAL DEPOSITION OF AMINO ACID PARTICLES FOR ANTIBODY PROFILING <u>A. Nesterov-Müller</u> , F.F. Loeffler, L. Hahn, A. Fischer, T. Förtsch, V. Bykovskaya, M. Sedlmayr, B. Ridder, D. Althoun, C. von Bojničić-Kninski, R. Popov, F.R. Bischoff and F. Breitling	96
SL-171	IMMUNE RESPONSES TO CANDIDATE VACCINE ANTIGENS DELIVERED THROUGH NAKED PLASMIDS AND MYCOBACTERIAL VECTORS <u>AbuSalim Mustafa</u>	96
SL-188	DESIGN AND SYNTHESIS OF NOVEL δ OPIOID AGONISTS AND THEIR PHARMACOLOGIES <u>Hiroshi Nagase</u> , Yoshikazu Watanabe, Kohei Hayashida, Daisuke Saito, Toshihiro Takahashi, Junichi Sakai, Eriko Nakata, Takashi Kanda, Shigeto Hiroayama, Takashi Iwai, Hideaki Fujii and Tomio Yamakawa	97
SL-318	SINGLE-STEP FORMATION AND CELLULAR RESPONSE OF VESICLES AND DISK-LIKE BICELLES Wafa Aresh, Ying Liu, Jessica Sine, Derek Thayerc, Anu Puri and <u>Mu-Ping Nieh</u>	98
SL-182	DISCOVERY AND DEVELOPMENT OF SMALL MOLECULE MODULATORS OF APOPTOSIS AS NEW ANTICANCER DRUGS <u>Zaneta Nikolovska-Coleska</u>	98
SL-203	FORMULATION OF FLOATING GASTRORETENTIVE METRONIDAZOLE MICROSPHERES USING CASSAVA STARCH (<i>MANIHOT ESCULENTA</i>) AS POLYMER <u>Oluwatoyin A. Odeku</u> , Aderemi A. Aderogba, Olufunke D. Akin-Ajani and Tolulope O. Ajala	99
SL-89	ANTICANCER, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME SYNTHESIZED URSOLIC ACID DERIVATIVES <u>Adebola Omowunmi Oyedeji</u>	99
SL-83	HYPOXIA ISCHEMIA IN THE NEONATAL RAT: THERAPEUTIC HYPOTHERMIA AND MAST CELL STABILIZATION AS ADJUNCT PHARMACOTHERAPY <u>Shyama D. Patel</u>	100
SL-255	IODOTHYRONINE DEIODINASES: DETERMINATION OF THEIR ACTIVITIES BY NOVEL RADIOMETRIC ENZYME ASSAYS <u>Stanislav Pavelka</u>	100

SL-131	GINKGOLIDE B REDUCES TLR4 AND INFLAMMATORY PROTEIN EXPRESSION IN GLUCOSE-TREATED HUVECS <u>Ruomei Qi and Wenjia Sun</u>	101
SL-11	CHEMOKINES AS DRUG TARGETS FOR CARDIOVASCULAR DISEASES <u>Krishna Rajarathnam</u>	101
SL-148	THE USE OF MICROSCALE THERMOPHORESIS, VIRTUAL SCREENING AND THERMAL SHIFT ASSAYS FOR THE RAPID IDENTIFICATION OF FRAGMENTS ACTIVE AGAINST HUMAN KINASE Dennis Breitsprecher, Kwame Amaning, Stefan Duhr and <u>Alexey Rak</u>	102
SL-69	DEVELOPMENT OF CIRCADIAN RHYTHM BASED FORMULATION OF ATOMOXETINE HYDROCHLORIDE FOR THE TREATMENT OF ATTENTION DEFICIT HYPERACTIVITY DISORDER <u>Akila Ramanathan, P. Chandran, Rao Gopal and T.K. Ravi</u>	102
SL-260	FROM PATIENT BIOSPECIMENS TO THERAPEUTIC SOLUTIONS: NEW DEPARTURE IN DRUG DISCOVERY <u>Araz A. Raoof and Jeroen Aerssens</u>	103
SL-242	MOLECULAR HYBRIDS: AN INNOVATIVE APPROACH IN DRUG DISCOVERY <u>Diwan S. Rawat</u>	103
SL-217(a)	NANOSCALE DRUG CARRYING SYSTEMS TO PREVENT PLACENTAL PASSAGE OF HARMFUL DRUGS TO THE FETUS IN A PREGNANT RODENT MODEL <u>Jerrie S. Refuerzo</u>	104
SL-151	SIMILARITY AND DIFFERENCE IN MECHANISMS OF ACTION OF ANTISTAPHYLOCOCCAL ANTIBIOTICS MUPIROCIN AND BATUMIN REVEALED BY <i>IN-SILICO</i> MOLECULAR DOCKING J.Y. Kim, V.V. Klochko, E.V. Zhuravel, M.A. Soldatkina and <u>O.N. Reva</u>	105
SL-261	COOPERATIVE RNA MODULATION OF NEURODEGENERATIVE DISEASE TRANSCRIPTS TO IMPROVE IRON HOMEOSTASIS WHILE ALSO PROVIDING ANTI-AMYLOID EFFICACY <u>Jack T. Rogers</u> , Kevin Hodgetts, Yanyan Liu, Qinqun Li, Catherine M. Cahill and Xudong Huang	105
SL-26	A HIGHLY EFFICIENT <i>IN VIVO</i> THERAPY FOR MELANOMA AND OTHER CANCER TYPES <u>Karli Rosner</u> , Fredric P. Manfredsson, Evangelia Kirou, Rhyomi Sellnow, Judith Abrams, Seongho Kim, Tal Rosner and Darius R. Mehregan	106
SL-322	PROLONGED ACTING NANOMEDICINE BASED ANTI-HIV DRUG DELIVERY TARGETING GUT ASSOCIATED LYMPHOID TISSUES <u>Upal Roy</u> , Hong Ding, Sudheesh P Kanthikeel, Andrea Raymond, Elena M. Kaftanovskaya, Elena Batrakova and Madhavan Nair	107
SL-143	RATIONALLY DESIGNED ALLOSTERIC EFFECTORS OF HEMOGLOBIN EXHIBIT SUPERIOR ANTISICKLING PROPERTIES <u>Martin K. Safo</u> , Tanvi Deshpande, Mohini Ghatge, Guoyan Xu, Apurvasena Parikh, Jurgen Venitz, Yan Zhang and Osheiza Abdulmalik	107
SL-25	NATURAL AND SYNTHETIC CAFFEIC ACID-BASED OLIGOMERS: ANTI-OXIDATIVE, ANTI-ELASTASE AND VEGF MODULATORY ACTIVITIES FOR TREATMENT OF EMPHYSEMA <u>Masahiro Sakagami</u> , Sneha Dhapare, Norbert F. Voelkel and Umesh R. Desai	108

<i>SL-76</i>	SOLUTE CARRIER 6A1 (SLC6A1; GAT1) TRANSPORTER SUBFAMILY AS A TARGET FOR NOVEL MEMORY-ENHANCING DRUGS <u>Kinga Sałat</u>	109
<i>SL-202</i>	USE OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING TO ASSESS DRUG INTERACTION POTENTIAL OF ANTIBODY-DRUG CONJUGATES (ADCs) <u>Divya Samineni</u> , Yuan Chen, Sophie Mukadam, Harvey Wong, Ben-Quan Shen, Dan Lu, Cornelis Hop, Sandhya Girish, Jin Yan Jin, Bert Lum and Chunze Li	110
<i>SL-110</i>	ULEINE <u>Cid Aimbiré M. Santos</u>	110
<i>SL-311</i>	THE EFFECT OF ULTRASOUND OF AMPLITUDE AND EXPOSE TIME ON THE PARTICLE SIZE AND SOLUBILITY OF NAPROXENE SODIUM <u>Gökhan Savaroğlu</u> , Lütfi Genç, Aygün Ceren İldaşer, Evrim Hür and Müjdat Çağlar	111
<i>SL-237</i>	DEVELOPMENT OF POTENT DUAL BROMODOMAIN-KINASE INHIBITORS FOR CANCER THERAPY <u>Ernst Schonbrunn</u>	112
<i>SL-288</i>	IMMUNOMODULATORY POTENTIAL OF ISOLATED CONSTITUENTS FROM <i>SALIX CAPREA</i> AND SIMULTANEOUS QUANTIFICATION, METHOD VALIDATION OF FIVE POLAR SECONDARY METABOLITES BY LC/MS <u>Syed Wajahat Amin Shah</u>	112
<i>SL-329</i>	MAGNETOFECTION ENHANCING SUICIDAL GENE THERAPY OF UTERINE FIBROID TUMOR CELLS <u>Shahinaz Shalaby</u> , Mostafa K. Khater, David Curiel, Micheal Diamond and Ayman A Al-Hendy	113
<i>SL-96</i>	EVALUATION OF ANTIOXIDANT POTENTIALS AND RADIOPROTECTIVE PROPERTIES OF TROPICAL GINGER IN ALBINO RATS <u>G.J. Sharma</u>	113
<i>SL-218</i>	<i>IN SILICO</i> IDENTIFICATION OF PROMISCUOUS CTL EPITOPES OF MYMA OPERON PROTEINS OF <i>MYCOBACTERIUM TUBERCULOSIS</i> FOR A EPITOPE BASED VACCINE DESIGN <u>Sadhna Sharma</u> , Monika Sharma, Iti Saraav, Kirty Pandey, Prasun Dutta and Anshu Bhardwaj	114
<i>SL-50</i>	UNDERSTANDING TRACER DIFFUSION IN TUMOR TISSUE FOR HYPOXIA IMAGING USING REACTION-DIFFUSION MODELING: MATCHING COMPUTATIONAL SIMULATION TO DYNAMIC [¹⁸ F]FMISO PET MEASUREMENTS IN TUMORS <u>Kuangyu Shi</u> , Christine Bayer, Constantin-Alin Maftei, Florian C. Gaertner, Sabrina T. Astmer, Jan J. Wilkens, Fridtjof Nüsslin, Peter Vaupel and Sibylle I. Ziegler	114
<i>SL-103</i>	ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF ENDOPHYTIC FUNGAL EXTRACT OF RAUVOLFIA SERPENTINA <u>Santosh Kumar Singh</u> , Madhu Verma, Amit Ranjan and Rajesh Kumar Singh	115
<i>SL-215</i>	ICCB-LONGWOOD: EXAMPLES OF HOW AN ACADEMIC SCREENING CORE CAN ENABLE INDUSTRY COLLABORATIONS <u>Jennifer Smith</u>	116

SL-270	INSIGHTS FROM MOLECULAR DYNAMICS STUDIES TO COMBAT ISONIAZID - RESISTANT TUBERCULOSIS <u>M. Elizabeth Sobhia and Vivek Kumar</u>	117
SL-299	GENERIC NANOMEDICINES: PHARMACOKINETIC AND BIOANALYTICAL EVALUATION <u>Stephan T. Stern</u>	117
SL-326	NANO-FORMULATION MODIFIED DRUG PHARMACOKINETICS AND THERAPY <u>Stephan Stern</u>	118
SL-144	NANOTECHNOLOGY-BASED TOOLS FOR THE STUDY OF THE AFFINITY AND SELECTIVITY OF ANTIGENS FOR SMALL MOLECULAR TARGETS UNDERLYING DRUG DISCOVERY AND THERAPEUTICS <u>Michael A. Strosio, Mitra Dutta, Xenia Meshik, Sidra Farid, Yi (Elaine) Lan, Souvik Mukherjee, Min Choi, Undarmaa Bat-Erdene, Devanshi Parikh, Saadia Ranginwala, Debopam Datta, Ke (Robin) Xu, Preeti Pratap and TsaiChin Wu</u>	119
SL-257	NOVEL INHIBITORS AND CHEMICAL PROBES TO STUDY THE PROTEIN ARGININE DEIMINASES (PADS) <u>Venkat Subramanian</u>	119
SL-73	STUDY ON ANTI-LUNG CANCER EFFICIENCY OF CENTIPEDE EXTRACTION <i>IN VITRO</i> AND <i>IN VIVO</i> <u>Xue-Fei Tian, Yuan Chen, Zhi-Qi Wang, Sha Tian, Qing Zhou and Gang Pei</u>	120
SL-137	INHIBITING C-REACTIVE PROTEIN FOR THE TREATMENT OF CARDIOVASCULAR DISEASE – WAY INTO CLINICAL STUDIES <u>Jan Torzewski, Oliver Zimmermann, Katharina Weber, Matthias Graf and Oliver Zimmermann</u>	121
SL-252	PEPTOID-COMBINATORIAL STRATEGIES TO TARGET NON-PROTEIN BIOMARKERS IN THE TUMOR MICROENVIRONMENT <u>D. Gomika Udugamasooriya</u>	121
SL-326(a)	A MEDICINAL CHEMISTRY APPROACH TO GAINING PHARMACOLOGICAL CONTROL OF IMMUNITY <u>Nicholas Mark Valiante</u>	122
SL-27	DEUTERATED POLYUNSATURATED FATTY ACIDS (dPUFAs) OVERCOME LIPID PEROXIDATION DAMAGE IN CNS DISORDERS <u>Lex Van der Ploeg, Charles Cantor, Robert De Jager, Harry Saal, Bob Molinari and Misha Shchepinov</u>	122
SL-150	PEPTIDE NANOTUBES FOR THE NON-INVASIVE DETECTION OF BIOMOLECULE SECRETION FROM CELLS <u>Noah S. Franklin, Christian Trippe, Lisa Perreault, Douglas Andrews, Raymond B. Lawton, Joshua Stuckey, Maricris Mayes, Vijaya B. Chalivendra and Milana C. Vasudev</u>	123
SL-35	APPLICATION OF PARAMETRIC IMAGING USING ¹⁸ F-FDG PET/CT DYNAMIC MULTI-BED SCANNING IN DIFFERENTIAL DIAGNOSIS OF PULMONARY LESIONS <u>Rong Fu Wang, Wang Qiang, Zhang Jian-Hua and Zhou Yun</u>	123
SL-43	SITUATION AND PROGRESS OF TUMOR ANGIOGENESIS TARGETED IMAGING AND THERAPY <i>VIA</i> RADIONUCLIDE TRACING TECHNIQUES <u>Rong Fu Wang</u>	124

SL-77	INJECTABLE AND BIODEGRADABLE POLYMERIC BIOMATERIALS FOR REGENERATIVE MEDICINE <u>Shanfeng Wang</u>	124
SL-284	PHARMACOLOGICAL MANIPULATION OF SHIP-1 MODULATES HUMAN T LYMPHOCYTE ADHESION AND MIGRATION Jennifer A Ball, William Wood and <u>Stephen G. Ward</u>	125
SL-220	ULEINE, A BOOSTER OF THE IMMUNE RESPONSE: ACTIVITY ON AIDS PATIENTS <u>A.M. Weffort-Santos</u> and J.D. Federlin	125
SL-316	STRESS GRANULES AND NEURODEGENERATION: NOVEL TARGETS FOR THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS AND ALZHEIMER'S DISEASE <u>Benjamin Wolozin</u>	126
SL-142	TARGETING CALCITONIN RECEPTOR EXPRESSED BY BRAIN TUMOUR INITIATING CELLS OF GLIOBLASTOMA MULTIFORME <u>Peter J. Wookey</u>	127
SL-245	EFFECTIVE AND LESION-FREE CUTANEOUS INFLUENZA VACCINATION <u>Mei X. Wu</u> , Ji Wang and Bo Li	127
SL-19	ANTI-TUMOR EFFECT OF THE NEWLY SYNTHESIZED HYDROXYCHLOROQUINE CONJUGATE-AHQ <u>Hong-tao Xiao</u> , Dong-ke Yu, Rong-sheng Tong, Jun-lan Chuan and Li-juan Zhang	128
SL-120	NANOPARTICLES FOR NEURAL REGENERATION <u>Qiaobing Xu</u>	128
SL-70	BIODEGRADABLE PHOTOLUMINESCENT POLYMERS AND MOLECULES FOR TISSUE ENGINEERING, DRUG DELIVERY, AND IMAGING <u>Jian Yang</u>	129
SL-75	RESIDUES OF ENROFLOXACIN IN TISSUES OF FRESHLY SLAUGHTERED BROILER CHICKENS AVAILABLE IN LOCAL MARKET OF JHANG CITY (PAKISTAN) <u>M. Younus</u> , T. Abbas, M. Zafar, S. Raza, A. Khan, M. Aslam, S. Jaleel and A. Idress	129
SL-176	DISCOVERY OF ORALLY ACTIVE FSHR ALLOSTERIC MODULATORS <u>Henry Yu</u>	130
SL-190	PRELIMINARY ASSESSMENT OF 99MTC-SALMETEROL XINAFATE AS A POTENTIAL BONE-IMAGING AGENT <u>Wafaa A. Zaghary</u> , Mohammed A. Motaleb and Manal M. Anwar	130
SL-15	COMPARISON OF TWO METHODS FOR SEMIQUANTITATIVE EVALUATION OF DATSCAN <u>Jianhua Zhang</u> , Esther Mena Gonzalez, Yan Fan, Rongfu Wang, Melin Vranesic, Zoltan Mari and Zsolt Szabo	131
SL-102	SEPIN-1, A NOVEL SEPARASE INHIBITOR, FOR BREAST CANCER THERAPY <u>Nenggang Zhang</u> , Ha Do, Lacey E. Dobrolecki, Silviya Demerzhian, Jessica T. Li, Michael T. Lewis, Scott R. Gilbertson, and Debananda Pati	131

SL-184	FIRST MULTI-SITE STUDY ON QUANTITATIVE DYNAMIC WHOLE-BODY FDG PET-CT IN ONCOLOGIC IMAGING <u>Sulei Zhang</u> , Li Huo, Changjing Zuo, Xueqi Chen, Wenchan Li, Bing Cui, Yubing Wang, Fang Li, Yun Zhou	132
SL-307	CHALLENGES AND CONSIDERATIONS IN NANO-DRUG CHARACTERIZATION <u>Jiwen Zheng</u>	133
SL-206	DEVELOPING SMALL-MOLECULE BAX ACTIVATORS FOR CANCER THERAPY <u>Jia Zhou</u>	133
SL-3	DEVELOPMENT OF SEROTONIN 2C RECEPTOR POSITIVE ALLOSTERIC MODULATORS FOR TREATMENT OF NEUROPSYCHIATRIC DISEASES Christopher Wild, Rachel M. Hartley, Chunyong Ding, Carrie McAllister, Noelle C. Anastasio, Robert G. Fox, Sonja Stutz, Suzanne Tomlinson, Kathryn A. Cunningham and <u>Jia Zhou</u>	134
SL-201	VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION COMPARED TO CLINICAL AND PATHOLOGICAL FEATURES IN OVARIAN CANCER Inbar Ben Shachar, Hasan Zedan, Svetlana Gandler and <u>Jamal Zidan</u>	134

POSTERS

PO-39	SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL COPPER COMPLEXES WITH BITHIAZOLE <u>Anita Abedi</u> , S. Nasser Ostad, Vahid Amani and Zohreh Mehri Lighvan	135
PO-100	DISCOVERY OF NEW AZO DYES AS POTENTIAL NON-GENOTOXIC FOOD COLORS <u>Olajire A. Adegoke</u>	135
PO-81	THROMBIN ALLOSTERISM: STRUCTURALLY SIMILAR MONOSULFATED BENZOFURANS DISPLAY VARIABLE REGULATION OF THE PROTEASE <u>Daniel Afosah</u> , Stephen Verespy, Rajesh Karuturi and Umesh R. Desai	136
PO-53	PATTERNS OF DRUG USE AND ADHERENCE IN THE MANAGEMENT OF HYPERTENSION IN A HEALTH CARE FACILITY IN WARRI, DELTA STATE, NIGERIA <u>Ogheneovo Clement Aghoja</u>	137
PO-87	PROFILE OF DRUG THERAPY PROBLEMS AND INTERVENTIONS IN HYPERTENSIVE PATIENTS <u>Rita O. Akonoghrere</u> , Azuka C. Oparah and Jeffrey S. Soni	137
PO-16	EVALUATION OF THE ANTI-INFLAMMATORY AND SEDATIVE EFFECTS OF LEAF AQUEOUS EXTRACT OF <i>WITHANIA SOMNIFERA</i> (L.) <i>DUNAL</i> (<i>SOLANACEAE</i>) IN RATS AND MICE Oluchi N Mbamalu, Munira Ismail, Nabeel Dudhia, Nadeem Noordien, Zukiswa Mabuya, Oatlohostse Kakutsi and <u>George Jimboyeka Amabeoku</u>	138
PO-65	CHOLESTEROL OXIDASE P450 ENZYMES IN <i>MYCOBACTERIUM TUBERCULOSIS</i> : STRUCTURE-GUIDED APPROACH FOR DRUG TARGETING AND SELECTIVE INHIBITION <u>Cecilia N. Amadi</u> , Kirsty J. Mclean, Colin W. Levy, Anthony G. Coyne, Chris Abell, David Leys and Andrew W. Munro	139

PO-82	EFFECTS OF PHARMACOLOGICALLY TARGETING THE REVERBs ON SLEEP AND WAKEFULNESS <u>Ariadna Amador</u> , Salvador Huitron-Resendiz, Amanda J. Roberts, Laura S. Solt and Thomas P. Burris	139
PO-70	NANOFUNCTIONALIZATION OF 3D CONSTRUCT HYDROGELS FOR TISSUE ENGINEERING APPLICATION Rana Kadri, Ali Tamayol, Laura Sanchez-Gonzalez, Stéphane Desobry, Michel Linder and <u>Elmira Arab-Tehrany</u>	140
PO-57	FLAVONOIDS; CONCRETE NUTRITIONAL COVERS AGAINST IMMUNOPATHOLOGIES <u>Lotfollah Behroo</u> , Farideh Shishehbor and Mehri Ghafouriyan/Ghafourian	140
PO-68	PEANUT-ALLERGENS EXTRACTION/CHARACTERIZATION: EVIDENCES FOR CONFORMITY WITH FOOD-LABELING <u>Lotfollah Behroo</u> , Farideh Shishehbor and Lotfollah Behroo	141
PO-61	ANALYSIS OF FAMOTIDINE AND RABEPRAZOLE IN COMBINED DOSAGE FORM BY RP – HPLC Kuldeep Singh, Ankur Singh, Prashant Musmade and <u>Krishnamurthy Bhat</u>	142
PO-48	AK301: A NOVEL MITOTIC ARREST AGENT THAT PROMOTES THE MITOSIS-TO-APOPTOSIS TRANSITION <u>Michael Joseph Bond</u> , Avijeet Chopra, Amy Anderson and Charles Giardina	142
PO-84	BIOASSAY-GUIDED ISOLATION AND STRUCTURAL ELUCIDATION OF ANTIDIABETIC PRINCIPLE OF METHANOL LEAF EXTRACT OF <i>NEWBOULDIA LAEVIS</i> (P. BEAUV) <u>Joel Aondohulugh Bosha</u> , Aruh Ottah Anaga and Isaac Uzoma Asuzu	143
PO-85	ORAL ADMINISTRATION OF MONTMORILLONITE-ILLITE CLAY MINERAL ALLEVIATES CLINICAL SYMPTOMS OF COLITIS INDUCED BY DEXTRAN SODIUM SALT IN BALB/C MICE <u>Anne Breitrück</u> , Claus Kerkhoff, Kai Sempert, Jacqueline Hofrichter and Steffen Mitzner	143
PO-59	EFFECTS OF METHYLSULFONYLMETHANE ON VIABILITY OF K562 CELL LINE <u>Zeliha Buyukbingol</u> and Arzu Z. Karabay	144
PO-36	DEVELOPMENT OF A STRUCTURAL MODEL FOR THE HUMAN DOPAMINE TRANSPORTER PROTEIN (hDAT) <u>Matthew C. Castellana</u> , Tarek M. Mahfouz and Andrea Castellar	145
PO-23	CHEMICAL IDENTIFICATION OF A PROANTHOCYANIDIN POLYMER FROM THE MEDICINAL HERB GRAPTOPETALUM PARAGUAYENSE FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA <u>Chia-Chuan Chang</u> , Li-Chen Wu, Chi-Ying F. Huang, Shih-Lan Hsu and Chia-Chuan Chang	145
PO-92	CREB BINDING PROTEIN (CBP) INHIBITION AND TARGET ENGAGEMENT <u>Eugene L. Piatnitski Chekler</u>	146
PO-90	Wnt/BETA-CATENIN SIGNAL REGULATS VASCULAR CALCIFICATION BY REGULATE ANK EXPRESSION <u>Beidong Chen</u> , Ruomei Qi, Cunyu Wang, Xian Wang and Gexin Zhao	146

PO-25	RE-CRYSTALLIZATION AND MICRONIZATION OF ACTIVE PHARMACEUTICAL INGREDIENTS USING THE SUPERCRITICAL FLUID TECHNOLOGY <u>Yan-Ping Chen</u> , Chun-Da Chen, Pei-Hua Chen and Muoi Tang	147
PO-5	BIODEGRADABLE INTERSTITIAL RELEASE POLYMER LOADING A NOVEL SMALL MOLECULE TARGETING AXL RECEPTOR TYROSINE KINASE AND REDUCING BRAIN TUMOUR MIGRATION AND INVASION <u>Tzzy-Wen Chiou</u> , Jeanne Hsieh, Ssu-Yin Yen, Syuan-Rong Chen, Shuang-En Chuang, Hong-Meng Chuang, Mao-Hsuan Huang, Shinn-Zong Lin and Horng-Jyh Harn	147
PO-105	COMPUTER AIDED FORMULATION AND CHARACTERIZATION OF PROPRANOLOL HCL BUCCAL TABLET USING POLYMERIC BLEND <u>Muhammad Zeeshan Danish</u> , Nadeem Irfan Bukhari, Sana Hanif, Nadeem Irfan, Nisaar Hussain, Zeeshan Danish, Muhammad Ali, Bushra Nasir, Javed Iqbal, Bilal Tahir, Hamid Saeed, Rubina Ali and Zikrya Saleem	148
PO-95	NOVEL TREATMENT DESIGNED TO STOP MULTIPLE SCLEROSIS PROGRESSION, BASED ON SYNCITIN INHIBITION <u>Enrique de Font-Réaulx</u>	148
PO-64	A NEW TREATMENT FOR CLINICAL STABILIZATION IN PARKINSON'S DISEASE <u>Enrique de Font-Réaulx</u>	149
PO-41	SPILANTHOL EXTRACTION FROM <i>ACMELLA OLERACEA</i> USING SUPERCRITICAL CARBON DIOXIDE <u>Verônica Santana de Freitas</u> , I.A. M. Zelioli, R.A.F. Rodrigues, J.E. Carvalho and F.A. Cabral	149
PO-53	ORAL DELIVERY OF BIOPHARMACEUTICS: CHARACTERIZATION AND EVALUATION OF MUCUS PERMEATING NANOPARTICLES <u>Irene Pereira de Sousa</u> , Beatrice Cattoz, Peter C. Griffiths and Andreas Bernkop-Schnürch	150
PO-33	CHARACTERIZATION AND PREPARATION OF BENZOTRIAZOLE - PALLADIUM COMPLEX LOADED SOLID LIPID NANOPARTICLES <u>Gökhan Dikmen</u> , Deniz Hür and Lütfi Genç	151
PO-7	EVALUATION OF THE METHANOLIC EXTRACT OF MISTLETOE (<i>TAPINANTHUS BANGWENSIS</i>) LEAVES GROWN ON ORANGE TREES FOR THE PHYTOCHEMICAL PROPERTIES AND ITS PHYSIOLOGICAL EFFECTS ON STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN LABORATORY ANIMALS <u>F.O. Ekhaize</u> , K.M. Agboh and S. Uanseoje	152
PO-35	A CASE OF INSULIN NEUROPATHY: RARE ADVERSE EFFECT OF INSULIN THERAPY <u>Bülent Erğün</u>	152
PO-44	MYCOTOXINS IN DRUG DISCOVERY: EXPOSURE ASSESSMENT BY <i>IN VITRO</i> BIOTRANSFORMATION FOR THE PREDICTION OF RELEVANT TEST CONCENTRATIONS <u>Christiane Kruse Fæste</u> , Silvio Uhlig and Lada Ivanova	153
PO-75	THE IONOPHORIC MYCOTOXIN ENNIATIN B INDUCES NON-APOPTOTIC CELL DEATH BY LYSOSOME DESTABILIZATION <u>Christiane Kruse Faeste</u> , Lada Ivanova and Anita Solhaug	153

PO-31	RATIONAL DESIGN OF NEW HIV REVERSE TRANSCRIPTASE INHIBITORS <u>T. Frączek</u> and <u>P. Paneth</u>	154
PO-76	<i>IN VITRO</i> SCREENING OF ANTICANCER ACTIVITY OF 38 SYNTHETIC QUINOID DERIVATIVES ON HT-29 COLORECTAL CANCER CELL LINE <u>L.A. Franco</u> , <u>J.F. Acuña</u> , <u>J.P. Castro</u> , <u>Y.C. Ocampo</u> , <u>D.E. Rivera</u> , <u>R. Gaitán</u> , <u>F.A. Aristizabal</u> and <u>K. Müller</u>	154
PO-77	COLOMBIAN CARIBBEAN PLANT EXTRACTS WITH CYTOTOXIC EFFECT AGAINST HUMAN CANCER CELLS <u>L.A. Franco</u> , <u>Daneiva Caro Fuentes</u> , <u>V. Teherán</u> , <u>Y.C. Ocampo</u> , <u>R. Salas</u> and <u>F. Díaz</u>	155
PO-52	PREPARATION AND CHARACTERIZATION OF CIS-PLATIN LOADED NANO LIPID PARTICLES AND INVESTIGATION OF THEIR ANTICANCER ACTIVITY <u>Lütfi Genç</u> , <u>Gökhan Dikmen</u> and <u>Şennur Görgülü Kahyaoğlu</u>	155
PO-18	IMMUNOMODULATION OF SOME SELECTED CYTOKINES IN WISTAR RATS BY A CRUDE EXTRACT OF <i>HALICLONA (SOESTELLA)</i> SP, A SRI LANKAN MARINE DEMOSPONGE <u>Varuni K Gunathilake</u> , <u>Wanigasekara D. Ratnasooriya</u> and <u>Preethi V. Udagama</u>	156
PO-51	EFFECT OF DIETARY EPA AND DHA SUPPLEMENTATION ON FATTY ACID METABOLISM IN STATIN-ADMINISTERED SHR.CG- <i>LEPR^{CP}</i> /NDMCR RATS, A METABOLIC SYNDROME MODEL <u>Michio Hashimoto</u> , <u>Abdullah Al Mamun</u> , <u>Masanori Katakura</u> , <u>Yoko Tanabe</u> , <u>Setsushi Kato</u> , <u>Kyoko Kato</u> and <u>Osamu Shido</u>	157
PO-63	AMINOPHOSPHONATES AS NOVEL ACTIVITY-BASED PROBES FOR MATRIPTASE-2 <u>D. Häußler</u> and <u>M. Gütschow</u>	158
PO-9	ANTIOXIDANT ACTIVITY AND FREE RADICAL SCAVENGING PROPERTIES OF CAPTOPRIL <u>Mohsen Asori</u> and <u>Akbar Hedayatizadeh-Omran</u>	159
PO-93	THE MAJORITY OF IRANIAN <i>ASPERGILLUS</i> ISOLATED FROM CLINICAL SPECIMENS HAS MIC NEAR THE MIC OF STANDARD STRAINS OF THESE FUNGI <u>Seyed Jamal Hashemi Hezave</u> , <u>Velashjerdifarahani Mohammadreza</u> , <u>Hashemi Farshad</u> and <u>Zakeri Abolfazl</u>	159
PO-66	MONTMORILLONITE-ILLITE CLAY MINERAL REDUCES UREMIC TOXINS AND VASCULAR PATHOLOGIES IN RATS WITH CHRONIC RENAL FAILURE <u>Jacqueline Hofrichter</u> , <u>Claus Kerkhoff</u> , <u>Anne Breitrück</u> , <u>Kai Sempert</u> and <u>Steffen Mitzner</u>	160
PO-32	CHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF <i>SIDERITIS SYRIACA</i> SUBSP. <i>NUSAIRENSIS</i> AND <i>SIDERITIS ARGYRAE</i> ESSENTIAL OIL AND METHANOLIC EXTRACTS <u>Gokalp Iscan</u> , <u>Yavuz Bulent Kose</u> , <u>Fatih Goger</u> , <u>Betül Demirci</u>	160
PO-26	IMMUNE RESPONSE AND MACROPHAGE POLARIZATION ON MINERALIZED CELL SHEETS SEEDED WITH HUMAN MESENCHYMAL AND ENDOTHELIAL PROGENITOR CELLS <u>Esmail Jabbari</u>	161

PO-29	COMPARISON BETWEEN HER2 EXTRACELLULAR DOMAIN IN SERUM AND HER2 OVEREXPRESSION IN BREAST CANCER TISSUE IN THE SAME PATIENTS <u>Zidan Jamal</u> , Mzalbat Raneen and Sharabi Adi	162
PO-4	MOLECULAR EXPRESSION AND TRANSPORT ACTIVITY OF A PROMISING TARGET FOR TUMOR SPECIFIC DRUG DELIVERY----OLIGOPEPTIDE TRANSPORTER 1 IN HUMAN HEPATOCARCINOMA <u>Xu Jian</u> , Yanxia Gong, Xiang Wu, Zhi Yao, Qingyu Zhang and Jia Zhao	162
PO-56	COMPARATIVE STUDY BETWEEN DIFFERENT EMULSIFICATION METHODS: MULTI-SCALE CHARACTERIZATION <u>Messaouda Kaci</u> , Elmira Arab Tehrani, Guillaume Gillet, Isabelle Lavissee and Stéphane Desobry	163
PO-96	A PROTEOMIC ANALYSIS OF HUMAN FOLLICULAR FLUID: YOUNGER <i>VERSUS</i> OLDER WOMEN <u>Hee-Gyoo Kang</u> , Hee-Joung Lim, AeEun Seok, Jiyeong Lee, Arum Park, JuHwan Lee and Yun-Seok Yang	163
PO-91	NEW DIETARY SUPPLEMENTS AND THEIR EVENTUAL USING FOR WEIGHT <u>Rabbia Khursheed</u>	164
PO-12	ATYPICAL ANTIPSYCHOTIC ARIPIRAZOLE FACILITATES FEAR EXTINCTION IN ADOLESCENT RATS D.E. Ganella, H.B. Madsen, L. Lee-Kardashyan, I.C. Zbukvic, S. Luikinga, R.W. Coulthard, A.J. Lawrence and <u>J.H. Kim</u>	165
PO-8	ELUCIDATION OF INVOLVEMENT OF 11- β HYDRXY STERIOD DEHYDROGENASE 1 IN DIABETIC CEREBRAL STROKE <u>Patel Mittalben Kirtikumar</u>	166
PO-37	ANTIBACTERIAL, ANTICANDIDAL AND ANTIOXIDANT PROPERTIES OF <i>TANACETUM ARGENTEUM</i> (LAM.) WILLD. SUBSP. <i>FLABELLIFOLIUM</i> (BOISS. & HELDR.) GRIERSON <u>Yavuz Bülent Köse</u> , Gökalp İşcan, Fatih Göger, Betül Demirci, Ceren Elmacı	167
PO-50	DETERMINATION OF PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF TWO <i>THYMUS</i> L. (LAMIACEAE) SPECIES FROM TURKEY <u>S. Küçük</u> and F. Göger	167
PO-88	ESSENTIAL OIL COMPOSITION OF ENDEMIC <i>TRIPLEUROSPERMUM</i> SPECIES FROM TURKEY <u>M. Kurkcuoglu</u> , H. Inceer and K.H.C. Baser	167
PO-69	A NOVEL TARGETING DRUG DELIVERY SYSTEM FOR SPECIFIC DIAGNOSIS AND THERAPY OF LIVER CANCER <u>Yaoxian Li</u> , Yuzhen Wang and Jingyuan Wang	168
PO-58	PREPARATION OF DOX-CONJUGATED DENDRIMER-MODIFIED MAGNETIC IRON OXIDE CONJUGATES FOR DRUG DELIVERY <u>Yaoxian Li</u> , Yuzhen Wang, Jingyuan Wang and Yapeng Li	168
PO-30	SYNTHESIS OF PHENYL THIAZOLE ACYL SHIKONIN ESTER DERIVATIVES AS ANTICANCER AGENTS THROUGH MICROTUBULE STABILIZATION <u>Hong-Yan Lin</u>	169

PO-45	DEVELOPMENT OF POLYMERIC NANOPARTICLES TO IMPROVE THE THERAPEUTIC EFFICACY OF IRON CHELATORS FOR IRON OVERLOAD DISEASES <u>Tianqing Liu</u> , Shanshan Guo, Guangjun Nie and Greg Anderson	169
PO-17	TARGETING CANCER STEM CELLS IN ESOPHAGEAL CANCER <u>Pei-Jung Lu</u> , Cheng-Han Lin, Pei-Fang Kuo and Wan-Chun Hung	170
PO-21	NEW FORMULATION BASED IN ANTI-ATROPHIC PEPTIDES AND DENDRIMERS FOR THE TREATMENT OF SKELETAL MUSCLE ATROPHY. MOLECULAR DYNAMICS STUDIES AND EXPERIMENTAL VALIDATION <u>Valeria O. Márquez-Miranda</u> , Ingrid D. Araya Durán, Nicolas C. Pacheco, Lars Ratjen, Claudio A. Cabello-Verrugio, Gabriela Morales, Juan C. Rivera and Fernando D. González-Nilo	170
PO-3	PREVALENCE OF PHANTOM VIBRATION SYNDROME AND PHANTOM RINGING SYNDROME (RINGXIETY): RISK OF SLEEP DISORDERS AND INFERTILITY AMONG MEDICAL STUDENTS Mehtab Alam, Muhammad Sameer Qureshi, Ayesha Sarwat, Zeba Haque, Muhammad Salman Masroor, Muhammad Abdullah Makki, Ibad-ur-Rehman Baig, Muhammad Saad Munir, Moiz Ehtesham and Saad Aslam	171
PO-79	IDENTIFICATION OF NATURAL LACTATE EFFLUX INHIBITORS FOR THE DEVELOPMENT OF NOVEL ANTIPROLIFERATIVE AGENTS <u>Samia S. Messeha</u> , Najla O. Zarmouh, Malak G. Kolta and Karam F. Soliman	172
PO-38	<i>IN VITRO</i> INHIBITORY ACTIVITIES OF PIPLARTINE ON HUMAN CYTOCHROME P450 <u>Fernanda Lima Moreira</u> , Máisa D.Habenschus and Anderson Rodrigo Moraes de Oliveira	172
PO-40	CARBOXYTHERAPY AND PLATELET RICH PLASMA: A NEW THERAPY FOR TRIGONITIS, ABACTERIAL AND INTERSTITIAL CYSTITIS <u>F. Muzi</u> , G. Delicato, D. D'Andria, G. Baffigo, E. Tartaglia, F. Corvese, S. Signore, A. Perla, G. Montagna and G. Tati	173
PO-10	THE SYNTHESIS AND EVALUATION OF 4-CHROMANONE DERIVATIVES AS DUAL INHIBITORS OF MONOAMINE OXIDASE AND ACETYLCHOLINESTERASE <u>Clarina I. N'Da</u> , Anél Petzer and Jacobus P. Petzer	173
PO-2	ANTIMICROBIAL PEPTIDES FOR TREATMENT OF BONE INFECTIONS <u>Ondřej Nešuta</u> , Pavel Melicherčík and Václav Čerovský	174
PO-15	CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF THE ESSENTIAL OIL OF <i>FOENICULUM VULGARE</i> (FENNEL) FROM SOUTH AFRICA (EASTERN CAPE) <u>M. Nyalambisa</u> , A.O Oyedeji, S.P Songca, O. O Aremu, B.N Nkeh-Chungag, O.O Oyedeji and S.O Oluwafemi	174
PO-1	IDENTITY OF COMPOUNDS RESPONSIBLE FOR ANTI ADHESION ACTIVITY IN CRANBERRY JUICE A.C. Neto, A.K. Penndrof, M. Feldman, S. Meron-Sudai, Z. Ronen, D. Steinberg, M. Fridman, Y. Kashman, I. Ginsburg, <u>I. Ofek</u> and I.E. Weiss	175
PO-94	CHEMICAL COMPOSITION, FREE RADICAL SCAVENGING AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL OF <i>MARISCUS ALTERNIFOLIUS</i> VAHL <u>Patricia A. Onocha</u> , Ganiyat K. Oloyede and Jamiu A. Akintola	176

PO-14	ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF SOME SEMI-SYNTHESIZED OLEANOLIC ACID DERIVATIVES <u>Sibusiso Rali, Opeoluwa O. Oyediji, Oluokaode O. Aremu, Benedicta N. Nkeh-Chungag and Adebola O. Oyediji</u>	176
PO-19	<i>IN SILICO</i> STUDIES AND EXPERIMENTAL BINDING ASSAYS OF POTENTIAL ANTI-PRION COMPOUNDS REVEAL AN IMPORTANT BINDING SITE FOR PRION INHIBITION FROM PrPC TO PrPSc <u>Natraj Sekar Pagadala, Rolando Perez-Pineiro, Trent C. Bjorndahl, Ritesh Kaur Saini, Li Li, Ebrima Gibbs, Neil Cashman and David S. Wishart</u>	177
PO-78	FREE RADICAL SCAVENGING AND DIFFERENTIAL RESPONSE OF TROXERUTIN TO NORMAL AND CANCER CELLS <u>Niranjan A. Panat, Dharmendra K. Maurya, Santosh K. Sandur and Saroj S. Ghaskadbi</u>	177
PO-58-a	RELATIONS BETWEEN LEPTIN AND THYROID HORMONES METABOLISM IN WHITE ADIPOSE TISSUE <u>S. Pavelka</u>	178
PO-58-b	THE INFLUENCE OF SUPPLEMENTAL N-3 PUFA IN DIET AND ALTERED THYROID STATUS OF THE RATS ON THEIR LIPID METABOLISM <u>S. Pavelka</u>	179
PO-24	ACTIVITY OF FRACTIONS OF <i>PIPER UMBELLATUM</i> HEXANE EXTRACT AGAINST <i>TRICHOPHYTON RUBRUM</i> <u>Rosemeire Cristina Linhari Rodrigues Pietro, Jolindo Alencar Freitas and Rodrigo Sorrechia</u>	179
PO-13	EFFECTS OF MELATONIN ON MICE TREATED WITH ANTIRETROVIRAL THERAPY <u>Áurea Regina Telles Pupulin, Julia B Barbosa and Miguel Spack Jr</u>	180
PO-106	FORMULATION AND <i>IN VITRO</i> EVALUATION OF CONTROLLED RELEASE MATRIX TABLETS OF DILTIAZEM HYDROCHLORIDE USING DIFFERENT RATE CONTROLLING POLYMERS <u>Ghulam Razaque, Gul Majid, Zeeshan Danish, Nisar Ahmed, Kamran Ahmad, Junaid Qureshi, Tahmina Rabbani and Mohammad Younis</u>	180
PO-99	<i>PHYSALIS ANGULATA</i> L. CALYCES AS A PROMISING NATURAL SOURCE OF POTENTIAL ANTI-INFLAMMATORY COMPOUNDS <u>D.E. Rivera, Y.C. Ocampo, J.P. Castro and L.A. Franco</u>	181
PO-49	DISCOVERY OF NOVEL ANTICONVULSANT ACTIVE GABA REUPTAKE INHIBITORS <u>Kinga Sałat, Adrian Podkowa, Anna Rapacz, Paula Zaręba and Katarzyna Kulig</u>	181
PO-62	ACTIVE SITE MAPPING OF HUMAN CATHEPSIN F WITH DIPEPTIDE NITRILE INHIBITORS <u>J. Schmitz, N. Furtmann, M. Ponert, U. Bartz, J. Bajorath and M. Gütschow</u>	182
PO-11	NOVEL METHOD IN SELECTION OF PRIMARY SETS LEADS TO IDENTIFICATION OF POTENT PIM-1 KINASE INHIBITORS USING LIGAND BASED DRUG DESIGN COMBINED WITH QSAR ANALYSIS TECHNIQUES AND VIRTUAL SCREENING <u>Rand Omar Shahin</u>	183

PO-43	DIRUTHENIUM-IBUPROFEN MODIFIED METALLODRUGS INVESTIGATED FOR THE ANTICANCER ACTIVITY IN HUMAN GLIOMA CELLS Hanif-Ur-Rehman, Tatiana E. Freitas, Alison Colquhoun and <u>Denise de Oliveira Silva</u>	183
PO-6	SYNTHESIS AND BIOLOGICAL EVALUATION OF DIHYDROARTEMISINYL-CHALCONE ESTERS Riëtte A. van Biljon, Lyn-Marie Birkholtz, David D. N'Da and <u>Frans Johannes Smit</u>	184
PO-80	SELECTIVE AND COMPETITIVE MONOAMINE OXIDASE-B INHIBITION BY THE FLAVANONE BAVACHININ <u>Najla O. Zarmouh</u> , Elizabeth A. Mazzio, Faisal M. Elshami, Samia S. Messeha, Suresh V. K. Eyunni and Karam F.A. Soliman	184
PO-42	INDUCED PRODUCTION OF HALOGENATED BIOACTIVE COMPOUND BY THE MARINE-DERIVED BACTERIA AND FUNGI <u>Byeng W. Son</u> and Keumja Yun	185
PO-67	MICROBIAL BROMINATION OF POLYKETIDE BY THE MARINE-DERIVED FUNGUS <i>ASPERGILLUS</i> SP. <u>Byeng W. Son</u> and Keumja Yun	185
PO-55	A NEW CLASS OF POTENT ANTIBACTERIAL AGENTS – 1,4 DISUBSTITUTED THIOSEMI-CARBAZIDE DERIVATIVES <u>Aleksandra Strzelczyk</u> , Agata Paneth, Magdalena Frysiak, Marlena Rasiuk and Paweł Stączek	186
PO-54	DETERMINATION OF MOLECULAR TARGET AND BIOLOGICAL ACTIVITY OF 1,2,4-TRIAZOLE - CIPROFLOXACIN HYBRIDS <u>Aleksandra Strzelczyk</u> , Barbara Kaproń and Tomasz Plech	187
PO-22	MILD CONCUSSIVE- AND BLAST-TRAUMATIC BRAIN INJURY (TBI) IN MICE: MODELS FOR DRUG DEVELOPMENT <u>Ian Tamargo</u> , David Tweedie, Lital Rachmany, Vardit Rubovitch, Kumar Sambamurti, Debomoy K. Lahiri, Barry J. Hoffer, Chaim G. Pick and Nigel H. Greig	187
PO-74	DEVELOPMENT OF PROTEIN-POLYMER CORE-SHELL NANOPARTICLES (PPCS-NPS) AS EFFICIENT VEHICLES TO DELIVER THERAPEUTIC AGENTS ACROSS BLOOD BRAIN BARRIER (BBB) <u>Napat Tandikul</u> and Qian Wang	188
PO-72	ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF <i>EMBLICA OFFICINALIS</i> IN EXPERIMENTAL ANIMALS <u>Isabella Topno</u> and Jesudoss Prabhakaran	189
PO-46	HYPOTHALAMIC PHOSPHOLIPIDS (LIPOSOMES FORTE) AND PLATELET-RICH PLASMA IN THE REHABILITATION OF PATIENTS WITH SPINAL CORD INJURY Saule T. Turuspekova, Anatoliy Ostapenko, Dmitry Mitrokhin, Bakhytzhaz Gaziz, Erlan Kudabaev, Amina Seydanova and Elmira Moldakulova	189
PO-60	HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF SUMATRIPTAN SUCCINATE IN RAT PLASMA Siddu Bhagawat, Krishnamurthy Baht and <u>S.G. Vasantharaju</u>	190
PO-102	SULFATED COUMARINS AS POTENT, ALLOSTERIC REGULATORS OF THROMBIN ACTIVITY <u>Stephen Verespy III</u> , Akul Y. Mehta, Daniel Afosah, Rami A. Al-Horani and Umesh R. Desai	190

PO-71	CLINICAL STUDY ON TC-99M-MDP WHOLE BODY BONE SCAN COMBINED WITH MRI ON THE DIAGNOSTIC VALUE OF BONE METASTASIS <u>Rong Fu Wang</u> and An Hui Zhu	191
PO-89	SOLID-STATE NMR CHARACTERIZATION OF S31N M2 TRANSMEMBRANE DOMAIN BOUND TO NOVEL ADAMANTANES WITH PERSISTENT <i>IN VITRO</i> EFFICACY <u>Anna K. Wright</u> , Ivan Hung, Christina Tzitzoglaki, Harris Ioannidis, David D. Busath, Antonios Kolocouris and Timothy A. Cross	192
PO-47	EVALUATION OF TOPICAL FORMULATIONS CONTAINING EXTRACTS OF <i>ACMELLA OLERACEA</i> (L.) R.K. JANSEN (JAMBU) AND <i>ACHYROCLINE SATUREIODES</i> LAM (MACELA) <u>Lais Thiemi Yamane</u> and Rodney Alexandre Ferreira Rodrigues	192
PO-86	STRUCTURAL AND DYNAMIC CHARACTERIZATION ON MUTATED Keap1 FOR VARIED AFFINITY TOWARD Nrf2: A MOLECULAR DYNAMICS SIMULATION STUDY Pei-Jung Lu and <u>Chia-Ning Yang</u>	193
PO-104	MICROPLATE-FORMATTED MAMMALIAN PROTEIN EXPRESSION FOR HIGH THROUGHPUT DISSECTING BROAD NEUTRALIZATION ANTIBODIES AND IMMUNOGENS <u>Yongping Yang</u> , Gabriel Lu and Peter D. Kwong	193
PO-83	ELUCIDATION AND CHARACTERIZATION OF A NOVEL ANTI-TUMOR PROTEIN FROM ARCA INFLATA <u>Rongmin Yu</u> , Jian Xu, Shuangshuang Lv, Zhiyan Chen, Jianhua Zhu, Yue Gao, Jingjing Gao, Jiachen Zi and Liyan Song	194
PO-73	VASCULAR NORMALIZATION INDUCED BY SINOMENINE HYDROCHLORIDE RESULTS IN SUPPRESSED MAMMARY TUMOR GROWTH AND METASTASIS <u>Huimin Zhang</u> , Yang Liu, Ligang Niu and Jianjun He	194
PO-34	THE ENHANCEMENT EFFECT OF DA4 ISOLATED FROM BAMBOO (<i>DENDROCALAMUS ASPER</i>) SHOOTS ON $\alpha 1\beta 2\gamma 2S$ GABA A RECEPTOR EXPRESSED IN XENOPUS OOCYTES <u>Jingli Zhang</u> , Habsah Mohamad, Muhammad Bilal, Jia Hui Wong, Abdul Hadi Bin Ismail, Amelia Jane Lloyd, Fatin Hilyan Mohamad, Nurnazihah Binti Mohd Noor, Abdul Aziz Mohamed Yusoff, Hasnah Osman, Koktong Wong, Zamzuri Idris and Jafri Malin Abdullah	195

e-POSTERS

e-PO-98	ACID-TRIGGERED CHARGE-REVERSAL POLYPEPTIDE MICELLE FOR OPTIMAL INTRACELLULAR DRUG DELIVERY Taoyuan He, <u>Jianxun Ding</u> and Yanan Yang	196
e-PO-97	PH POTENTIAL-MEDIATED INTRACELLULAR TARGETING DRUG DELIVERY FOR UPREGULATED ANTITUMOR EFFICACY <u>Jianxun Ding</u> , Jixue Wang, Jinjin Chen and Xuesi Chen	196
e-PO-103	TARGETING THE LEISHMANIA MEXICANA CYSTEINE PROTEASE CPB2.8 BY DECORATED FUSED BENZO[B]THIOPHENE SCAFFOLD <u>Angela Scala</u> , Nicola Micale, Anna Piperno, Antonio Rescifina, Tanja Schirmeister and Giovanni Grassi ¹	197

<i>e-PO-28</i>	COMPUTATIONAL SIMULATIONS ON STRUCTURAL CHANGES AND DOMAIN INTERACTIONS BETWEEN FLAVIN MONONUCLEOTIDE AND HEME IN MURINE INDUCIBLE NITRIC OXIDE SYNTHASE <u>Yinghong Sheng</u> , Linghao Zhong and Jordan Wirth	198
<i>e-PO-20</i>	NOVEL OXAZOLIDINES AND IMIDAZOLIDINES: DEVELOPMENT OF NEW METHOD FOR SYNTHESIS, AND DOCKING STUDY FOR ANTIVIRAL ACTIVITY <u>Sharada Prasanna Swain</u> and Jih Ru Hwua	199
<i>e-PO-101</i>	DESIGN AND SYNTHESIS OF FUNCTIONAL SUPRAMOLECULE BY SELF-ASSEMBLY OF DIMERIC (Zn^{2+} -CYCLEN) COMPLEX, ORGANIC ANION AND METAL IONS IN AQUEOUS SOLUTION <u>Nur Amina Zainal</u> , Hamizah M. Zaki, Siti Azma Jusoh, Minaketan Tripathy, Hadariah Bahron, Shin Aoki and Mohd Zulkefeli	200

Global Biotechnology Congress - 2015

Welcome Note



Atta-ur-Rahman, FRS
Co-President

The “**Global Biotechnology Congress 2015**” would provide eminent scientists the opportunity to present their cutting edge researches in the field of biotechnology and its applications in medicine. Three Nobel Laureates and leading researchers are

participate in this important conference.



Ferid Murad, NL
Co-President

It is our pleasure to welcome the participants to GBC 2015. We expect that they will enjoy the lectures delivered by leading authorities in their respective fields.

Global Biotechnology Congress 2015

CONTENTS

PLENARY LECTURES

PL-293	CURRENT STATUS OF OBESITY AND THE NEED FOR A PARADIGM SHIFT <u>Richard L. Atkinson</u>	201
PL-122	APPLICATION OF NITRIC OXIDE RESEARCH TO DRUG DEVELOPMENT <u>Ferid Murad</u>	202
PL-123	EVOLUTIONARY DYNAMICS AND TREATMENT OF CANCER <u>Martin Nowak</u>	203
PL-224	EMERGING PHARMACO-MPE (MOLECULAR PATHOLOGICAL EPIDEMIOLOGY) PARADIGM FOR GLOBAL PRECISION MEDICINE <u>Shuji Ogino</u>	203
PL-222	RECONSTRUCTION, MODELING AND USE OF GENOME-SCALE NETWORKS IN BIOLOGY <u>Bernhard Palsson</u>	204
PL-223	NOVEL PRO-RESOLVING LIPID MEDIATORS IN INFLAMMATION & INFECTION LEADS FOR RESOLUTION PHYSIOLOGY AND PHARMACOLOGY <u>Charles N. Serhan</u>	204
PL-2	MICRORNAs, SMALL INTERFERING RNAs AND MODULAR THERAPEUTICS <u>Phillip A. Sharp</u>	205
PL-1	DESIGNING BIOLOGY FOR A HEALTHY WORLD <u>Pamela A. Silver</u>	205
PL-291	ADVANCED THERAPEUTICS WHILE PRESERVING THE MICROBIOME <u>Ada Yonath</u>	206
PL-292	SPECIFIC ANTI-TUBERCULIN (IgG) HIGH LEVELS AND LYMPHOCYTE <i>IN VITRO</i> PROLIFERATION WITH TUBERCULIN ARE BETTER INDICATORS OF LTBI THAN TST Edmond J. Feris, Joaquin Zuñiga, Liliana Encinales, Joel N.H. Stern, Julio Granados, Marcelo Fernandez-Vina and <u>Edmond J. Yunis</u>	206

KEYNOTE LECTURES

KNL-82	CREATING BIOACTIVE CHEMICAL SPACE BY BIOTRANSFORMATION <u>M. Iqbal Choudhary</u> and Atta-ur-Rahman	208
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INVITED LECTURES

IL-169	NOVEL APPROACH FOR BIG DATA MANAGEMENT AT THE LEVEL OF INTRACELLULAR SIGNALIZATION AND ITS APPLICATIONS TO BIOMEDICINE, DRUG DISCOVERY AND PERSONALIZED THERAPY Nicolas Borisov, Alexander Aliper, Ksenia Lezhnina, Denis Shepelin, Michael Korzinkin, Artem Artemov, Qinsong Zhu, Alex Zhavoronkov and <u>Anton Buzdin</u>	209
IL-129	STEM CELL THERAPY FOR THE TREATMENT OF SEVERE TISSUE DAMAGE AFTER RADIATION EXPOSURE <u>A. Chapel</u> , A. Semont, N. Mathieu, C. Linard, L. Douay, N.C. Gorin, M. Mothy, J.M. Simmon, J.J. Lataillade, J. Voswinkel, R. Tamarat and M. Benderitter	209
IL-58	MEDICINAL PLANTS USED BY IMMIGRANTS FROM THE CARIBBEAN <u>Brahmadeo Dewprasad</u> , Kwame Amin, Maria Greene, Latifa Hadir, Richard Hendriks, Anthony Bradford, Joel Moroccho, Vishnu Tiwari and Sheuli Zakia	210
IL-229	IDENTIFICATION THE BOTANICAL ORIGIN OF NATURAL WOODS USED IN WINE AGING <u>Ignacio Díaz-Maroto</u> , M. Elena Alañón, Pablo Vila-Lameiro, Consuelo Díaz-Maroto and M. Soledad Pérez-Coello	210
IL-140	PERSONALIZED HEALTHCARE: FROM RESEARCH TO REALITY <u>Jeffrey P. Harrison</u> and Debra A Harrison	211
IL-267	MICROVESSEL ADAPTATION AND PLASTICITY IN MICROVASCULAR REGENERATION <u>James B. Hoying</u>	211
IL-162	CHARACTERISATION OF AN EXCEPTIONALLY LARGE <i>FAD2</i> GENE FAMILY AND DEVELOPMENT OF SUPER HIGH OLEIC GENOTYPE IN SAFFLOWER <u>Qing Liu</u> , Craig Wood, Shijiang Cao, Matthew Taylor Shoko Okada, Xue-Rong Zhou, Allan Green and Surinder Singh	212
IL-9	PSP94, PROSTASIN AND PSP94 TO PROSTASIN SIGNALING IN CANCER <u>Jin-xia Ma</u> and Bing-xue Yan	212
IL-14	VASCULAR TISSUE REGENERATION: A BIOPHYSICAL PERSPECTIVE ON THE ROLE OF MECHANICAL, MOLECULAR AND ELECTRIC INTERACTIONS Toloo Taghian, Abdul Sheikh, Andrei Kogan and <u>Daria Narmoneva</u>	213
IL-272	THE RATIONAL BIOLOGICAL DESIGN OF REGENERATIVE THERAPIES AND DEVICES <u>Nancy L. Parenteau</u>	214
IL-134	THE NEIL ARMSTRONG SPACE SYNDROME <u>William J. Rowe</u>	214
IL-297	NEW PERSPECTIVES IN NANOMEDICINE AND NANOPHARMACOLOGY <u>Hans-Christian Siebert</u>	215

SESSION LECTURES

<u>SL-244</u>	GENETIC STABILITY AND PERSISTENCE OF MORPHOLOGICAL FEATURES OF TRANSGENIC PEA (<i>PISUM SATIVUM</i> L.) HARBORING NA ⁺ /H ⁺ ANTIporter AGAINST SALT STRESS <u>Zahid Ali</u> , Saadia Naseem, Nighat Yasmeen, Hans Joerg Jacobsen and Fauzia Yusuf Hafeez	216
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<u>SL-186</u>	RAPD- PCR ANALYSIS OF INDIGENOUS IRAQI SINORHIZOBIUM MELILOTI ISOLATES DIFFERING IN THEIR ABILITY TO DROUGHT TOLERANCE Rana A. Hameed Nidhal N. Hussain and <u>Abed Aljasim M. Jasim Almanssoori</u>	216
<u>SL-305</u>	WHOLE CELL BIOCATALYST FOR SOYASAPOGENOL B PRODUCTION FROM SOYBEAN SAPONIN <u>Hala A. Amin</u> , Mostafa M. Abo Elsoud, Ahmed. F. Sahab	217
<u>SL-180</u>	EVIDENCE OF METABOLIC COMPETITION BETWEEN METHIONINE AND GLUTATHIONE BIOSYNTHETIC PATHWAYS Yael Hacham, Jong Yoon, Ifat Matityahu, Alex Kaplan, Lilach Kachan, David Oliver and <u>Rachel Amir</u>	217
<u>SL-121</u>	CONVERGENCE: A TRANSFORMATIVE APPROACH TO ADVANCED RESEARCH AT THE INTERSECTION OF THE LIFE, PHYSICAL AND ENGINEERING SCIENCES <u>Amanda Arnold</u> and Melvin Greer	218
<u>SL-21</u>	NATIVE EXTRACELLULAR MATRIX – A PARADIGM FOR TRANSLATABLE REGENERATIVE MEDICINE <u>Nathaniel Bachrach</u>	218
<u>SL-100</u>	<i>IN VITRO</i> PROLIFERATIVE EFFECT OF ALOE VERA EXTRACTS ON HAIR FOLLICLES ISOLATED FROM THE ALOPECIA ARETA PATCH Mohite, S. L. and <u>Bagde, U.S.</u>	219
<u>SL-289</u>	TARGETED DELIVERY OF LIPOSOMES AND NANOPARTICLES IN HIGH GRADE GLIOMA CELLS <i>IN VITRO</i> AND <i>IN VIVO</i> <u>Vladimir P. Baklaushev</u> , Svetlana E. Gelperina, Pavel A. Melnikov, Yulia Malinovskaya, Anna N. Gabashvily and Vladimir P. Chekhonin	219
<u>SL-42</u>	SCARLESS SURGERY: ARE WE THERE YET? <u>Swathi Balaji</u>	220
<u>SL-119</u>	FINITE ELEMENT ANALYSIS IN ORAL IMPLANTOLOGY <u>Nilufer Balcioglu</u> and H. A. Balcioglu	221
<u>SL-113</u>	TEMPOROMANDIBULAR JOINT DISORDERS, BIOTECHNOLOGY AND EVOLVING TRENDS <u>Huseyin Avni Balcioglu</u>	222
<u>SL-192</u>	THE DECONSTRUCTION OF SEED MUCILAGES <u>Rachel A. Burton</u>	222
<u>SL-119(a)</u>	VARIATION OF ALGAE OCCURRENCE BEFORE AND AFTER WEIR CONSTRUCTION AT MULGEUM SITE IN DOWNSTREAM THE NAKDONG RIVER, SOUTH KOREA Ji-Sung Lim, Jae-Ho Lee, Young-Woo Kim, Sang-Hoon Lee, Hyang-Gi Kim, Tae-Joo Park and <u>Im-Gyu Byun</u>	223
<u>SL-286</u>	GENETICALLY SHAPING MORPHOLOGY OF THE FILAMENTOUS FUNGUS ASPERGILLUS GLAUCUS FOR PRODUCTION OF ANTITUMOR POLYKETIDE ASPERGIOLIDE A <u>Menghao Cai</u> , Ying Zhang, Wei Hu, Wei Shen, Zhenzhong Yu, Weiqiang Zhou, Tao Jiang, Xiangshan Zhou and Yuanxing Zhang	223
<u>SL-281</u>	X-RAY SYNCHROTRON PHASE CONTRAST TOMOGRAPHY FOR THE INVESTIGATION OF REGENERATIVE TREATMENT IN NEURODEGENERATIVE DISEASES Inna Bukreeva, Michela Fratini, Gaetano Campi, Raffaele Spanò, Valentina Petrosino, Ranieri Cancedda, Nicole Kerlero de Rosbo, Antonio Uccelli, Alberto Bravin, Maddalena Mastrogiacomo and <u>Alessia Cedola</u>	224

<u>SL-193</u>	ANTIBACTERIAL ACTIVITY, MECHANISM OF ACTION AND PHYTOCHEMICAL STUDIES OF CYNODON DACTYLON (L.) PERS. SOLID PHASE EXTRACT (SPE) AGAINST SOME BACTERIAL PATHOGENS Syahriel Abdullah, Januarius Gobilik and <u>Khim Phin Chong</u>	224
<u>SL-174</u>	HIGHLY EFFICIENT GENOME EDITING FOR CROP IMPROVEMENT Feng Zhang, William J. Haun, Benjamin Clasen, Thomas Stoddard, <u>Zachary Demorest</u> , Jin Li, Song Luo, Dan Voytas and Luc Mathis	225
<u>SL-228</u>	TRANSCUTANEOUS APPLICATIONS OF FOAM METALS <u>Ian D. Dickey</u>	225
<u>SL-34</u>	SYNER-III: AN <i>IN VIVO</i> METHOD TO INDUCE PANCREATIC BETA CELL FORMATION IN THE ADULT PANCREAS <u>Bruno Doiron</u> and Ralph A DeFronzo,	226
<u>SL-91</u>	NUMERICAL MODELING OF MECHANOTACTIC INFLUENCE ON CELL MORPHOLOGY <u>Mohamed Hamdy Doweidar</u> and Seyed Jamaledin Mousavi	227
<u>SL-274</u>	EVALUATION OF HIGH BETA-CAROTENE CASSAVA GENOTYPES AT ADVANCED TRIAL IN NIGERIA <u>Ngozi Edo</u> , Ogbokiri, H., Njoku, D.N and C.N. Egesi	228
<u>SL-152</u>	SCALE-UP OF IMMUNE CELL THERAPIES FOR ORGAN TRANSPLANTS <u>Dr Marianne Ellis</u> , Dr Fadi Issa and Mr Jon Pleat	228
<u>SL-85</u>	SPECIFIC RECOGNITION OF THE HIV-1 GENOMIC RNA BY THE GAG PRECURSOR <u>Ekram W. Abd El- Wahab</u> , Redmond P. Smyth, Elodie Mailler, Serena Bernacchi, Valérie Vivet-Boudou, Marcel Hijnen, Fabrice Jossinet, Johnson Mak, Jean-Christophe Paillart and Roland Marquet	229
<u>SL-30</u>	PLANT-BASED NUTRITION ADHERENCE STRATEGY HALTS CORONARY DISEASE <u>Caldwell B. Esselstyn</u> and Mladen Golubić	229
<u>SL-141</u>	LONG ACTING ANALOGS OF GLYCOPROTEIN HORMONES DESIGNED BY SITE-DIRECTED MUTAGENESIS AND GENE FUSION <u>Fuad Fares</u>	230
<u>SL-157</u>	MOLECULE DRUG CANDIDATES – PROSPECTING, DEVELOPMENT, OPTIMIZATION AND CLINICAL TRIALS: TWO BRAZILIAN SUCCESSFUL CASES <u>Rui Seabra Ferreira</u> , Daniel Carvalho Pimenta, Ricardo de Oliveira Orsi, Silvia Regina S. Barraviera, Luciana Patrícia F. Abbade, Alexandre Naime Barbosa, Luis Eduardo Ribeiro da Cunha, Benedito Barraviera	231
<u>SL-210</u>	PLANT-BASED BIOPHARMACEUTICALS FOR EMERGENCY RESPONSES AND ALTERNATIVE RAPID PRODUCTION OF NOVEL BIOPHARMACEUTICALS <u>Rainer Fischer</u>	232
<u>SL-287</u>	SYNTHETIC BIOLOGY OF CYANOBACTERIA FOR BIOETHANOL PRODUCTION <u>Pengcheng (Patrick) Fu</u>	233
<u>SL-16</u>	PRODUCTION OF HYDROGEN AND METHANE FROM FOOD PROCESSING WASTE IN INDUCED BED REACTORS: EXPERIMENT AND MODELING <u>Conly Hansen</u> and Jianming Zhong	233
<u>SL-56</u>	AUTOLOGOUS BRAIN SOURCES FOR PERSONALIZED CELL THERAPY IN NEUROLOGICAL DISEASE Hu Xu, Cleusa DeOliveira, Hon Leong, Susanne Schmid, Paula Foster and <u>Matthew O. Hebb</u>	234

<u>SL-28</u>	<i>IN VIVO</i> MODEL OF HUMAN INTESTINE Smail Meziani and <u>M. A. Helmrath</u>	234
<u>SL-290</u>	BIO-SALINE AGRICULTURE OF SALICORNIA BEGELOVII, NEW APPROACHES FOR BIODIESEL PRODUCTION <u>Mohamed M. Ibrahim</u> , Ibrahim Arifl and Gehan A. El-Gaaly	236
<u>SL-216</u>	HARNESSING MICROBIAL INTERACTION FOR INCREASING CROP PRODUCTIVITY IN SEMIARID PRAIRIE AGRICULTURAL REGION OF WESTERN CANADA <u>Jamshid Jazestani</u> , Lobna Abdellatif and Chantal Hamel	236
<u>SL-198</u>	A WHOLE PLANT APPROACH FOR NITROGEN-FIXATION IN NON-LEGUMINOUS CROPS Munusamy Madhaiyan and <u>Lianghui Ji</u>	237
<u>SL-57</u>	PROTECTIVE EFFECTS OF LOW DOSES RADIATION AGAINST DOXORUBICIN-INDUCED CARDIAC TOXICITY IN BALB/C MICES <u>Hong-Yu Jiang</u> , Gui-Ru Wang, Ya-Qiong Hong, Hong-Mei Zhang and Miao-Miao Li	238
<u>SL-168</u>	THE ROLE OF CELLULOSE-SYNTHASE-LIKE CSLF6 IN 1,3;1,4- β -D-GLUCAN BIOSYNTHESIS AND OVEREXPRESSION IN WHEAT GRAIN TO INCREASE SOLUBLE DIETARY FIBRE LEVELS. <u>S.A. Jobling</u>	239
<u>SL-71</u>	EVALUATION OF ADVANCED GLYCATION END PRODUCTS (AGES), CIRCULATING SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (SRAGE) AND OXIDIZED LOW-DENSITY LIPOPROTEINS (OXLDL) IN HEMODIALYSIS PATIENTS Adel M. A. Assiri, <u>Hala F. M. Kamel</u> and Abeer A. EL-Refai	239
<u>SL-65</u>	ANTIOXIDANT PROPERTIES OF PRUNES (<i>PRUNUS DOMESTICA</i> L.) AND THEIR CONSTITUENTS <u>Shin-ichi Kayano</u> , Hiroe Kikuzaki, Takahiko Mitani and Nobuji Nakatani	240
<u>SL-258</u>	THE NEED FOR OPTIMIZATION: OVERVIEW OF EXTRACELLULAR VESICLES (EVS) ISOLATION TECHNIQUES FROM SMALL VOLUMES OF SERUM AND REPRODUCTIVE BIOLOGICAL FLUIDS <u>Shlomit Kenigsberg</u> , Shlomit Kenigsberg, Arshia Azizeddin, Bahar Behrouzi, Nicole Legro, Christina Lee, Parshvi Vyas, Kevin Quach, Paula Mackie and Clifford Librach	240
<u>SL-325</u>	PRODUCTION OF HIGH MOLECULAR WEIGHT FUNGAL PECTINASE FROM THE NOVEL STRAIN OF <i>ASPERGILLUS</i> SPP <u>Samina Khan</u> , Saima Saleem and Abid Azhar	241
<u>SL-107</u>	THE ROLE OF STEM-LIKE GLIOMA CELLS IN GBM PROGRESSION AND POST-TREATMENT RECURRENCE. <u>Ella L. Kim</u>	241
<u>SL-239</u>	METABOLIC ENGINEERING OF CAROTENOIDS IN TRANSGENIC <i>SWEETPOTAO</i> FOR SUSTAINABLE DEVELOPMENT ON GLOBAL MARGINAL LANDS Sun Ha Kim, Sung-Chul Park, Haeng-Soon Lee, Jae Cheol Jeong and <u>Sang-Soo Kwak</u>	242
<u>SL-78</u>	BRING SANGER SEQUENCING TO THE COMMUNITY CLINICAL LABORATORIES WORLDWIDE <u>Sin Hang Lee</u> and Guofan Hong	243

<u>SL-114</u>	EFFECTS OF STRONG ACID AND IONIC MATERIALS ON MICROWAVE-ASSISTED THERMO-CHEMICAL HYDROLYSIS OF CATTLE MANURE Jeongmin Lee, <u>Jaeho Lee</u> , Hyanggi Kim, Sanghoon Lee, Youngwoo Kim, Imgyu Byun and Taejoo Park	243
<u>SL-92</u>	BLOCKING THE FUNCTION OF INFLAMMATORY CYTOKINES AND MEDIATORS BY USING IL-10 AND TGF-B: A POTENTIAL BIOLOGICAL IMMUNOTHERAPY FOR INTERVERTEBRAL DISC DEGENERATION IN A BEAGLE MODEL <u>Wei Li</u> , Chun Chen, Zhiwei Jia, Xuedong Bai and Dike Ruan	244
<u>SL-283</u>	CO-DISPLAY OF SYNERGETIC RHIZOPUS ORYZAE LIPASE AND CANDIDA RUGOSA LIPASE 1 BIODIESEL PRODUCTION Zhang Yan, <u>Xu Li</u> , Huang Zhengnan and Yan Yunjun	244
<u>SL-269</u>	NOVEL APPLICATION OF SARGASSUM AS A POTENTIAL WASTE DERIVED CATALYST FOR LOW TEMPERATURE SELECTIVE CATALYTIC REDUCTION OF NITRIC OXIDES <u>Sujing Li</u> , Shan Tan, Yun shi and Wei Li	245
<u>SL-199</u>	INHIBITION OF THE IGG-FC γ RIIIA INTERACTIONS IN MSOD1 MICE PROLONGING MOUSE SURVIVAL. Meital Edri-Brami, Inga Shengof, Shirley Tzalik and <u>Rachel G. Lichtenstein</u>	245
<u>SL-248</u>	ENGINEERING OF NOVEL DOMAIN ANTIBODY SCAFFOLD USING <i>IN VIVO</i> PROTEIN FOLDING PATHWAY <u>Hyung-Kwon Lim</u>	246
<u>SL-63</u>	TRACKING REGENERATION OF THE BRAIN AFTER GENE THERAPY USING TARGETED GUIDED MRI <u>Philip Liu</u>	246
<u>SL-221</u>	MICROSPORIDIA AND <i>ZINGIBER OFFICINALE</i> (GINGER); AN EMERGING PARASITE AND AN OLD MEDICINAL PLANT: <i>IN VIVO</i> TRIAL. <u>Rasha F. Mady</u> , Radwa G. Diab and Doaa A. Abdel Monsef	247
<u>SL-135</u>	THE EVALUATION OF CERCARIAL TRANSFORMATION FLUID USED SINGLY AND IN COMBINATION WITH CRUDE CERCARIAL ANTIGEN IN EXPERIMENTAL SCHISTOSOMIASIS MANSONI. Mervat Zakaria El Azzouni, <u>Rasha fadly Mady</u> , Maha Reda Gaafar, Fadwa Arafa and Abeer Elhadidi	247
<u>SL-204</u>	INCREASING THE EFFICIENCY OF NITROGEN COMPOUNDS REMOVAL FROM AGRICULTURAL AREAS THROUGH THE OPTIMIZATION OF CONDITIONS FOR MICROBIAL ACTIVITY <u>Joanna Dorota Mankiewicz-Boczek</u> , Bednarek A., Zaborowski A., Gągała I., Serwecińska L., Kolate E., Dziadek J. and Zalewski M.	248
<u>SL-211</u>	PAOT TECHNOLOGY® NEW APPROACH FOR DETERMINATION OF OXIDATIVE STRESS STATUS OF BIOLOGICAL LIQUIDS AND TISSUES. <u>Smail MEZIANI</u>	248
<u>SL-317</u>	TOWARDS A UNIVERSAL METHOD FOR PROTEIN REFOLDING <u>Catherine Michaux</u> and Eric A Perpète	249
<u>SL-44</u>	INFLUENCE OF BOVINE AND CAPRINE MILK-BASED PEPTIDES ON EXOPOLYSACCHARIDE SYNTHESIS BY <i>STREPTOCOCCUS THERMOPHILUS</i> AND <i>LACTOBACILLUS DELBRUECKII</i> SSP. <i>BULGARICUS</i> <u>Adela Mora-Gutierrez</u>	249

<u>SL-233</u>	TARGETING CD28 SIGNAL FOR STEROID RESISTANT ASTHMA <u>Akio Mori, Satoshi Kouyama, Miyako Yamaguchi, Yo Iijima, Akemi Ohtomo-Abe, Arisa Kinoshita, Hiroaki Hayashi, Kentaro Watarai, Chihiro Mitsui, Chiyako Oshikata, Kiyoshi Sekiya, Takahiro Tsuburai, Mamoru Ohtomo, Yuma Fukutomi, Masami Taniguchi, Takayuki Ohtomo and Osamu Kaminuma</u>	250
<u>SL-64</u>	ASSESSMENT OF CRUDE EXTRACT OF HELIX ASPERSA ON CHEMO-INDUCED COLITIS IN RAT <u>Dalila Naimi, Kouachi Meriem, Djadouri Jallal, Bendahra Ismahane</u>	250
<u>SL-105</u>	ANTI-INFLAMMATORY REGENERATIVE ACTIVITY OF A CD44-DERIVED PEPTIDE <u>David Naor, Lora Eshkar Sebban, Keren Or Amar and Shmuel Cohen</u>	252
<u>SL-36</u>	FINANCIAL VALUATION ALGORITHM FOR THE ASSESSMENT OF THE FUTURES SALES OF A NEW, INNOVATIVE MEDICINAL PRODUCT <u>Mark Nuijten</u>	252
<u>SL-147</u>	HYPOLIPIDEMIC AND HYPOGLYCEMIC ACTIVITIES OF ETHANOL EXTRACT OF CYPERUS ROTUNDUS RHIZOME <u>Gloria N. Okwu, S.E. Abanobi, U.V. Nnadi, N. Nwachukwu, C.O. Ujowundu and A.C. Ene.</u>	253
<u>SL-312</u>	EFFECT OF TRANSGENE COPY NUMBER ON VECTOR BACKBONE (VBB) INTEGRATION IN TRANSGENIC CASSAVA GENOME <u>Ihuoma Okwuonu</u>	253
<u>SL-23</u>	FOOD SCIENCE AND BIOTECHNOLOGY: INNOVATIVE APPLICATIONS OF NON THERMAL TECHNOLOGIES IN FOODS: HIGH PRESSURE PROCESSING FOR INCREASING THE SHELF LIFE AND SENSORIAL ANALYSIS OF FISH AND SEA FOOD. <u>Ivana Orlando and Luigi Palmieri</u>	254
<u>SL-300</u>	INHIBITION OF <i>CHILO PARTELLUS</i> (LEPIDOPTERA: PYRALIDAE) GUT PROTEASES BY COMPOUNDS FROM <i>IPOMOEA BATATA</i> <u>Balaji Panchal and Manvendra Kachole</u>	254
<u>SL-304</u>	PLANT STRESS ADAPTATION, SIGNALING AND THE REGULATORY SMALL RNA PATHWAYS: EVOLUTION AND FUNCTIONAL SPECIALIZATION <u>Shree Prakash Panday</u>	255
<u>SL-234</u>	A NEW PLANT OIL PRODUCTION PLATFORM: SEED-LIKE OIL YIELD FROM BIOMASS <u>James Petrie, Thomas Vanhercke, Allan Green and Surinder Singh</u>	255
<u>SL-268</u>	ATPASE 6/8 GENE ASSISTED GENETIC VARIABILITY STUDIES AMONG THE POPULATION OF LABEO ROHITA <u>Fayyaz Rasool, Shafat Hussain and Shakeela Parveen</u>	256
<u>SL-282</u>	METALLOGENIC INFORMATION EXTRACT AND PROGNOSIS RESEARCH OF SEAFLOOR POLYMETALLIC SULFIDE RESOURCES IN SOUTHWEST INDIAN OCEAN <u>Mengyi Ren, Jianping Chen and Ke Shao</u>	256
<u>SL-153</u>	ENDOPHYTIC FUNGUS AS A SOURCE OF BIOACTIVE SECONDARY METABOLITES: RESVERATROL AND VINIFERIN <u>Chetana Roat and Shailesh R. Dave</u>	257

<u>SL-313</u>	PAPER-ATTACHED YEAST BIOFILMS. AN ALTERNATIVE FOR LIVING BIOCATALYSTS GROWTH <u>Edvaldo Antonio Ribeiro Rosa</u> , Rosimeire Takaki Rosa, Alinne Ulbrich Mores Rymovicz, Cristina Rauen Ribas, Marcela Cardoso Selow	257
<u>SL-175</u>	PHARMACOKINETICS AND IMMUNOGENICITY OF PLANT-DERIVED BROADLY NEUTRALIZING HIV MONOCLONAL ANTIBODIES IN MACAQUES <u>Yvonne J. Rosenberg</u>	258
<u>SL-253</u>	BIOTECHNOLOGICAL APPROACH OF MEMS MICROROBOT SYSTEM WITH ARTIFICIAL NEURAL NETWORKS INTEGRATED CIRCUIT <u>Ken Saito</u> , Minami Takato, Yoshifumi Sekine and Fumio Uchikoba	258
<u>SL-249</u>	BIOAUGMENTATION OF CRUDE OIL SPILLS: BIOREMEDIATION WITH A MICROBIAL FORMULA TAILORED WITH SELECTED SOLVENT TOLERANT NATIVE STRAIN <u>Seema Sambrani</u> , Bharat Shinde and Shailesh Dave	259
<u>SL-217</u>	NON-INVASIVE DELIVERY OF BIOTECHNOLOGICAL DRUGS <u>Jun Shao</u>	259
<u>SL-259</u>	BIOREMEDIATION OF HEAVY METAL POLLUTED AND SALT AFFECTED SOILS EMPLOYING ARBUSCULAR MYCORRHIZAL FUNGI <u>Satyawati Sharma</u> , Kalpana Arora, Abhishek Sharma and Ashwani Kumar	260
<u>SL-84</u>	NOVEL SMOOTH MUSCLE CELL PROGENITORS ARE PRIMED TO MUSCULARIZE IN DISEASE <u>Abdul Q. Sheikh</u> , Ralf H. Adams and Daniel M. Greif	260
<u>SL-273</u>	BOOSTING THE FOOD FUNCTIONALITY (<i>IN VIVO</i> AND <i>IN VITRO</i>) OF SPIRULINA GROWN IN BANGLADESH BY GAMMA RADIATION: AN INSPIRING APPROACH <u>Hossain Uddin Shekhar</u> , Emran Kabir Chowdhury and Jahid M M Islam	261
<u>SL-187</u>	MTDLS DESIGN ON ACETYLCHOLINESTERASE (ACHE) AND B-SECRETASE (BACE-1): 3D-QSAR AND MOLECULAR DOCKING STUDIES <u>Jiancheng Shi</u> , Wentong Tu and Chusheng Huang	261
<u>SL-264</u>	MULTI-OMICS STUDY ON ANTHOCYANIN AND RESVERATROL ACCUMULATIONS IN GRAPE BERRY SKIN AND CULTURE CELLS <u>Katsuhiro Shiratake</u> , Mami Suzuki, Hiroshi Sakamoto, Moeko Taki, Ryo Nakabayashi, Yoichiro Fukao, Yoshiyuki Ogata, Nozomu Sakurai, Toshiaki Tokimatsu, Susumu Goto and Kazuki Saito	262
<u>SL-99</u>	APPLICATION OF NEWER MOLECULAR METHODS FOR DIAGNOSING AND DRUG RESISTANCE DETECTION IN <i>MYCOBACTERIUM TUBERCULOSIS</i> : EVALUATION IN INDIAN SETTING <u>Sarman Singh</u> , Beenish Syed Rufai, Parveen Kumar and Jitender Singh	262
<u>SL-296</u>	METABOLIC ENGINEERING OF LONG CHAIN OMEGA-3 FATTY ACIDS FOR THE OILSEED INDUSTRY <u>Surinder Singh</u> , James Petrie and Malcolm Devine	263
<u>SL-51</u>	MICROBIAL RESPONSE(S) TO THE 2010 DEEPWATER HORIZON GULF OIL SPILL: A 5-YEAR STUDY OF A COASTAL ALABAMA SALT MARSH <u>P. A. Sobecky</u>	263
<u>SL-98</u>	INITIAL RESULTS OF GENE THERAPY FOR B THALASSEMIA MAJOR <i>VIA</i> TRANSPLANTATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSDUCED <i>EX-VIVO</i> WITH A LENTIVIRAL BA T87Q GLOBIN VECTOR <u>Sandeep Soni</u> , Alexis A. Thompson, Mark C. Walters, Philippe Leboulch and Marina Cavazzana,	264

<u>SL-279</u>	ENZYMATIC DE-EPOXIDATION OF VIOLAXANTHIN AND DIADINOXANTHIN IN THE XANTHOPHYLL CYCLE REQUIRES PRESENCE OF NON-BILAYER LIPIDS <u>Kazimierz Strzałka</u> , Dariusz Latowski, Susann Schaller, Malgorzata Jemioła-Rzeminska, Christian Wilhelm and Reimund Goss	264
<u>SL-86</u>	THE FIRST EXTENSIVE CASE-STUDY ON THE EFFECTS OF ARTIFICIAL MYCORRHIZATION OF ACER BUERGERIANUM MIQ. IN HUNGARY <u>Krisztina Szabó</u> , Vilmos Lovas and Zsolt Erős-Honti	265
<u>SL-263</u>	HELIO-GERO PROTECTED DRINK-WATER AND FOOD: THE NEW POSSIBILITIES AND PERSPECTIVES OF THE PREVENTIVE MEDICINE <u>Alexander Trofimov</u>	265
<u>SL-72</u>	BIOTECHNOLOGICAL APPROACH TO INCREASING NUTRITIONAL QUALITY IN HIGH YIELDING RICE <u>Herry Utomo</u> , Steve Linscombe and Ida Wenefrida	267
<u>SL-238</u>	THE EXPRESSION OF PLASMA MIR-199A-5P, MIR-200C-3P IN GASTRIC CARCINOMA AND ITS CLINICAL SIGNIFICANCE Tang Jinli, <u>Xiaoming Wang</u> , Ge Mengyuan, Gui Zhen, Li Jinchang, Zhu Mingchen and Yan Feng	267
<u>SL-277</u>	NEUROPROTECTIVE DRUG DISCOVERY AND NEURAL STEM CELL RESEARCH IN ALS <u>Xin Wang</u>	268
<u>SL-205</u>	THE DEVELOPMENT OF A HIGH RESOLUTION LC-MS PEPTIDE MAPPING METHOD FOR AUTOMATED QUANTIFICATION OF N-LINKED GLYCANS IN IMMUNOGLOBULINS <u>Tian Wang</u> , Lily Chu, Wenzhou Li, Ken Lawson, Izydor Apostol and Tamer Eris	268
<u>SL-79</u>	NUTRITIONAL QUALITY ENHANCEMENT IN A NON-GMO HIGH YIELDING RICE <u>Ida Wenefrida</u> , Herry S. Utomo and Steve D. Linscombe	269
<u>SL-146</u>	HUMAN THYMUS DERIVED PROGENITOR/STEM CELLS IMMUNOTHERAPY IN AUTOIMMUNE DISEASES Nawrot I, <u>Wozniiewicz BM</u> , Maruszewski B, Janas R, Wozniiewicz M and Nazarewski S.	269
<u>SL-243</u>	THE PHYSICAL PROPERTIES AND BIOCOMPATIBILITY OF BIOMEDICAL POLYURETHANE REINFORCED BY NATIVE SILK FIBROIN POWDER <u>Weilin Xu</u> , Hongjun Yang, Yuping Pi, Ruiqing Hu, Mei Zhou, Chenxi Ouyang, Dujin Wang and Xia Dong	270
<u>SL-49</u>	MESENCHYMAL STEM CELL TREATMENT IMPROVES DIABETIC WOUND HEALING THROUGH MODULATION OF MICRORNA EXPRESSION <u>Junwang Xu</u> , Carlos Zgheib, Junyi Hu and Kenneth W. Liechty	270
<u>SL-106</u>	PHOTOCROSS LINKABLE MALEIC ANHYDRIDE ACYLATED CHITOSAN/METHACRYL POLY (VINYL ALCOHOL) NANOFIBROUS MEMBRANE AS POTENTIAL WOUND DRESSING FOR SKIN REGENERATION <u>Hongjun Yang</u> , Xin Liu, Yingshan Zhou, Shaojin Gu, Shuoshuo Yang, Qi Dong and Weilin Xu	271
<u>SL-181</u>	HUMAN CANNABINOID RECEPTOR CB2: EXPRESSION, FUNCTIONAL AND STRUCTURAL STUDIES <u>Alexei A. Yeliseev</u>	272
<u>SL-254</u>	THE SIGNIFICANCE OF DOUBLED HAPLOID PRODUCTION <i>VIA</i> MICROSPORE EMBRYOGENESIS IN PLANT BIOTECHNOLOGY AND CROP BREEDING <u>Ming Y. Zheng</u>	272

- SL-278** DETERMINE THE ROLE AND MECHANISM OF GENE CODON USAGE IN REGULATING PROTEIN EXPRESSION AND FOLDING 273
Mian Zhou, Jinhu Guo, Joonseok Cha, Michael Chae, She Chen, Jose M. Barral, Matthew S. Sachs and Yi Liu

POSTERS

- PO-31** ETHANOL PRODUCTION USING RED BEET JUICE BY SACCHAROMYCES *CERVISIAE* ATCC 9763 274
Venegas-Sánchez Josué Addiel and Jiménez Islas Donaji
- PO-76** HETEROGENEOUS NANOBIOCOMPOSITES ON THE BASIS OF CARBONIZED SORBENTS 274
Nuraly Akimbekov, Abdieva Z. Gulzhamal, Ualyeva S. Perizat, Kaiyrmanova K. Gulzhan, Digel Ilya and Zhubanova A. Azhar
- PO-27** PHYSIOLOGICAL AND GROWTH PERFORMANCE OF CYANOBACTERIUM, PHORMIDIUM FRAGILE UNDER EXPOSURE TO DIFFERENT LIGHT CONDITIONS 275
Jehan Saud AL-Abraham, Mudawi Mukhtar Elobeid and Afrah Eltayeb Mohammed
- PO-4** MOLECULAR ANALYSIS OF ACETOLACTATE SYNTHASE LOCUS REVEALS POTENTIAL NEW ALS- RESISTANT ALLELES AMONG SAUDI LOLIUM RIGIDUM POPULATIONS 275
Abdullah A. Al-Doss, Abdelhalim I. Ghazy, Sulieman A. Al-Faifi, Hussein M. Migdadi, Muhammad A. Khan, Megahed H. Ammar and Mohammad I. Althamrah
- PO-20** ANTIMICROBIAL EFFECT OF MELITTIN ISOLATED FROM SYRIAN HONEYBEE (*APISMELLIFERA*) VENOM AND ITS WOUND HEALING POTENTIAL 276
Omran Alia, Massouh Laila and Al-DaoudeAntonious
- PO-41** STUDIES ON GENETIC FIDELITY OF CRYOPRESERVED CALLI OF DATE PALM CULTIVARS (*PHOENIX DACTYLIFERA*) FROM SAUDI ARABIA 276
Fahad Al-Qurainy, Salim Khan, Mohammad Nadeem and Mohamed Tarroum
- PO-43** STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE OXYGENASE DOMAIN (ENOSOXY) ON LIPID NANODISCS 277
Ghaith Altawallbeh, Mohammad Mahfuzul Haque, Dennis J. Stuehr and Mekki Bayachou
- PO-42** STUDY OF THE EFFECT OF MOBILE PHONE RADIATION ON ANTIBIOTIC SENSITIVITY IN MICRO ORGANISMS 277
Am Arunkumar G
- PO-26** BLOCKADE OF NMDA RECEPTOR AFFECTS PROLIFERATION AND DIFFERENTIATION OF HUMAN CORTICAL PROGENITORS 278
Inseyah Bagasrawala, Nevena Radonjic and Nada Zecevic
- PO-79** BIOTRANSFORMATION OF EXISTING DRUGS INTO NEW ANALOGUES-AN APPROACH TOWARDS COST-EFFECTIVE DRUGS 279
Elias Baydoun, Saira Bano, Hina Mahmood and M. Asif Iqbal
- PO-30** DENITRIFICATION BARRIERS – AS A BIOREMEDIATION TOOL FOR NITROGEN REMOVAL FROM POINT SOURCE AND DIFFUSED POLLUTION IN RURAL AREAS 279
Bednarek A., Szklarek S., Gągała I., Dziedziczak K., Serwecińska L., Zaborowski A., Dziadek J., Mankiewicz–Boczek J. and Zalewski M.

PO-64	MODULATION OF ENDOPROTEASES BY TRICHOPHYTON RUBRUM GROWTH ON ELASTIN AND KERATIN SUBSTRATES <u>Tamires Aparecida Bitencourt</u> , Matheus Eloy Franco, Mozart Marins and Ana Lúcia Fachin	280
PO-11	FERMENTATION OF BACILLUS LICHENIFORMIS TO PRODUCE POLY (GLUTAMIC ACID) AT HIGH PRESSURE AND HIGH OXYGEN CONCENTRATION Alejandra Hernández-Valencia, Jose R. Cascante-Alpizar and <u>Jordi J. Bou</u>	280
PO-37	STUDY OF THE EFFECT OF POLYPHENOLS EXTRACTED FROM GREEN ALGAE <i>CAULERPA PROLIFERA</i> IN PREVENTING CELL DAMAGE BY IRI <u>Bover, E. A.</u> , Sanchez, D.A, Belda, M.A, Padrón, C. A, Cejalvo, D.A and Lloris, J.M.B	281
PO-39	ANTINOCICEPTIVE ACTIVITY OF BYRSONIMA DUCKEANA W. R. ANDERSON (MALPIDGHIACEAE) - AN AMAZONIAN "MURICI" Maria Christina dos Santos Verdam, Fernanda Guilhon-Simplicio, Kleyton Cardoso de Andrade, Karina Lorena Meira Fernandes, Marilis Dallarmi Miguel, Obdúlio Gomes Miguel and <u>Patricia M. Stuelp Campelo</u>	282
PO-68	INVESTIGATION OF THE ESSENCE OF PLATELET-RICH FIBRIN (EPRF): CYTOKINE DISTRIBUTION IN RELATION TO THREE-DIMENSIONAL STRUCTURE <u>Wing P. Chan</u> , Meng-Yi Bai, Ching-Wei Wang, Jyun-Yi Wang and Ming-Fang Lin	283
PO-8	DIMETHOXYFLAVONE, A FLAVONOID FROM <i>STEREOSPERMUM KUNTHIANUM</i> STEM BARK WITH ANALGESIC AND ANTIINFLAMMATORY ACTIVITIES <u>Ching, F. P</u> and Falodun, A.	283
PO-23	EFFECTS OF UNIFORM MAGNETIC FIELD REVERSAL ON ARABIDOPSIS THALIANA <u>Albert M. Chung</u>	284
PO-36	EXTRACTING AND TRANSPIRING METALS FROM CONTAMINATED SOILS <u>Jenna Forte</u> , <u>Megan Corley</u> , Sam Mutiti, and Alyssa Thomson	284
PO-71	THE NEUTRALIZATION OF ACID MINE DRAINAGE BY MIXED CULTURE 'ALGAL' BIOMASS Rosemary Falcon and <u>Evanie Devi Deenanath</u>	285
PO-82	<i>EX-VITRO</i> ADAPTATION AND SCREEN HOUSE EVALUATION OF GENETICALLY MODIFIED CASSAVA GENOTYPES FOR CMD RESISTANCE IN UMUDIKE, NIGERIA <u>Edoh N.L.</u> , Okwuonu I., Onyeka J. and C.N. Egesi	285
PO-61	WATER QUALITY CONTROL FOR INTENSIVE SHRIMP CULTURE PONDS IN DEVELOPING COUNTRIES USING INORGANIC NITROGENS UPTAKE BY SEAWEED <u>Ryuichi Egashira</u> , Hiroaki Habaki, Kosuke Sato, Shin Tajiri, Yuta Ueno and Tippabust Eksangsri	286
PO-77	LACTIC ACID BACTERIAL EXTRACT AS AN ALTERNATIVE CHOICE IN THE TREATMENT OF <i>C. ALBICANS</i> INFECTION: AN APPROACH <u>Hala El-Adawi</u> , Mahmoud Taha, Abdel Fattah Abdel Fattah and Nagwan Youssef	286
PO-78	EFFECT OF GAMMA IRRADIATION ON THE BIOACTIVITY OF <i>ARTHROSPIRA FUSIFORMIS</i> <u>Hala El-Adawi</u> , Mahmoud Taha, Abdel Fattah Abdel Fattah and Nagwan Youssef	287
VP-05	AN EFFECTIVE BCG VACCINATION SCHEME-CONTAINING PRIME AND ANNUAL PERIODIC SUBUNIT BOOST SELECTS AND ACTIVATES TEM AND TH17 CD4+ SUBSETS IN NATURALLY PREINFECTED DAIRY CATTLE Miranda-Hernández D.U., Ramírez-Cerda E.L. and <u>Estrada-Chávez C</u>	287

PO-47	LAVANDULA OFFICINALIS ESSENCIAL OIL AGAINST HAEMONCHUS CONTORTUS <u>Ferreira, L.E.</u> , França, S.C., Taleb-Contini, S.H., Chagas, A.C.S. and Beleboni, R.O.	289
PO-32	LIPID REMODELING AND LIPOPROTEIN FORMATION BY SERUM AMYLOID A: POTENTIAL FUNCTIONAL IMPLICATIONS <u>Nicholas Frame</u> , Shobini Jayaraman and Olga Gursky	289
VP-03	RAPID AND LOW COST PRODUCTION OF HGM-CSF CYTOKINE IN INDUSTRIAL TOBACCO – PROOF OF CONCEPT <u>Lea Vojta</u> , Lana Ljuma-Skupnjak, Ankica Budimir, Slobodan Vukičević and Hrvoje Fulgosi	290
PO-83	IMPACT OF WATER PH CHANGES ON FRESH WATER AQUATIC LIFE <u>Debolina Ghosh</u>	290
PO-48	RESEARCHES REGARDING THE USE OF COACERVATES ESSENTIAL OILS IN SEED TREATMENT IN ECOLOGICAL CROP PRODUCTION <u>Mihai Gidea</u> , Emil Stepan, Lenuta Iulia Epure, Mihaela Niculesu, Carmen Gaidau and Doru Gabriel Epure	291
PO-9	PRODUCTION OF BIOHYDROGEN BY COMBINING A TWO STAGE FERMENTATION PROCESS USING SALT-RICH SUBSTRATES <u>Dayana Muzziotti Gil</u> , Alessandra Adessi, Andrea Sanchini, Laura Dipasquale, Giuliana d'Ippolito, Angelo Fontana and Roberto De Philippis	291
PO-51	AGROBACTERIUM MEDIATED TRANSFORMATION OF SOMATIC EMBRYOS OF PERSIAN WALNUT (JUGLANS REGIA L.) USING FLD GENE FOR OSMOTIC STRESS TOLERANCE <u>Mohammad Ali Sheikh Beig Goharizi</u> , Atta Dejahang, Koroush Vahdati, Masoud Tohidfar, Ali Izadi Darbandi, Carrillo Nestor and Mohammad-Reza Hajirezaei	292
PO-21	DEVELOPMENT AND EVALUATION OF A GENETICALLY MODIFIED MICROORGANISM FOR HUMAN EPIDERMAL GROWTH FACTOR EXPRESSION USING GATEWAY® TECHNOLOGY Isaura Patricia Torres G, Sinar David Granada G, Ana María Aristizábal O, Juan Sebastian Herrera, Yuri Maritza Zapata, Cesar Segura L, Carlos Uribe, Orville Hernández R, Ligia Luz Corrales G, Ana María García, C, <u>Oscar Mauricio Gómez</u>	292
PO-58	IMMUNOLOGICAL & RECEPTOR BINDING CHARACTERIZATION OF BEETAL RECOMBINANT CAPRINE GROWTH HORMONE <u>Roquyya Gul</u> , Farkhanda Ghafoor, Sumbal Mehmood, Rabail Alam and Muhammad Waheed Akhtar	293
PO-13	ENGINEERING OF CYTOKINE GENE FOR CONDITIONAL ACTIVATION <u>Iram Gull</u> , Muhammad Shahbaz Aslam and Muhammad Amin Athar	293
PO-66	ROLE OF WOMEN STUDIES IN INHIBITORY EFFECTS OF GLORIOSA SUPERBA EXTRACT ON RHEUMATOID ARTERITIS <u>Veena Megaravalli Krishnappa Hegde</u> and H. N. Girish	294
PO-16	HYBRID DETECTION, DNA BARCODING AND IDENTIFICATION OF RETROTRANSPOSONS IN GENOME OF THAILAND'S NOTABLE WATERLILIES, <i>NYMPHAEA</i> SPP. <u>V. Hongtrakul</u> , C. Khensri, B. Sangsri, R. Tuntipaiboonana and P. Putanyawiwat	294
PO-6	EMULSIFICATION AND DIESEL OIL DEGRADATION ABILITY OF ACINETOBACTER HAEMOLYTICUS ZN01 ISOLATED FROM PETROLEUM HYDROCARBON CONTAMINATED RIVER WATERS <u>Bulent Içgen</u> , Gozde Onur and Fadime Yilmaz	295

PO-19	TRANSCRIPTOME ANALYSIS OF WHEAT GLUME UNDER DROUGHT STRESS Changxin Liu and <u>Jinyin Lyu</u>	295
PO-17	NEW THERAPEUTIC POTENTIAL OF LOW-DOSE FARNESYLTRANSFERASE INHIBITORS IN ATHEROSCLEROSIS, SEVERE INFECTION, MAJOR TRAUMA, FULMINANT HEPATITIS AND OBESITY <u>Masao Kaneki</u>	296
PO-57	POPLAR <i>GIGANTEAS</i> REGULATE CROSS-TALK BETWEEN FLOWERING TIME AND SALT RESPONSE <u>Qingbo Ke</u> , Zhi Wang, Ho Soo Kim, Seyeon Park, Jae Cheol Jeong, Haeng-Soon Lee and Sang-Soo Kwak	297
VP-01	ENCAPSULATION-DEHYDRATION TECHNIQUE EXPLORATION FOR CHRYSANTHEMUM 'LADY SALMON' CRYOPRESERVATION <u>Dariusz Kulus</u> , Monika Rewers, Agnieszka Abratowska and Anna Mikula	298
PO-73	<i>LENTINULA EDODES</i> LACCASES: RECOMBINANT ENZYME PRODUCTION AND APPLICATION IN SOFTWOOD HYDROLYSIS <u>Hoi-Shan Kwan</u> , Kin-Sing Wong, Valdeir Arantes and Jack N. Saddler	299
PO-45	<i>IN VIVO</i> ANTI-HYPERGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF THE LEAF EXTRACTS OF HOLARRHENA ANTIDYSENTERICA ON ALLOXAN- INDUCED DIABETIC RATS <u>Latha K.P.</u> and Shwetha C	299
PO-55	MORPHOLOGY ENGINEERING OF BASIDIOMYCETES FOR IMPROVED LACCASE BIOSYNTHESIS Anna Antecka, Michał Blatkiewicz, Marcin Bizukojć and <u>Stanisław Ledakowicz</u>	300
PO-56	BIOCHEMICAL METHANE POTENTIAL OF MAIZE HYBRIDS <u>Ledakowicz S.</u> , Pazera A. and Krzystek L.	300
PO-81	IMPROVEMENT OF THE ANAEROBIC DIGESTION EFFICIENCY OF WASTE ACTIVATED SLUDGE THROUGH CHEMICALLY-ASSISTED MICROWAVE IRRADIATION <u>Jaeho Lee</u> , Jeongmin Lee, Sanghoon Lee, Hyanggi Kim, Youngwoo Kim, Taejoo Park and Imgyu Byun	301
PO-25	THE C-TERMINUS OF CORE B-LADDER DOMAIN IN FLAVIVIRUS NONSTRUCTURAL PROTEIN 1 IS FLEXIBLE FOR ACCOMMODATION OF A HETEROLOGOUS EPI TOPE FUSION Li-Chen Yen, Yi-Ling Lin, Hwei-Jen Lee, Jia-Teh Liao and <u>Ching-Len Liao</u>	301
PO-14	LONG TERM EVALUATION OF ALGAE OCCURRENCE IN DOWNSTREAM THE NAKDONG RIVER, SOUTH KOREA: 1998~2014 <u>Ji- Sung Lim</u> , Jae-Ho Lee, Young-Woo Kim, Sang-Hoon Lee, Hyang-Gi Kim, Tae-Joo Park and Im-Gyu Byun	302
PO-50	NORMAL TISSUE COMPLICATION PROBABILITY MODELING FOR CONTROLLING THE PERCENTAGE OF IPSILATERAL LUNG IRRADIATED- VOLUME TO AVOID CAUSING SYMPTOMATIC RADIATION PNEUMONITIS FOR BREAST CANCER PATIENTS <u>Ming-Hsiang Liou</u> , Tsair-Fwu Lee, Pei-Ju Chao, Hsiao-Yi Lee and Yu-Jie Huang	302
PO-49	NORMAL TISSUE COMPLICATION PROBABILITY MODELING FOR COCHLEA CONSTRAINTS TO AVOID CAUSING TINNITUS AFTER HEAD-AND-NECK INTENSITY-MODULATED RADIATION THERAPY <u>Ming-Hsiang Liou</u> , Tsair-Fwu Lee, Shyh-An Yeh, Pei-Ju Chao, Hui-Min Ting, Yu-Jie Huang, Hsiao-Yi Lee	303

PO-15	<i>RHODOCOCCLUS ERYTHROPOLIS</i> NTU-1: FROM BIO-DEGRADATION TO BIO-FLOCCULATION AND BIO-DEMULSIFICATION <u>Hwai-Shen Liu</u>	304
PO-1	REMOVAL OF NITROGEN AND PHOSPHORUS FROM WASTEWATER IN AN OSMOTIC MEMBRANE PHOTOBIOREACTOR USING MICROALGAE Prashant Praveen and <u>Kai-Chee Loh</u>	304
PO-12	DESTABILIZING INFLUENCE OF MICROORGANISMS ON EFFICIENCY OF IMMOBILIZATION OF ARSENIC BY BOG IRON ORES Lukasz Drewniak, Katarzyna Kucharek and <u>Pola Lomza</u>	305
PO-22	MAQUI (ARISTOTELIA CHILENSIS) AMELIORATES ENDOTHELIAL DYSFUNCTION IN RATS FED WITH HIGH FAT/CHOLESTEROL DIET. <u>Oscar Fuentes Mardones</u> , Susana Badilla Wagner and Carlos Céspedes Acuña	305
PO-53	REAL TIME PCR TO ELUCIDATE ENDOPHYTIC STAGE OF RAMULARIA COLLO-CYgni IN BARLEY SEED <u>Pavel Matusinsky</u> , Leona Svobodova-Leisova, Pavel Marik and Ludvik Tvaruzek	306
PO-10	SYNAPSE FORMATION IN EMBRYONIC NEURAL STEM CELLS TRANSPLANTED INTO ISCHEMICALLY INJURED MOUSE BRAIN Nina Kosi, Ivan Alić, Srećko Gajović and <u>Dinko Mitrečić</u>	306
PO-72	PHYSIOLOGICAL EFFECT OF SODIUM ALGINATE DECOMPOSED PARTIALLY BY VIBRIO ALGINOLYTICUS SUN53 ON THE INHIBITION TO DISACCHARIDASE AND GLUCOSYLTRANSFERASE <u>Sadako Nakamura</u> , Michiru Hashiguchi, Kenichi Tanabe and Tsuneyuki Oku	307
PO-52	A NEW ASSAY FOR MEASURING TRACE ELEMENTS IN SERUM FOR THE ESTIMATION OF BREAST CANCER RISK <u>Naoyuki Okamoto</u> , Haruo Mikami, Youko Nakamura, Hiroki Nagase, Katsura Katou, Seiichi Inagaki, Hideo Tanaka and Yohei Miyagi	307
PO-67	BIOAVAILABILITY AND AVAILABLE ENERGY OF NEWLY DEVELOPED DIETARY FIBRE MATERIALS, RESISTANT GLUCAN AND HYDROGENATED RESISTANT GLUCAN, IN RATS AND HUMANS <u>Tsuneyuki Oku</u> , Kenichi Tanabe, Norihisa Hamaguchi, Fumio Shimura and Sadako Nakamura	308
PO-74	EFFECTS OF OLEUROPEIN AND PERACETIC ACID AS SANITIZING AGENTS FOR INACTIVATION OF LISTERIA <i>MONOCYTOGENES</i> BIOFILMS Laura C. C. Dominciano, Sarah H. I. Lee, Roice E. Rosim, Carlos H. Corassin and <u>Carlos A. F. Oliveira</u>	308
PO-75	COMPARISON OF THE INACTIVATION EFFECT OF PERACETIC ACID AND ATMOSPHERIC PLASMA TREATMENTS ON STAPHYLOCOCCUS <i>AUREUS</i> AND LISTERIA <i>MONOCYTOGENES</i> STRAINS Sarah. H. I. Lee, Julia Jacob, Antje Fröhling, Oliver Schlüter and <u>Carlos A. F. Oliveira</u>	309
PO-33	FORMIC ACID REINFORCED AUTOHYDROLYSIS OF WHEAT STRAW FOR HIGH YIELD ENZYMATIC SUGAR PRODUCTION AND MINIMAL LIGNIN PRECIPITATION DURING PRETREATMENT <u>Omer Ozyurek</u> and Adriaan van Heiningen	310
PO-63	PHYLOGENETIC ANALYSIS AND MARKER DEVELOPMENT USING COMPLETE CHLOROPLAST GENOME SEQUENCES OF SOLANACEOUS SPECIES <u>Tae-Ho Park</u> and Kwang-Soo Cho	310

PO-38	PHARMACOLOGY METABOLIC CHANGES ASSOCIATED WITH ISCHEMIA AND REPERFUSION <u>Beatriz Prieto-Moure</u> , Dolores Cejalvo-Lapeña, Lajara-Romance J.M., Elena Bover-Arbona, Daniel López-Malao Carolina Padrón-Sanz, Javier Torres Gavilá and José Miguel Lloris-Carsí	311
PO-29	USE OF MEDIUM DENSITY SNP PANELS FOR DEVELOPING A MULTIVARIATE BREED ASSIGNMENT APPROACH IN CATTLE AND SHEEP <u>Pulina G.</u> , Dimauro C., Stella A., Ciani E., Moiola B.5, Pilla F., Crepaldi P. and Maciotta N.P.P.	312
PO-44	INCREASED EXPRESSION OF HUMAN INTERFERON GAMMA THROUGH SELECTIVE PRESSURE ON THE ADJACENT HIS4 GENE IN PICHIA PASTORIS <u>Ali Razaghi</u> , Roger Huerlimann, Leigh Owens, Kirsten Heimann	312
PO-18	REDUCING THE TOXIC EFFECT OF WINE INTAKE (NEUTRALISATION OF THE MOST TOXIC ETANOL DERIVATE ACETALDEHYD WITH ESSENTIAL AMINOACIDS L-LYSINE AND L-ARGININ) Gy. Kóródi, <u>J. Rikk</u> and T. Szarvas	313
VP-2	INCLUSION OF RICE BRAN EXTRACT AND ITS EFFECT ON NUTRITIONAL QUALITY OF SUGARCANE BAGASSE HEMICELULOSIC HYDROLYSATE TO XYLITOL PRODUCTION BY YEAST <u>Rita C.L.B. Rodrigues</u> , Maria das Graças A. Felipe, Arnaldo M. R. Prata and Adalberto Pessoa Jr.	314
PO-3	<i>IN VITRO</i> RESTORATION OF SMN PROTEIN BY HOMOLOGOUS RECOMBINATION METHOD <u>Afroz Rashnonejad</u> , Cumhur Gündüz, Sunde Süslüer, Hüseyin Onay, Burak Durmaz, Mojgan Bandehpour and Ferda Özkinay	314
PO-2	EXPERIMENTAL BURN WOUND HEALING USING MOUSE EMBRYONIC STEM CELLS DERIVED KERATINOCYTES Gulinnaz Ercan, Yiğit Tiftikcioglu, Seda Vatansever, Elgin Turkoz, <u>Afroz Rashnonejad</u> and Cafer Seyid	315
PO-34	LIPID-BASED SIGNALING IN ARABIDOPSIS LOCAL DEFENSE RESPONSES <u>Stephen B. Ryu</u> , Jihye Jung and Sunghee Jung	315
PO-35	APPLICATION OF VARIOUS COLLOIDAL SERS APPROACHES FOR DETECTION OF TRACES OF URIC ACID Martynas Velicka, Milda Pucetaite, Justinas Ceponkus and <u>Valdas Sablinskas</u>	316
VP-06	INDUCTION OF DNA DAMAGE IN BLOOD CANCER CELLS BY LOW INTENSITY ULTRASOUND <u>Timur Saliev</u> , Dinara Begimbetova, Aibobek Seitak and Dinara Baiskhanova	316
PO-5	STUDY OF GLYCOSYLATION CHANGES WITH LECTIN-BASED PROTEIN MICROARRAY <u>Alena Šedivá</u> , Martina Zámorová, Dragana Robajac, Goran Miljuš, Miloš Šunderić Olgica Nedic, Jaroslav Katrlík	317
PO-28	BIOGENIC SYNTHESIS, OPTIMIZATION AND ANTIBACTERIAL EFFICACY OF EXTRACELLULAR SILVER NANOPARTICLES USING NOVEL FUNGAL ISOLATE ASPERGILLUS NIGER MA Vikas Sarsar, Manjit K. Selwal and Krishan <u>K. Selwal</u>	317
PO-70	BIOTECHNOLOGICAL POTENTIAL OF INDUSTRIAL RESIDUES AS SUBSTRATES FOR BIONEMATICIDE DEVELOPMENT <u>Satyawati Sharma</u> and Abhishek Sharma	318

PO-7	APOPTOTIC EVENTS INDUCED BY PROTOTYPE FOAMY VIRUS INFECTION Md. A. Hossain, Jinsun Kim, Faysal Bin Hamid and <u>Cha-Gyun Shin</u>	318
VP-04	ETHICAL AND LEGAL FACETS OF BIOFEEDBACK IN ICT-BASED ASSISTIVE TECHNOLOGIES FOR SENIORS <u>Luiza Spiru</u> and Stefan Alexandru Sterea	319
PO-60	EFFECT OF DIFFERENT SOUND GENRES ON IN VITRO SEED GERMINATION OF GRAMMATOPHYLLUM HYBRID AND GRAMMATOPHYLLUM STAPELIIFLORUM ORCHIDS <u>Noor Anilizawatima Sulong</u> , Noor Izzah Mohd Khalil, Mohd Ikhwan Dahari and Anis Athirah Zakaria	319
PO-62	A GENOME-WIDE ASSOCIATION STUDY ON BODY COMPOSITION OF JINGHAI YELLOW CHICKEN <u>ZHANG Tao</u> , FAN Qing-can, ZHANG Xiang-qian, ZHANG Gen-xi, WANG Jin-yu and GU Yu-ping	320
PO-24	A TECHNIQUE FOR CONTINUOUSLY IMAGING PANCREATIC ISLETS WITHIN A ROTATING WALL VESSEL BIOREACTOR <u>Lauren E. Getz</u> , S. Dan Dimitrijevič, Jeffrey L. Coffey and <u>Tristan J. Tayag</u>	320
PO-40	APPLICATION OF PRACTICAL MINIATURIZED PROTOCOL TO FACILITATE ENUMERATION OF <i>ESCHERICHIA COLI</i> AND COLIFORMS FOR FOOD INDUSTRY Wipavadee Sangadkit, Anat Deepatana and <u>Aluck Thipayarat</u>	321
PO-65	<i>IN VITRO</i> ANTIOXIDANT AND ANTITUBERCULAR ACTIVITY OF LEUCAS MARRUBIODES DESF. ROOT EXTRACTS <u>Vagdevi H.M.</u> , Gowrish and Rajashekar	321
PO-80	TUMOR TARGETING DUAL IMAGING PROBE AND DRUG DELIVERY <u>J. Y. Wang</u> , Y. Z. Wang, Y. X. Li and Y. P. Li	322
PO-54	BROAD EXISTENCE OF PLURIPOTENCY FACTOR REGULATED TRANSCRIPT ISOFORMS WITH STAGE-SPECIFIC ALTERNATIVE FIRST EXONS (SAFE) IN MOUSE EMBRYONIC STEM CELL <u>Xiu-Jie Wang</u>	322
PO-46	ESTABLISHMENT OF A NOVEL TUMOR ENDOTHELIAL CELL LINE DERIVED FROM HUMAN HCC <u>Liping Yang</u> , Yixin Zhang, Wenjing Zhao, Chen Xudong, Qian Hongyan and Shao Jingjing	323
PO-59	ISOLATED AND FUNCTION ANALYSIS OF GAPC GENES AND THEIR PROMOTERS IN DIFFERENT WHEATS Fenling-Liu1, Min-Zhang, <u>Shushen-Yang</u> , Xiping-Deng and Lun-Shan	323
PO-69	BIOREMEDIATION OF WASTEWATER BASED ON MICROALGAE STRAIN - PRODUCER OF FATTY ACIDS, PROMISING FOR BIODIESEL <u>Bolatkhan K. Zayadan</u> , Asem K. Sadvakasova, Fariza K. Sarsekeyeva, Aizhan A. Userbayeva and Dmitry A. Los	324
	CUMULATIVE AUTHOR INDEX	325
	CUMULATIVE SUBJECT INDEX	339

PLENARY LECTURES

PL-293**CURRENT STATUS OF OBESITY AND THE NEED FOR A PARADIGM SHIFT****Richard L. Atkinson**

Virginia Commonwealth University, Obetech Obesity Research Center, 800 East Leigh St., Suite 50, Richmond, VA 23219, USA; E-mail: ratkinson2@vcu.edu



There is a worldwide epidemic of obesity that is creating great health problems for individuals and massive economic costs for governments. More than 68% of American adults are overweight or obese. Many government and professional society guidelines for the treatment of obesity state that “diet and exercise are the primary treatment of obesity.” If the search terms “obesity and lifestyle and treatment and human” are used in PubMed, there are 8819 references. None have shown significant long term success of lifestyle treatment of obesity – the 5 year failure rate is >95%. It seems highly unlikely that the 8820th study of lifestyle is suddenly going to prove successful. Only in 2013 did the American Medical Association adopt a statement that obesity is a “disease.” Before the founding of the American Obesity Association, a lay advocacy group, in 1995, the NIH spent about \$30 million on obesity research out of a total budget of about \$12 billion. This was less than 1% of the budget for a disease affecting almost 30% of American adults at that time. Currently, the NIH states on its website that it spends about \$857 million on obesity research, but since the total budget figure is inflated by 5 fold, the true expenditure probably is about \$171 million. A quite significant percentage of this amount is spent on lifestyle research. In contrast to more established diseases, the early research into obesity has not focused on basic science, but on treatment (e.g., \$220 million for the “LOOK AHEAD” trial). We are in our infancy of the understanding of obesity. Since pre-history, humans have thought they knew the etiology of obesity – too much diet and too little exercise. It seems logical, but just because a perturbation affects a variable, does not mean that it is the cause of the variable. It seems quite possible that diet and exercise account for only a very small percentage of obesity. There is no doubt that body weight, or at least body fat, are regulated by the body. Why some people regulate at a high percentage of body fat vs others is not clear. Recent research has identified a number of etiologies of obesity that are NOT the “Big Two” of diet and exercise. This talk will summarize the research on some of these alternate etiologies of obesity and focus on several that may be responsible for large portions of obesity. Genetic factors are very important. At least 60 genes have been shown to contribute to or prevent obesity. Calculating the factorial (60 x 59 x 58, etc.), there are more combinations of genes for obesity than there are people on Earth. Next is virus-induced obesity. Human adenovirus 36 (Adv36) causes obesity in animals and in multiple countries that have been studied, about 30% of obese humans have been infected compared to about 10%-20% of non-obese humans. Scientists have postulated that Adv36 first appeared in the 1970s, just before the prevalence of obesity dramatically increased across the world. Another etiology of obesity that is being recognized as a major contributor to the epidemic are presumably epigenetic factors affecting women of child bearing age. If a woman has the following factors before and during pregnancy, the risk of obesity in her child is 20-40 fold higher than if none of the factors are present: obese at conception, increased weight gain, smoking, eating a high fat or high carbohydrate diet, lack of exercise, older age, taking certain drugs, and perhaps one of the most important, developing gestational diabetes during pregnancy. Almost all of these factors may be avoided or removed, and some evidence suggests that this will prevent a great deal of obesity in her offspring. Other factors that may play a role in causing obesity are certain drugs that are taken much more commonly in the last 30 years, certain industrial pollutants in the environment, and alteration of gut microbiota by changes in the diet favoring processed or refined foods and beverages. The etiology of obesity is so complex that a concerted effort must be made to identify basic biochemical and molecular factors leading to obesity. This information must be used to identify new drugs for treating obesity. Finally, in contrast to most “cookie cutter” treatment of obesity today, individualized treatment must be developed. No two patients are alike and it seems likely that obesity treatment options will be very numerous in the future.

PL-122**APPLICATION OF NITRIC OXIDE RESEARCH TO DRUG DEVELOPMENT****Ferid Murad**

Department of Biochemistry & Molecular Medicine, George Washington University, 2300 Eye Street NW, Suite 530, Washington, DC 20037, USA; E-mail: jsreed83@gwmail.gwu.edu



The role of nitric oxide in cellular signaling in the past three decades has become one of the most rapidly growing areas in biology. Nitric oxide (NO) is a gas and a free radical with an unshared electron that can regulate an ever-growing list of biological processes. Nitric oxide is formed from L-arginine by a family of enzymes called nitric oxide synthases. These enzymes have a complex requirement for a number of co-factors and regulators including NADPH, tetrahydrobiopterin, flavins, calmodulin and heme. The enzymes are present in most cells and tissues. In many instances, nitric oxide mediates its biological effects by activating the soluble isoform of guanylyl cyclase (SGC) and increasing cyclic GMP synthesis from GTP. Cyclic GMP, in turn, can activate cyclic GMP-dependent protein kinase (PKG) and can cause smooth muscles and blood vessels to relax, decrease platelet aggregation, alter neuron function, *etc.* These effects can decrease blood pressure, increase blood flow to tissues, alter memory and behavior, decrease blood clotting, *etc.* The list of effects of nitric oxide that are independent of cyclic GMP formation is also growing at a rapid rate. For example, nitric oxide can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Some of these reactions result in the oxidation of nitric oxide to nitrite and nitrate to terminate the effect and perhaps act as NO reservoir for future NO formations; while other reactions can lead to altered protein structure function and/or catalytic capacity. These effects of (NO) probably regulate bacterial infections, inflammation of tissues, tumor growth, and other disorders. These diverse effects of nitric oxide that are cyclic GMP dependent or independent can alter and regulate numerous important physiological events in cell regulation and function. Nitric oxide can function as an intracellular messenger, an autacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects. Thus, it is a unique molecule with an array of signaling functions. However, with any messenger molecule, there can be too little or too much of the substance, resulting in pathological events. Some of the methods to regulate either nitric oxide formation, metabolism, or function have been in clinical use for more than a century as with the use of organic nitrates and nitroglycerin in angina pectoris that was initiated in the 1870's. Inhalation of low concentrations of nitric oxide can be beneficial in premature infants with pulmonary hypertension and increase survival rates. Ongoing clinical trials with nitric oxide synthase inhibitors and nitric oxide scavengers are examining the effects of these agents in septic shock, hypotension with dialysis, inflammatory disorders, cancer therapy, *etc.* Recognition of additional molecular targets in the areas of nitric oxide and cyclic GMP research will continue to promote drug discovery and development programs in this field. Current and future research will undoubtedly expand the clinician's therapeutic armamentarium to manage a number of important diseases by perturbing nitric oxide formation and metabolism. Such promise and expectations have obviously fueled the interests in nitric oxide research for a growing list of potential therapeutic applications. There have been and will continue to be many opportunities from nitric oxide and cyclic GMP research to develop novel and important therapeutic agents. There are presently more than 150,000 publications in the areas of nitric oxide research. The lecture will discuss our discovery of the first biological effects of nitric oxide and how the field has evolved since our original reports in 1977. The possible utility of this signaling pathway to facilitate novel drug development and the creation of numerous projects in the Pharmaceutical and Biotechnology industries will also be discussed.

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PL-123**EVOLUTIONARY DYNAMICS AND TREATMENT OF CANCER****Martin Nowak**

Program for Evolutionary Dynamics, Harvard University, Cambridge, MA 02138, USA;

E-mail: martin_nowak@harvard.edu

Cancer is an evolutionary process. Cancer initiation and progression are caused by somatic mutation and selection of dividing cells. The mathematical theory of evolution can therefore provide quantitative insights into human cancer. I will discuss the role of chromosomal instability (CIN) and the accumulation of drivers and passengers in growing tumors. I will study success and failure of targeted therapy including combination of different drugs and evolution of resistance. A simple conclusion is that combination treatment can succeed, if the cancer requires at least two point mutations to gain resistance. From the perspective of preventing resistance, simultaneous therapy is highly recommended whereas sequential therapy is a recipe for almost certain treatment failure.

FURTHER READINGS

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PL-224**EMERGING PHARMACO-MPE (MOLECULAR PATHOLOGICAL EPIDEMIOLOGY) PARADIGM FOR GLOBAL PRECISION MEDICINE****Shuji Ogino**

Harvard Medical School, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard T.H. Chan School of Public Health; E-mail: Shuji_Ogino@dfci.harvard.edu

This lecture introduces the evolving paradigm of "Molecular Pathological Epidemiology (MPE)" (= Molecular Pathology + Epidemiology) (S Ogino *et al.* J Natl Cancer Inst 2010; S Ogino *et al.* Nat Rev Clin Oncol 2011; A Field *et al.* JAMA 2013; *etc.*) as simply as possible. Any given human disease represents fundamentally heterogeneous process, as implicated by the "Unique Disease Principle". MPE dissects complex interplay between environmental, dietary, lifestyle factors, molecular pathogenic alterations, and disease occurrence and progression. MPE is a logical next step of genome-wide association studies (GWAS), termed "GWAS-MPE Approach". MPE has proven itself to be a promising approach to identify biomarkers for precision medicine (A Chan *et al.* NEJM 2007; X Liao *et al.* NEJM 2012; R Nishihara *et al.* NEJM 2013, *etc.*). Recently, the pharmaco-MPE paradigm has been utilized to uncover unanticipated effects of medications on health and diseases, using large population-based MPE databases. It is increasingly possible to design MPE database worldwide using routine molecular testing data, as molecular pathology testing is becoming routine clinical practice. It is essential to build large-scale population-based databases including medication use, lifestyle factors, molecular pathology, and clinical outcome. Such databases can generate novel information on potential chemopreventive or therapeutic benefits of drugs, which can be further tested by experimental models and clinical trials. To expand opportunities and address challenges, the "International Molecular Pathological Epidemiology (MPE) Meeting Series" was established in 2013, and the Third International MPE Meeting will be held in Boston on May 12-13, 2016. Because disease heterogeneity is a ubiquitous phenomenon, the MPE and pharmaco-MPE paradigms should become routine to advance biomedical and population health sciences in the 21st century, and move us towards personalized prevention and treatment.

PL-222**RECONSTRUCTION, MODELING AND USE OF GENOME-SCALE NETWORKS IN BIOLOGY****Bernhard Palsson**

*Departments of Bioengineering and Pediatrics University of California, San Diego, San Diego, USA;
E-mail: palsson@eng.ucsd.edu*

Following the availability of full genome sequences in the mid 1990s, an effort was initiated to reconstruct, on a genome-scale, the biochemical reaction networks that underlie cellular functions. After 15 years of intense efforts, we now have highly curated network reconstructions, their experimental validation, and the generation of mathematical and modeling procedures available that allow the computation of cellular functions from genome- and bibliome-wide data sets. This effort has put a mechanistic basis into the most fundamental relationship in the life sciences; the genotype-phenotype relationship. This effort has started with simple organisms and the best characterized cellular functions and it is steadily growing in scope and biological complexity.

**PL-223****NOVEL PRO-RESOLVING LIPID MEDIATORS IN INFLAMMATION & INFECTION LEADS FOR RESOLUTION PHYSIOLOGY AND PHARMACOLOGY****Charles N. Serhan**

*Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women's Hospital & Harvard Medical School, Boston, Massachusetts, 02115, USA;
E-mail: cnserhan@zeus.bwh.harvard.edu*

Endogenous mechanisms controlling inflammation are of paramount importance because persistent and chronic inflammation can impact all organs and tissues throughout the body and are involved in many widely occurring diseases. Recent advances in our appreciation of the molecular mechanisms in resolution of acute inflammation (RoI) and ischemia-reperfusion injury systematically uncovered a novel genus of potent pro-resolving autacoid families, each biosynthesized from essential polyunsaturated fatty acids (PUFA) to activate potent responses not shared by the substrate. These include the *resolvins* (Rv), *protectins* (PD) and *maresins* (MaR), collectively termed *specialized proresolving mediators* (SPM) that act in pico-nanogram range. SPM are temporally and spatially biosynthesized by resolving-inflammatory exudates, which proved to evoke potent anti-inflammatory and pro-resolving actions as well as enhance microbial clearance. The potent SPM actions and complete structures are confirmed, which also permitted use of LC-MS-MS-based metabololipidomics to identify SPM in human and murine tissues (i.e. peripheral blood, breast milk, adipose, lymphoid, placenta), isolated human cells types (e.g. apoptotic human neutrophils, microparticles and macrophage phenotypes M1, M2), fish and diminished SPM in human pathologies e.g. breath condensates, Alzheimer brain, and synovial fluids from rheumatoid patients (CN Serhan Nature June vol 510, 2014 doi:10.1038/nature13479). Specific SPM demonstrate potent and stereoselective actions that involve specific G-protein-coupled receptors and are not immunosuppressive. Lipid mediator-metabololipidomics with self-limited resolving inflammatory exudates and human tissues demonstrated temporal orchestration of the SPM, i.e. RvD1 and RvD2 antecede RvD3, and MaR1 in mice and human tissues (Colas *et al.* AJP 2014). Many of the SPM born in inflammation-resolution are now shown to have potent actions and roles within host defense against bacteria and virus, pain, organ protection, tissue regeneration, exercise and neurobiology/cognitive function. This Plenary Lecture will update advances in SPM mechanisms in RoI, their new sites of formation and novel actions that opened the door for their role(s) in resolution physiology and pharmacology. Together, these new SPM families provide opportunities for resolution-based pharmacology and resolution physiology.



PL-2**MICRORNAs, SMALL INTERFERING RNAs AND MODULAR THERAPEUTICS****Phillip A. Sharp**

Koch Institute for Integrated Cancer Research and Department of Biology, MIT, USA; E-mail: marg@mit.edu

RNA interference was discovered a little over ten years ago and subsequently was shown to be mediated by intracellular double stranded small RNAs approximately 21 nucleotides in length (siRNA). In mammals, these RNAs enter the microRNA pathway, present in all cell types, are loaded into a complex containing the critical Argonaute protein, and target its activity to cleave and cause degradation of specific complimentary mRNA. Thus, in principle with the appropriate design of the siRNA any target gene could be silenced. Over the past years, the challenge of effective delivery of the hydrophilic siRNAs to cells has been advanced to where it is now possible in a therapeutically attractive fashion to silence genes expressed in the liver of humans with a sugar-based conjugate of a chemically modified siRNA. These therapeutic agents are modular in structure, where one constituent provides a gene-specific component whose modulation of expression is beneficial, while the other agent targets and facilitates delivery to the inside of cells. This modular property greatly reduces the time required to develop therapeutics to new disease modifying genes and is an

example of future developments in pharmaceuticals. Examples of these types of agents will be discussed. The activities of small RNA based agents will be set in the context of the known biology of small non-coding RNAs.

PL-1**DESIGNING BIOLOGY FOR A HEALTHY WORLD****Pamela A. Silver**

Department of Systems Biology, Harvard Medical School and The Wyss Institute for Biologically Inspired Engineering, Harvard University, USA; E-mail: Pamela_Silver@hms.harvard.edu

The engineering of Biology presents infinite opportunities for therapeutic design, diagnosis, and prevention of disease. Towards these goals, we seek to make the engineering of Biology faster, more predictable and cheaper. This 'Synthetic Biology' has deep practical and social consequences for the pharmaceutical as well as the commodity industry. Here, I will present concepts and experiments that begin to address how we approach these problems in a systematic way.



By one strategy, we seek to predictably engineer mammalian cells to produce novel compounds that could potentially act as new therapeutics. For example, we have developed an algorithm for biosynthesis of new steroids that could have increased specificity towards their respective targets. This has implications in treatment of inflammation and clean production of other chemicals of interest.

By a second strategy, we design chimeric proteins to act as specific therapeutics. Specificity in biologics remains one of the outstanding issues in their use. We have again developed an algorithm based on coarse grain modeling for the predictable design on new proteins. Some have been tested in animals and show the predicted effects.

Lastly, we engineer components of the microbiome to act as both diagnostics and therapeutics. In one example, we have engineered natural gut bacteria to record the exposure of animals to antibiotics and to count the number of cell divisions as the bacteria passes through the gut. We can engineer the same bacteria to secrete toxins that could result in localized killing of pathogens and to act in a communal manner. Taken together, these experiments have far-reaching implications for the use of biology to prevent and treat disease in the future.

PL-291**ADVANCED THERAPEUTICS WHILE PRESERVING THE MICROBIOME****Ada Yonath**

*Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel;
E-mail: Ada.Yonath@weizmann.ac.il*



Ribosomes, the universal cellular machines for translation of the genetic code into proteins, possess spectacular architecture accompanied by inherent mobility, allowing for their smooth performance as polymerases that translate the genetic code into proteins. The site for peptide bond formation is located within a universal internal semi-symmetrical region. The high conservation of this region implies its existence irrespective of environmental conditions and indicates that it may represent an ancient RNA machine. Hence, it could be the kernel around which life originated. The mechanistic and genetic applications of this finding will be discussed.

Owing to the key role played by ribosomes in life cycles, almost half of the clinically useful antibiotics paralyze ribosomes by binding to their functional sites. By investigating the three dimensional structures of ribosomes from non-pathogenic bacteria as models for genuine pathogens, common features were identified. Thus, the antibiotics binding modes, inhibitory actions and synergism pathways have been determined for almost all ribosomal antibiotics. These indicated the principles of differentiation between patients and pathogens and suggested common principles of mechanisms leading to bacterial resistance.

The incredible global increase in resistance to antibiotics that we are witnessing recently is a serious medical threat. It seems that the world is approaching a post-antibiotic era, in which common infections and minor injuries that have been treatable for decades could become fatal once again.

As species specific diversity was detected in susceptibility to infectious diseases and in developing specific resistance mechanisms, our structural studies have been extended to ribosomes from genuine pathogens. By determining the high resolution structure of the first and only ribosomal particle from a genuine pathogen with several antibiotics, we identified subtle, albeit highly significant structural elements that can account for the species specificity in resistance, thus could paved ways for improvement of existing antibiotics as well as for the design of advanced therapeutics capable of minimizing antibiotics resistance.

PL-292**SPECIFIC ANTI-TUBERCULIN (IgG) HIGH LEVELS AND LYMPHOCYTE *IN VITRO* PROLIFERATION WITH TUBERCULIN ARE BETTER INDICATORS OF LTBI THAN TST**

Edmond J. Feris^{1,§}, Joaquin Zuñiga^{2,§}, Liliana Encinales³, Joel N.H. Stern^{4,5}, Julio Granados⁶, Marcelo Fernandez-Vina⁷ and Edmond J. Yunis³

¹*Department of Pharmacology and Toxicology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03765, USA*

²*Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico*

³*Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA;
E-mail: edmond_yunis@dfci.harvard.edu*

⁴*Department of Science Education, Hofstra North Shore-LIJ School of Medicine, Hempstead, NY 11549, USA*

⁵*Department of Neurology, North Shore- LIJ, Manhasset, NY 11030, USA*

⁶*Immunology and Rheumatology Department, Instituto Nacional de Ciencias Medicas y Nutricion México City, Mexico*



⁷Department of Pathology, Stanford University School of Medicine, Stanford, CA 94304, USA

In previous report, we reported that anti tuberculin (IgG) antibodies and *in vitro* proliferation assays against tuberculin are better indicators of latent tuberculosis infection (LTBI) than the tuberculin skin test (TST). We concluded that these tests are markers of LTBI regardless of the level of exposure and TST results. We now report that, the blocking of proliferation responses was only produced in the cultures with tuberculin and not *Candida* suggesting a crosstalk between the two antigens influence cellular immunity and not humoral immunity. Most important is the fact that although cellular immunity plays a central role in the pathogenesis of active and latent tuberculosis, humoral immunity is dominant in LTBI. In this regard, recent evidence demonstrated that conserved genes of *Mycobacterium tuberculosis* coding the epitopes that induce T cell dependent are naturally selected; the immune response benefits the mycobacteria. Therefore, we emphasize the need to use humoral immune mechanisms in the diagnosis and control of mycobacterium infection.

[§]These authors contributed equally.

KEYNOTE LECTURES

KNL-250

Track: Pharmaceutical Research & Development

SCIENTIFIC MISCONDUCT IN BIOMEDICAL SCIENCES**Rathnam Chaguturu**

iDDPartners, Princeton Junction, NJ 08550, USA; E-mail: mchaguturu@gmail.com



Discovering safe and effective medicines, one of the greatest gifts to humanity, relies intensely upon the availability of detailed, unassailably accurate biomedical scientific knowledge. Unfortunately, such critical technical endeavors are being jeopardized by an epidemic of false-science unfolding around us. There has been an alarming increase in the number of scholarly articles retracted, and almost two thirds of the retractions have been traced to scientific misconduct and fraud, not error. Although science, like Wall Street, is self-correcting to a certain extent, the outcome of the recent housing bubble reminds us vividly how much pain and destruction can be incurred by passively awaiting organic self-correction. The talk centers on research malfeasance in the biomedical arena, characterizes some of the key forms of deliberate misconduct, including falsification of results, peer-review rigging, data over-interpretation and improper or willfully selective sampling practices. The discussion also explores problematic grey areas such as choice of inappropriate analytical protocols, the failure to retract erroneous findings and the use of textual plagiarism for manuscript assembly. The investigator, the publisher, the institution, the funding agencies, and the national policy-makers have the imperative, the ability and the resources to identify research improprieties and prevent such misconduct from inflicting negative consequences on their research priorities. It is good to be reminded of the quotes from Mark Twain, which was true then, certainly now, and surely also for eternity:

“It ain’t what you don’t know that gets you into trouble. It’s what you know for sure that just ain’t so.”

KNL-53

Track: Cancer Targeted Drug Delivery

ATP SYNTHESIS BY CANCER CELLS: BEYOND WARBURG**Gautam Dhar, Suvajit Sen and Gautam Chaudhuri**

*Departments of Obstetrics and Gynecology and Molecular and Medical Pharmacology & Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at University of California at Los Angeles, USA;
E-mail: GChaudhuri@mednet.ucla.edu*

Aggressive cancers exhibit high glucose to lactate conversion accompanied by acid secretion, a phenomenon popularly known as the Warburg effect. The acidic microenvironment and the alkaline cytosol create a proton-gradient (acid gradient) across the plasma membrane that represents proton-motive energy. There is strong *in vivo* physiological evidence which indicate that acid gradient can stimulate proliferation of tumors and is also supportive of their energy needs. However, direct biochemical evidence linking extracellular acid gradient to the generation of intracellular ATP have been lacking. We demonstrated that cancer cells can synthesize significant amounts of phosphate- bonds from phosphate in response to acid gradient across the plasma membrane. This phenomenon was observed even in the absence of glycolysis and mitochondrial ATP synthesis, and is unique to cancer. Assays with viable cancer cells and plasma membrane vesicles utilizing radioactive phosphate confirmed new phosphate-bond synthesis from phosphate (P_i), as well as localization of this activity to the plasma membrane. In addition to ATP, predominant formation of pyrophosphate (PP_i) from P_i was observed when plasma membrane vesicles from cancer cells were subjected to trans-membrane acid gradient. Cytosol from cancer cells were found capable of converting PP_i to ATP, and also stimulating ATP synthesis from P_i from the vesicles. Acid gradient created through glucose metabolism by cancer cells, as observed in tumors, also proved critical for phosphate-bond synthesis. The findings suggest a role of acidic tumor milieu as a potential energy source. This pathway can be a prospective therapeutic target.

KNL-82

Track: Hot Topics in Natural Products

NATURE-BASED DRUG DISCOVERY – REEMERGING PARADIGM**M. Iqbal Choudhary and Atta-ur-Rahman**

International Center for Chemical and Biological Sciences, (H.E.J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research) University of Karachi, Karachi-75270, Pakistan; E-mail: iqbal.choudhary@iccs.edu



Modern drug development is an expensive and lengthy process, which requires over \$ 1.8-2.0 billion worth of investment, and focused work of a large interdisciplinary team of scientists, involving years of studies, and screening of a large chemical space. Unfortunately this situation has out-numbered and out-resourced the academic institutions and pharmaceutical R & D of developing nations. The role of academic institutions in drug development, particularly in developing countries, is gradually diminishing. As a result, several diseases, affecting the lives of poor population of the South remain untreated. This situation demands a major *soul searching* by pharmaceutical scientists who wish to serve the humanity through the skills they possess. Overall change in paradigm in drug development is required, which create space for academic researchers and R & D workers of developing nations to contribute in the discovery and development of drugs against diseases affecting their regions. This change must involve the effective use of indigenous knowledge and resources. Natural products and their traditional uses can play a very important role in drug development for poor by researchers of developing world. During the presentation, results of our studies on natural products will be presented to support the argument that the knowledge-based research on the natural products is a key to discover potential drug candidates at low cost.

Multidrug resistance is a challenging problem for the healthcare sector and is very common in familiar pathogens, such as vancomycin-resistant enterococci and *Staphylococcus aureus*. Exposure and inappropriate use of the antibiotics is the measure cause of MDR, both in developed and developing regions. Our study, focusing on the discovery of natural and synthetic compounds, active against multidrug resistant bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* have resulted in the identification of several novel and potent inhibitors of MDR *Staphylococcus aureus*, (EMRSA-17, EMRSA-16, MRSA-252, and Pak clinical isolates) from natural sources. Resistance-reversal studies at molecular level were carried out by employing flow cytometric and microscopic techniques. Synergistic and partial synergistic effects of these compounds, in combination with antibiotics, were investigated. This work has so far resulted in the identification of several novel “helper molecules”, which can increase the efficacy of existing antibiotics to over 1000-fold in some cases.

Diabetes is one of the largest threats for public health in the new millennium, and its impact is felt most severely in developing countries. It is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common feature of uncontrolled diabetes which leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Diabetic patients are prone to long-term complications, such as retinopathy, cataract, atherosclerosis, neuropathy, nephropathy and impaired wound healing. Most of these complications are related to standard changes in tissues proteins by their non-enzymatic bindings with sugars molecules. Current treatments of diabetes are largely ineffective in achieving the normal sugar levels and delaying the onset of late diabetic complication. Therefore, there is an urgent need for new strategies for the treatment of diabetes, and its prevention against complications.

Among different therapeutic interventions, the discovery of effective α -glucosidase inhibitors and antiglycating agents are considered to be the most important one, based on modern knowledge about the disease at molecular level. Primary focus of these studies has been to discover lead molecules by using appropriate conventional and mechanism-based biological screening techniques. As a result, a large number of potent antiglycation agents, and α -glucosidase inhibitors of natural origin were discovered and structure-activity relationship studies were conducted. Many of these compounds represent new examples of inhibitors of α -glucosidase and protein glycation.

KNL-5

Track: Cancer Targeted Drug Delivery

DIFFERENT STRATEGIES TO TARGET RESISTANT CANCER CELLS OVEREXPRESSING MULTIDRUG ABC TRANSPORTERS: INHIBITION AND COLLATERAL SENSITIVITY**Attilio Di Pietro**

BMSSI UMR5086 CNRS-University of Lyon, Institute of Protein Biology and Chemistry, Passage du Vercors 7, 69367 Lyon, France; E-mail: a.dipietro@ibcp.fr



Multidrug ABC (“ATP-binding cassette”) transporters are involved, upon overexpression, in chemoresistant tumors by pumping anticancer drugs out of the cells. For early discovered ABCB1/“P-glycoprotein”, third-generation drug-efflux inhibitors are under clinical development. For more recently identified ABCG2/“breast cancer resistance protein”, we have screened different series of flavonoids and derivatives, such as flavones, rotenoids and acridones, and more recently chalcones [1, 2], chromones [3, 4], and indenoindoles [5], as inhibitors of mitoxantrone efflux from transfected HEK293 human cells and chemosensitizers of cell proliferation, to establish 3D-Quantitative Structure-Activity Relationships. Two types of selective, non-competitive, inhibitors have been characterized, either inhibiting or stimulating the basal ATPase activity. The most potent one is indeed efficient *in vivo* on *SCID* mice, xenografted with human ABCG2-transfected cells, by chemosensitizing tumor growth to the drug-substrate irinotecan [6]. These selective inhibitors constitute good drug candidates, with low intrinsic toxicity, as sensitizers of cell proliferation to conventional chemotherapeutics.

The “Multidrug Resistance Protein 1” ABCC1 is able to catalyze the efflux of either glutathione conjugates or free glutathione together with hydrophobic substrate drugs. We have identified modulators such as verapamil [7, 8] mimicking substrates and inducing a fast and massive efflux of intracellular glutathione from ABCC1-overexpressing cells, leading to a selective cell death through apoptosis, due to “*collateral sensitivity*”, or hypersensitivity. The overexpressed transporter then constitutes the *Achilles’ heel* of such resistant cancer cells. Since verapamil is known for its cardiotoxic effects, we investigated other types of modulators such as xanthenes, flavones [9] and flavonoid dimers. Glutathione efflux appeared to be necessary, but not sufficient alone, to trigger apoptosis, indicating the contribution of other partner(s) or signaling pathway(s). Such apoptosis inducers may constitute a new type of anticancer drugs operating through an original strategy aimed at selectively targeting and eliminating multidrug-resistant tumors overexpressing the ABCC1 transporter [10].

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KNL-12

Track: Cancer Targeted Drug Delivery

THERAPEUTIC MONOCLONAL ANTIBODIES AGAINST CANCER AND VIRUSES**Dimitar S. Dimitrov**

Frederick National Lab, Building 469, Room 150B, Frederick MD-21702-1201, USA;

E-mail: dimitrdi@mail.nih.gov

Therapeutic monoclonal antibodies (mAbs) are highly successful and currently enjoy unprecedented recognition of their potential. Hundreds of mAbs including bispecific mAbs and multispecific fusion proteins, antibody drug conjugates (ADCs) and mAbs with optimized pharmacokinetics are in clinical trials. However, challenges remain and deeper understanding of mechanisms is needed to overcome major problems including resistance to therapy, access to targets, complexity of biological systems and individual variations. We have discovered a number of candidate therapeutic mAbs against cancer-related proteins including components of the IGF system, mesothelin, folate receptor beta, CD22, DR4 and DR4, which are at various stages of preclinical and clinical development. We have been also developing candidate therapeutic mAbs against viruses including HIV-1, SARS-CoV, Hendra and Nipah viruses (henipaviruses), and dengue, as well as against MERS-CoV, which have been tested in animal models, and the mAb against henipaviruses was administered to humans who were exposed to those viruses. Most of these mAbs are full-size in IgG1 format. To overcome problems of target access we have been also developing engineered antibody domains (eAds) based on VH-, CH2-, monomeric CH3- and monomeric Fc-derived scaffolds. We constructed large libraries of eAds from which binders against HIV-1 and cancer-related proteins including components of the IGF system were selected and used for construction of mono- and bispecific fusion proteins targeting nonoverlapping epitopes on the same molecule. Some of them have potential as candidate therapeutics against cancer and HIV-1.

**KNL-167**

Track: Academic CRO/Industrial Collaborations in Drug Discovery

VBP15 FOR DMD: A COLLABORATIVE PUBLIC/PRIVATE PARTNERSHIP ENABLED BY VENTURE PHILANTHROPY SUPPORT FROM THE INTERNATIONAL STAKE-HOLDER COMMUNITY**Eric Hoffman**

Center for Genetic Medicine Research, Children's National Medical Center and Department of Integrative Systems Biology, George Washington University, Washington, DC 20010, USA;

E-mail: ehoffman@cnmcresearch.org

Drug development is a lengthy and time consuming process, where it has been suggested that it typically costs \$500 million and takes 15 years to bring a drug to market. The high costs become problematic with orphan drugs, where there may be very few patients to prescribe the new drug and distribute costs. Also, there are increasing opportunities for highly targeted drugs, but often in ever more stratified patient populations, so the proportion of orphan drugs is expected to increase dramatically. Methods to decrease the costs and time associated with orphan drug development include early de-risking (reducing late stage expensive failures), and accelerated approval (moving larger efficacy trials to the post-marketing space). VBP15 is a first in human drug developed for Duchenne muscular dystrophy (DMD). Intellectual property for the program was transferred from Children's National Medical Center to ReveraGen Biopharma. Initial seed funding for lead compound selection was accomplished through support of the Department of Defense CDMRP, and early de-risking of the lead (VBP15) was done in partnership with the National Institutes of Health TRND program. Pre-clinical studies and Phase 1 trials were funded by venture philanthropy support of seven DMD foundations through a risk sharing and profit sharing model



based on later drug sales. Phase 2a and 2b trials in DMD patients are to run by international academic networks, with the possibility of accelerated approval after a short-term (3-6 month) trial with a measure of strength as the primary outcome measure. VBP15 is a steroidal compound built on a delta 9,11 backbone, where the drug shows a high affinity for the glucocorticoid receptor, but is effective in dissociating anti-inflammatory transrepression subactivities (retention of efficacy), from the transactivation subproperties associated with side effects (growth stunting, adrenal suppression, immune suppression). VBP15 has shown efficacy in pre-clinical models of multiple chronic inflammatory states, including allergic lung disease, arthritis, multiple sclerosis, inflammatory bowel disease, and sickle cell anemia. Thus, VBP15 holds potential for replacing traditional glucocorticoids in multiple indications, including DMD.

KNL-321

Track: Regenerative Medicine

ENGINEERED HYDROGELS FOR REGENERATIVE MEDICINE APPLICATIONS

A. Khademhosseini

*Harvard-MIT Division of Health Sciences and Technology, Brigham and Women's Hospital and Harvard Medical School, Cambridge MA 02139, USA;
E-mail: akhademhosseini@rics.bwh.harvard.edu*



Engineered materials that integrate advances in polymer chemistry, nanotechnology, and biological sciences have the potential to create powerful medical therapies. Our group aims to engineer tissue regenerative therapies using water-containing polymer networks, called hydrogels, that can regulate cell behavior. Specifically, we have developed photocrosslinkable hybrid hydrogels that combine natural biomolecules with nanoparticles to regulate the chemical, biological, mechanical and electrical properties of gels. These functional scaffolds induce the differentiation of stem cells to desired cell types and direct the formation of vascularized heart or bone tissues. Since tissue function is highly dependent on architecture, we have also used microfabrication methods, such as microfluidics, photolithography, bioprinting, and molding, to regulate the architecture of these materials. We have employed these strategies to generate miniaturized tissues. To create tissue complexity, we have also developed directed assembly techniques to compile small tissue modules into larger constructs. It is anticipated that such approaches will lead to the development of next-generation regenerative therapeutics and biomedical devices.

KNL-60

Track: Cancer Targeted Drug Delivery

TARGETING MTA1 MASTER CHROMATIN REMODELER IN CANCER AND STEM CELL BIOLOGY

Rakesh Kumar

*Department of Biochemistry and Molecular Medicine, School of Medicine and Health Sciences, George Washington University, 2300 Eye Street, N.W., Washington, DC 20037, USA;
E-mail: bcmrxk@gwu.edu*



Fundamental to the process of cancer progression and metastasis is the ability of cancer cells to respond to both extra- and intra-cellular milieu and translate resulting signals for the benefit of gene expression in an orderly manner through chromatin remodeling complexes. The functional outcome of the chromatin remodeling complexes is primarily influenced by the nature of enzymes and coregulatory proteins and its combinatorial post-translational modifications, among other

mechanisms. One of such families of master chromatin modifiers is the metastasis tumor antigen (MTA) family, which are an integral part of nucleosome remodeling and histone deacetylation and nucleosome remodeling factor complexes.

The MTA family regulates cellular pathways in both normal and cancer cells by influencing the chromatin status and expression of target genes while influencing nucleosome landscape *via* interacting with various modified histones. The MTA1, one of the most frequently upregulated oncogenes, is a nodular molecule in the process of tumorigenesis by a number of oncogenes. In general, MTA1 upregulation correlates with an aggressive and invasive phenotypes of tumors and an unfavorable outcome for cancer patients. At the cellular level, MTA1-containing chromatin remodeling complexes regulate a range of processes including, cell survival, invasiveness, transformation, DNA-damage response, angiogenesis, metastasis and therapeutic sensitivity of tumor cells. In addition, MTA1-containing chromatin remodeling complexes represent emerging modifiers of gene expression with functions in embryonic stem-cell differentiation, reprogramming of pluripotent stem cells and mesenchymal nature of stem cells as well as cancer stem cells. For example, MTA1 status has been proposed to be one of critical modifiers of chemo-sensitivity of cancer cells due to MTA1's ability to support cancer stem cell-like phenotypes. The lecture will provide an overview of the current and future functions of MTA1 in cancer and stem cell biology and underlying molecular mechanisms.

KNL-226

Track: Neurodegenerative Disorders

SEEING A ROAD FROM THE CAR: COULD EFFECTIVE MICRORNA "BRAKES" SLOW DOWN THE TRIP TO ALZHEIMER'S DISEASE?

Debomoy K. Lahiri¹, Justin M. Long¹, Nipun Chopra¹, Xudong Huang², Nigel H. Greig³, Kumar Sambamurti⁴, Jack T. Rogers², Weihong Song⁵ and Bryan Maloney¹

¹*Department of Psychiatry, Neuroscience Research Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA; E-mail: dlahiri@iupui.edu*

²*Departments of Psychiatry and Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA*

³*National Institute on Aging, NIH, Baltimore, MD 21224, USA*

⁴*Medical University South Carolina, Charleston, SC 29425, USA*

⁵*Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada*

Alzheimer's disease (AD) is the most common form of dementia. Advances in "Research on Alzheimer's disease" (ROAD), as reported in Current Alzheimer Research' (CAR) and other journals, unveil many opportunities to tackle the disease but also unmask many potholes in our understanding of the disease process. Safe driving requires sensitive and responsive "brakes". What are such brakes on the path down neurodegeneration? We argue for microRNA (miRNA). Several specific miRNAs regulate expression of AD pathway proteins, such as amyloid- β (A β) peptide precursor protein (APP), β -site APP-cleaving enzyme (BACE1) and neprilysin (MME). AD is characterized by excessive A β -loaded neuritic plaques and may result from misregulation of production or clearance of A β . The rate-limiting step in production of A β is processing of APP by the β -secretase, BACE1. MME is a major A β -clearing enzyme. We demonstrate miRNA-mediated regulation of APP and BACE1. We have recently reported specific microRNA species that regulate APP or BACE1 expression (Long *et al.*, J. Biol. Chem., 2012; 2014). For the present work, we prepared either APP-, BACE1- or MME 3'-UTR plus the reporter vector by inserting the respective 3'-UTRs downstream from a Renilla luciferase gene and delivered the construct into human primary cultures, along with miRNAs predicted to target the respective 3'-UTRs. miR-153 reduced APP, and miR-298 and miR-339-5p reduced BACE1 reporter expression. Western analysis revealed reduction of native APP or BACE1 levels following specific miRNA-delivery. Specific interaction was confirmed by delivery of respective antagomirs and target protectors in primary human brain cultures. Notably, miR-339-5p levels were reduced in brains from AD patients *vs* age-matched controls. Our results reveal novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species. These interactions may serve as novel and superior therapeutic targets against AD to conventional drug strategies. miRNA "slows" protein expression like a chicane in Grand Prix and does not completely block it, which may reduce interference in other physiological functions.

This work is supported by grants from Alzheimer's Association and NIH.

KNL-93

Track: Pharmaceutical Biotechnology

SYNTHETIC AND BIOENGINEERED HEPARIN-BASED THERAPEUTICS

Robert J. Linhardt

Departments of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, Biotech Center 4005, 110 8th Street, Troy, NY 12180-3590, USA; E-mail: linhar@rpi.edu

Heparin, a highly sulfated polysaccharide anticoagulant, commands worldwide market of ~\$7B. Currently, heparin and related low molecular weight heparins (LMWHs) require an extraction from pig intestine. The ultra-low molecular weight heparin (ULMWH), a chemically synthesized pentasaccharide, is expensive and has limited clinical applications. This presentation describes the efficient chemoenzymatic synthesis of heparin, LMWH, and ULMWH. Our goals are to prepare ULMWH at reduced cost, LMWH with improved pharmacological properties (*i.e.*, defined pharmacokinetics, liver clearance, reduced heparin-induced-thrombocytopenia, and protamine reversibility), and heparin without the use of animal derived starting material. Our approach relies on chemoenzymatic synthesis chemical relying on Golgi-derived biosynthetic enzymes, including heparosan synthases, sulfotransferases and epimerase. We are also exploring metabolic engineering for heparin production. Such biotechnological processes should allow the cGMP preparation of these critical drugs, affording improved products reducing impurities, contaminants and adulterants that resulted in the 2008 heparin contamination crisis.

KNL-225

Track: Anti-Infectives

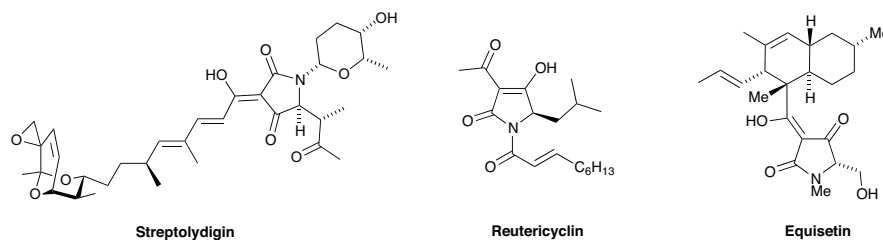
EQUISETIN, REUTERICYCLIN AND STREPTOLODYGIN: NATURAL PRODUCT LEAD STRUCTURES FOR NOVEL ANTIBIOTICS

Mark G. Moloney

*University of Oxford, Department of Chemistry, Chemistry Research Laboratory, Mansfield Rd., UK;
E-mail: mark.moloney@chem.ox.ac.uk*

The discovery of new antibiotics has become urgent as a result of the emergence of resistance and new pathogenic bacterial strains. However, this need has coincided with unprecedented lowering of levels of productivity in the drug discovery process and consequent reduced investment from large pharma. New strategies for antibacterial drug discovery are required, and a renewed understanding of the value of a natural products' guided approach has emerged. Our focus has been on the use of antibacterial natural products containing tetramate core structures, and using equisetin and reutericyclin as inspiration, we have developed novel chemistry that uses suitable serine, threonine and cysteine-derived oxazolidine templates for highly chemo- and diastereoselective ring closure reactions leading to tetramic acid derivatives. Although simple unsubstituted pyrrolidines and tetramates appear to be intrinsically devoid of activity, application of these templates for fragment-based synthesis, has permitted access to several compound series which possess high levels of antibacterial activity, SAR analysis has permitted some optimization of the initial activity and MOA and other pharmacokinetic data been obtained.





This lecture will illustrate the potential of natural products to guide antibacterial drug discovery, the role of synthetic organic chemistry in the construction of libraries which mimic these natural products, and suggest a possible way forward for more efficient drug discovery strategies.

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KNL-127

Track: In-Silico Drug Design and In-Silico Screening

ON THE ORIGIN AND COMPLETENESS OF LIGAND BINDING POCKETS WITH APPLICATIONS TO DRUG DISCOVERY

Jeffrey Skolnick

Center for the Study of Systems Biology, Georgia Institute of Technology, 250 14th Street NW Atlanta, GA 30318, USA; E-mail: jessica.forness@biology.gatech.edu



The intrinsic ability of protein structures to exhibit the geometric and sequence properties required for ligand binding without evolutionary selection is shown by the coincidence of the properties of pockets in native, single domain proteins with those in computationally generated, compact homopolypeptide, artificial structures, ART. The library of native pockets is covered by a remarkably small number of representative pockets (~400), with virtually every native pocket having a statistically significant match in the ART library, suggesting that the library is complete. When sequences are selected for ART structures based on fold stability, pocket sequence conservation is coincident to native. The fact that structurally and sequentially similar pockets occur across fold classes combined with the small number of representative pockets in native proteins implies that promiscuous interactions are inherent to proteins. Based on comparison of PDB and ART structures and pockets, the widespread assumption that the co-occurrence of global structure, pocket similarity, and amino acid conservation demands an evolutionary relationship between proteins is shown to significantly underestimate the random background probability. Indeed, many features of biochemical function arise from the physical properties of proteins which evolution likely fine-tunes to achieve specificity. This study suggests that a repertoire of thermodynamically (marginally) stable proteins could engage in many of the biochemical reactions needed for living systems without selection for function, a conclusion with significant implications for the origin of life. Finally, examples of experimental validation of promising small molecule hits that exploit the degeneracy of ligand binding pockets are presented.

KNL-40

Track: Enabling Technologies

NEW TECHNOLOGIES FOR IMPROVED VACCINES AGAINST INFECTIOUS DISEASES AND CANCER**Jeffrey B. Ulmer**

*Vaccines Research, Chiron Corporation, 4560 Horton Street, Emeryville, California 94608, USA;
E-mail: jeffrey.ulmer@novartis.com*

Vaccines are without a doubt the most successful of mankind's medical interventions. However, despite more than two centuries of effective use of vaccines, many substantial challenges remain. These include: 1) improvement of existing but suboptimal vaccines (*e.g.*, tuberculosis, influenza), 2) discovery and development of new vaccines against targets to address large unmet medical needs (*e.g.*, HIV, malaria, cancer), and 3) rapidly responding to new pathogens (*e.g.*, newly emerging microbes, bioweapons). Recent advancements have demonstrated proof of concept for active immunization in the treatment of cancers. Taking full advantage will require the application of new technologies and paradigms in the areas of tumor antigen identification and optimization, novel potent and safe adjuvants, and enhanced vaccine delivery systems.

**KNL-158**

Track: Drug Discovery in Preclinical Research

PREVENTION OF EGFR-TYROSINE KINASE INHIBITOR COMPLICATIONS BY TREATMENT WITH NK-1 RECEPTOR BLOCKADE**William B. Weglicki, J.H. Kramer, J.J. Chmielinska, C.F. Spurney and I.T. Mak**

*George Washington University, School of Medicine and Health Sciences, Washington D.C., USA;
E-mail: wweg@gwu.edu*

Prior reports of hypomagnesemia in patients receiving the epidermal growth factor receptor (EGFR)-blocking drug, Cetuximab, suggested that EGFR-tyrosine kinase inhibitors (TKI), like erlotinib (Tarceva), may have a similar side effect. In addition, the possibility of preventing complications of therapy by blockade of neurogenic inflammation was studied. Rats were treated with erlotinib for up to 9 weeks and were investigated for hypomagnesemia, inflammation and cardiac dysfunction. Plasma magnesium decreased progressively (-9 to -26%) between 3-9 weeks, and this was associated with modest increases (+27% at 3 and +25% at 9 weeks) in circulating levels of the neuropeptide, substance P (SP). In previous work the TKI, tyrphostin AG-1478, caused similar effects. The superoxide-generating activity of circulating neutrophils from erlotinib-treated rats increased 3-fold, and the plasma oxidative marker, 8-isoprostane rose 2.1-fold, together with appearance of cardiac peri-vascular nitrotyrosine. The SP receptor blocker, aprepitant (Emend), attenuated erlotinib-induced hypomagnesemia by 42% and completely prevented the rise in circulating SP; the increases in neutrophil superoxide activity and 8-isoprostane were also suppressed. Echocardiography revealed mild to moderate systolic dysfunction by 7 weeks of erlotinib treatment, with slight decreases in left ventricular ejection fraction (LVEF: -11%) and % fractional shortening (%FS: -17%); the mitral valve E/A ratio was significantly reduced (-17.5%, week 9), suggesting diastolic dysfunction. Also thinning of the left ventricular posterior wall (LVPWd & s in diastole and systole) was detected by 7 - 9 weeks. Most interestingly, the co-administration of aprepitant attenuated all of the erlotinib-induced effects on LV systolic, diastolic and anatomical parameters: At 9 weeks, LVEF improved 87.2%; LV % FS improved 86.4%; and mitral valve E/A ratio improved 84.6%.

In conclusion, prolonged erlotinib treatment in rats induced moderate hypomagnesemia, along with SP-mediated oxidative/inflammation stress, and mild to moderate cardiac dysfunction. These changes were substantially prevented by SP receptor blockade implicating the novel role of neurogenic inflammation due to EGFR-TK inhibition.



INVITED LECTURES

IL- 148

Track: Diabetes and Obesity and Drug Discovery & Therapy

NOVEL BIOMEDICAL AGENTS FROM ABORIGINAL MEDICINALLY IMPORTANT PLANTS OF CANADA

Athar Ata

Department of Chemistry, College for the Environmental and Science Complex, The University of Winnipeg, 599 Portage Ave. Winnipeg, MB, Canada; E-mail: a.ata@uwinnipeg.ca

Natural products exhibit interesting anti-microbial, anti-viral, and anti-inflammatory activities. These bioactivities make them an important source for the discovery of new pharmaceuticals. Currently, more than 60% of the drugs available on the market are of natural product origin. One of the aspects of drug discovery process is the identification of small molecules with enzyme-inhibiting activities. Enzymes are essential to human life, mediating biochemical processes including metabolism, cellular signal transduction, cell cycling, and development. Malfunction in these biochemical systems often leads to disease that can be caused either by the dysfunction, overexpression, or hyper-activation of the enzymes involved. An understanding of diseases at the molecular level has provided several enzyme inhibitors in clinics. For instance, galanthamine, a potent acetylcholinesterase (AChE) inhibitor, is used to treat early symptoms of Alzheimer's disease. α -Glucosidase (EC 3.2.1.20) is a membrane bound enzyme and lies at intestinal cells. This enzyme catalyzes the final step of carbohydrates digestion by hydrolyzing the glycosidic bonds in carbohydrates to liberate free glucose. The resulting glucose is a source of an exaggerated rise in blood sugar causing postprandial hyperglycemia. This causes type 2 diabetes mellitus and affects approximately 2.1 billion people worldwide. The potent α -glucosidase inhibitors prevent the breakdown of carbohydrates in small intestine and prolong the absorption of glucose or carbohydrates in blood. These compounds may be used as chemotherapeutic agents in clinics for the treatment of diabetes and obesity. Due to the catalytic role of α -glucosidase in carbohydrate digestion, these inhibitors may also be used as therapeutic target for other carbohydrate mediated diseases including viral infections, cancer, HIV and hepatitis. Our recent chemical investigation of Aboriginal medicinal plants of Canada and fungi resulted in the identification of natural products exhibiting potent bioactivities including anti-microbial, anti- α -glucosidase and anti-AChE activities. In this presentation, isolation, structure elucidation of new bioactive natural products and their structure-activity relationships will be discussed.

IL- 231

Track: Neurodegenerative Disorders

PRESENILINS ARE TRANSCRIPTIONAL TARGETS OF PARKIN

Frédéric Checler, Eric Duplan, Jean Sévalle, Julien Viotti, Thomas Goiran, Charlotte Bauer, Paul Renbaum, Ephrat Levy-Lahad, Clément A. Gautier, Olga Corti, Nathalie Leroudier and Cristine Alves da Costa

IPMC-CNRS UMR 7275, 660 route des Lucioles, 06560, Valbonne, France; E-mail: checler@ipmc.cnrs.fr

Medical Genetics Institute, ShaareZedec Medical Center, Hebrew University Medical School, Jerusalem, Israel

INSERM U679, Hôpital de la Pitié-Salpêtrière, 47 boulevard de l'Hôpital, Paris 75013, France

Parkin is associated to autosomal recessive early-onset Parkinson's disease. Parkin acts as an E3-ubiquitin ligase involved in the proteasome-mediated degradation of various substrates. It has been suggested that pathogenic mutations of parkin, abolishing its ubiquitin-ligase activity, could explain the accumulation of proteins and lead to neuronal death by apoptosis. However, besides this function, additional parkin-dependent cellular pathways exist. We demonstrated that parkin is a direct transcriptional repressor of the tumor suppressor p53 [1]. p53 regulates the expression and functions of presenilin-1 (PS1) and presenilin-2 (PS2), two members of the gamma secretase complex involved in the production of the amyloid β peptide (A β) and parkin could control the homeostasis of intracellular A β . These findings prompted us to

investigate whether parkin could control presenilins and if so, whether it is *via* a direct transcriptional control of PS promoters or indirectly, *via* p53.

We show that parkin controls presenilin 1 and 2 expressions, promoter activity, and mRNA levels *ex vivo* and in mouse brains. This regulation impacts on PS-dependent γ -secretase activity and presenilin-mediated control of cell death. This control is independent of parkin ubiquitin-ligase activity, does not involve p53 and is not affected by PS1 and PS2 functional interplay. Parkin binds to presenilins promoters *via* a consensus binding sequence that we identify and validate by functional analysis [2].

This study delineates a putative interplay between Parkinson's and Alzheimer's diseases. Furthermore, we identified a common putative parkin responsive element on p53 that should help identifying novel transcriptional targets of parkin and unravel putative additional functions.

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IL- 124

Track: Drug Discovery in Preclinical Research

TISSUE SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs): A PATH TO A CLINICAL CANDIDATE

Eugene L. Piatnitski Chekler

Project Leader, Senior Principal Scientist, Pfizer Inc., 610 Main St., Cambridge, MA 02139, USA; E-mail: Eugene.Chekler@Pfizer.com

A novel series of Selective Androgen Receptor Modulators (SARMs) which shows excellent biological activity and physical properties is presented. High quality SARM compounds have been identified using structure based design to derive potent anabolic agents using an assay predictive of androgenic activity (N/C termini interaction assay), and by designing chemical matter in good property space. Representative compounds demonstrate diminished activity in promoting the intramolecular interaction between the AR carboxyl (C) and amino (N) termini which proved to be useful as a biomarker to decouple undesired androgenic responses from anabolic activity. In castrated rats, the daily administration of a lead compound shows anabolic activity by increasing *levator ani* muscle weight. Minimal effects were observed on the prostate, and seminal vesicles along with minimal effects repressing circulating luteinizing hormone (LH) levels. A lead compound completed a two week rat toxicology study, and it was well tolerated at dosages up to 100 mg/kg/day for 14 consecutive days, the highest dosage evaluated.

IL- 173

Track: Academic CRO/Industrial Collaborations in Drug Discovery

“OPEN INNOVATION” IN DRUG DISCOVERY & TRANSLATIONAL RESEARCH IN ACADEMIA**Thomas D.Y. Chung**

Conrad Prebys Center for Chemical Genomics at Sanford Burnham Medical Research Institute, La Jolla, CA, USA; E-mail: tchung@sanfordburnham.org



The high costs (>\$1B per NCE) and long duration (10-15Y) of traditional R&D in "big Pharma" are under assault from disease advocacy groups, government and investment communities as being too high and long, stagnant, and unsustainable. Academia has more recently been championed as the beacon of innovation, however, there is still a gap from basic research into translational research. Leveraging our Prebys Center's experience in "open innovation" through our 6-yr experience as a Comprehensive Screening Center of the NIH's Molecular Libraries Probe Production Centers Network (MLPCN), we have established new models of academic drug discovery and development to leverage government funding with clinical translational partners. In this talk, we provide examples of our working operational models and our strategic alliance and partnerships.

IL- 236

Track: Neurodegenerative Disorders

BUTYRYLCHOLINESTERASE AS A DIAGNOSTIC AND THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE**Sultan Darvesh**

Departments of Medical Neuroscience, Medicine (Neurology and Geriatric Medicine), Dalhousie University, Halifax, Nova Scotia, Canada; E-mail: Sultan.Darvesh@Dal.Ca

Butyrylcholinesterase (BChE) is a serine hydrolase that is coregulator of the neurotransmitter acetylcholine and also has other functions in the brain and elsewhere. In Alzheimer's disease (AD), cholinesterase activity, particularly BChE, becomes associated with neuropathological structures such as β -amyloid ($A\beta$) plaques, serving a function that is not clear at present. To develop a truly effective diagnostic or therapeutic agent for AD it will be important to understand more about the mechanism of BChE involvement in AD pathology.

To that end we have developed an AD animal model, that is unable to synthesize BChE, for comparison with the related animal model that has the ability to synthesize the enzyme. We observed diminished fibrillar $A\beta$ plaque deposition in the 5X FAD/BChE-KO mouse strain compared to the 5X FAD mouse, an effect that is influenced by the sex of the animals. This provides a clue that BChE represents a target for therapeutic approaches in AD. Additionally, that BChE is associated with $A\beta$ plaques in AD suggests that BChE is also an appropriate diagnostic target for the disease.

Although $A\beta$ plaques can accumulate in the brains in up to 30% of all individuals without dementia, the association of BChE with $A\beta$ plaques in AD could provide a marker for definitive diagnosis of AD during life and a target for disease-modifying drugs for this condition. We have been developing radiolabelled BChE ligands that, in autoradiographic analysis, clearly differentiated Alzheimer brain tissues from those of individuals with $A\beta$ plaques without dementia.

Derivatives of phenothiazine have been used as well-tolerated drugs against a variety of human ailments. For example, chlorpromazine treatment of psychosis is related to its interaction with dopaminergic receptors. Many phenothiazines are also potent inhibitors of cholinesterases, especially BChE. However, antagonistic action of such drugs on cholinergic receptor systems would be counter-productive for treatment of AD. In a search for phenothiazines that are inhibitors of cholinesterases, especially BChE, with potential to treat AD, we wished to ascertain that such molecules could be devoid of neurotransmitter receptor interactions. A number of our synthetic N-10-carbonyl phenothiazine derivatives, with

cholinesterase inhibitory activity were tested for interaction with a wide variety of neurotransmitter receptor systems. We demonstrated that phenothiazines can be prepared without significant neurotransmitter receptor interactions while retaining high potency as cholinesterase inhibitors for potential treatment of AD.

IL- 295

Track: Innovative Drug Discovery and Nanotechnology

NANOSCIENCE APPROACHES TO DIAGNOSIS AND THERAPY OF HUMAN DISEASES

Silvia De Paoli

U.S. Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER), Office of Blood Research and Review (OBRR), FEDERAL RESEARCH CENTER AT WHITE OAK, 10903 New Hampshire Avenue, Building 52, Room 4207, Silver Spring, MD 20993-0002, USA; E-mail: silvia.depaoli@fda.hhs.gov

Advances in nanoscience are underpinning breakthroughs in biomedical research and nanotechnology based approaches hold substantial potential for improving healthcare. Metal, carbon, polymer, hybrid and nature-inspired nanoparticles are being developed for use in imaging diagnostics, drug delivery, implant engineering and regenerative medicine. Nanoscience and nanotechnology also provide means by which the interaction of cells with their environment can be studied thereby increasing the understanding of disease processes. Although the potential is clear, nanotechnology poses several challenges especially regarding nanomaterial characterization and biocompatibility. The aim of this talk is to provide a review of technical aspects of nanoscience and to provide a view of how nanotechnology can be applied to advance different arenas of research and clinical fields with emphasis on nanomaterial safety.

IL- 4

Track: Cardiovascular Drug Discovery & Therapy

DISCOVERING ANTAGONISTS OF GLYCOSAMINOGLYCAN-BINDING PROTEINS FOR TREATING THROMBOSIS, CANCER, AND OTHER DISEASES

Umesh R. Desai

Institute for Structural Biology and Drug Discovery and Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23219, USA; E-mail: urdesai@vcu.edu

Glycosaminoglycans (GAGs) are ubiquitously present in nature from hydra to humans, from embryonic stem cells to mature cells and from a fully secreted state to a cell surface-bound state in the form of proteoglycans. Structurally, most GAGs are polymers of alternating glycosamine and uronic acid residues, which may be incompletely sulfated and acetylated along the chain. GAGs including heparan sulfate, heparin, chondroitin sulfate, and dermatan sulfate, are biosynthesized in a template-free manner through a series of enzymatic reactions, which generates phenomenal number of sequences. As a result, GAGs modulate a large number of physiological and pathological responses including growth and cancer, hemostasis and thrombosis, immune response and inflammation, and microbial invasion and defense, which are brought about by their interaction with proteins. It is generally recognized that there is an element of selectivity in GAG recognition of different proteins. The bounty of GAG-protein interactions offered by nature remains largely unexploited by medicinal chemists. The primary reason for this state-of-art is that discovery of a small GAG-like sequences is difficult because of non-availability of a library of GAG sequences for high-throughput screening. Nearly a decade ago, we began with a fundamental hypothesis that non-saccharide GAG mimetics (NSGMs) could be designed to mimic the functions of GAGs and yield novel drug-like clinically relevant molecules. To date several hundred NSGMs have been synthesized and studied for their *in vitro* and *ex vivo* biological activities. Of special note is the recent discovery of sulfated pentagalloyl glucopyranoside that selectively inhibits human factor XIa by binding to the heparin binding site on its catalytic domain and preventing whole blood clotting *in vivo*. Likewise, a specific NSGM was found

to selectively inhibit cancer stem cells (colorectal and pancreas) and has the potential to show excellent activity *in vivo*. The NSGMs are attractive from the drug development perspective because they are easy to synthesize, highly water soluble and essentially non-toxic to normal cells. NSGMs preferentially bind proteins in their GAG-binding sites, which opens up a major avenue for discovering allosteric drugs. Finally, NSGMs are also very attractive from the intellectual property perspective. This talk will focus on NSGMs as a new frontier in drug discovery.

IL- 241

Track: Neurodegenerative Disorders

NEW PHARMACOLOGICAL TOOLS TO UNDERSTAND AND EXPERIMENTAL DRUGS TO TREAT MILD TRAUMATIC BRAIN INJURY AND RELATED DISORDERS

Nigel H. Greig, David Tweedie, Yazhou Li, Ian Tamargo, Barry J. Hoffer, Kumar Sambamurti, Debomoy K. Lahiri and Chaim G. Pick

NIA, NIH, USA; Case Western Reserve Univ., USA; Medical Univ. S Carolina, USA; Indiana Univ., USA; Tel-Aviv Univ., Israel; E-mail : greign@mail.nih.gov

Mild traumatic brain injury (mTBI) represents a major and increasing public health concern worldwide. It is jointly the most frequent cause of disability/mortality in young adults and a chief cause of morbidity in the elderly. Whereas mTBI patients do not show clear structural brain defects or require hospitalization following either concussive or blast injury - of civilian and military relevance, respectively - they frequently suffer from long-lasting cognitive, behavioral, and emotional problems. No effective pharmaceutical therapy is available, and existing treatment chiefly involves intensive care management after injury. Recently, it has become apparent that several neurological diseases share common terminal features of neuronal cell death, and that biochemical cascades triggered by mTBI can lead into pathways associated with chronic neurodegenerative disorders, epitomized by Alzheimer's disease and associated dementias, and Parkinson's disease. Significant interest has hence focused on the development of immediately translatable pharmacological treatments for TBI. Our prior studies developing anti-apoptotic, anti-inflammatory and neurotrophic/protective agents for neurodegenerative disorders provide valuable pharmacological tools to understand time-dependent neuronal loss and neuroinflammation instigated by mTBI to generate new experimental drugs to mitigate mTBI-induced neuronal dysfunction, demise and ensuing cognitive impairment. These studies are reviewed, together with molecular pathways associated with mTBI and its amelioration.

Keywords: Traumatic brain injury, neurodegeneration, drug development.

IL- 196

Track: Drug Metabolism

SYNTHESIS OF HYDROLYSABLE NANOGELS FOR CONTROLLED TEMPORAL DELIVERY OF VASCULOGENIC FACTORS IN PATTERNED CELLULAR CONSTRUCTS

Esmail Jabbari

Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA;

E-mail: jabbari@mailbox.sc.edu



Rapid and controlled induction of vascularization in engineered constructs is crucial to the successful regeneration of large skeletal defects. The process of lumen and microtube formation by endothelial progenitor cells in a cellular construct requires spatial and temporal control over the release of vasculoinductive factors. The objective of this work was to synthesize self-assembled polyethylene glycol (PEG) based nanogels (NGs) for controlled temporal delivery of vascular endothelial growth factor (VEGF) in cell-patterned constructs in regeneration of large skeletal defects. PEG macromers were chain-extended with lactide-co-glycolide (LG) segments by sequential ring-opening polymerization of PEG with L/G monomers. Next, the chain ends of the GL-PEG-LG macromers were functionalized with a succinimide group and self-assembled to NGs by dialysis. Then, the VEGF protein was grafted to the NGs by the reaction succinimide-amine reaction. The temporal release of the protein from the NGs was investigated with VEGF and bovine serum albumin (BSA) by ELISA. Effect of the released VEGF on the extent of vasculogenesis was assessed *in vitro* with endothelial progenitor cells (EPCs). The rate of release was independent of protein size but dependent on the L/G ratio of the NGs. The protein release rate increased with increasing the G/L molar ratio and increasing PEG molecular weight. Further, Composite gels with microchannels seeded with EPCs and loaded with VEGF-grafted NGs and the matrix seeded with mesenchymal stem cells and loaded with NG-grafted osteogenic factors results in the highest extent of vascularized osteogenesis compared with those without NGs. In conclusion, PEG based LG-chain extended hydrolysable NGs can potentially be used to control the temporal delivery of growth factors over a wide range of release times from a few days to a few weeks in regeneration of large tissue defects.

ACKNOWLEDGEMENTS

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Keywords: Nanogel, self-assembly, protein grafting, temporal release, vasculogenesis, regenerative medicine.

IL- 62

Track: CNS Drug Discovery and Therapy

CO-SUPPRESSION OF INTRACELLULAR CALCIUM AND OXIDATIVE STRESS, A KEY STRATEGY FOR DEVELOPMENT OF NEUROPROTECTIVE DRUGS**Zhi-Gang Jiang**

Neurodegenerative Diseases, Panacea Pharmaceuticals, Inc., Gaithersburg, Maryland, USA;

E-mail: ZGJiang@panaceapharma.com

Neurodegenerative diseases (NDs) are types of hereditary and sporadic disorders that demonstrate with progressive dysfunction and often destructive morphology of nervous system. Acute neurodegeneration is associated with traumatic head, spinal cord injury and stroke, and chronic neurodegeneration is typified by Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). It is estimated that within 30 years more than 12 million Americans will suffer from NDs, if NDs are not well controlled. Many of these diseases are fatal. There are no neuroprotective drugs available now for acute NDs. Although several drugs are used clinically for chronic NDs, the therapeutic efficacy is very limited.

It is of increasing urgency to find effective treatments for neurodegenerative diseases. However, discovery and development of neuroprotective drugs have been hindered either by inefficiency in therapy or severe side effects. Neurodegeneration is a complicated process. In excitatory neurotoxicity, for example, a toxic cascade is downstream of accumulation of intracellular free calcium $[Ca^{2+}]_i$. When one path way is blocked, other ways are still available in the toxic cascade. Antagonists of the NMDA receptors can efficiently inhibit neurotoxicity by blocking upstream of the toxic cascade, but these commonly have severe side effects in the body. Thus, neutralization of influx of $[Ca^{2+}]_i$ upstream of the toxic cascade is a key point for blockage of excitatory neurotoxicity.

PAN-811 is a small organic molecule with a molecular weight of 195.24 Daltons. PAN-811 exerts its function in cancer suppression *via* its role in inhibition of ribonucleotide reductase by chelating ferrous ion (Fe^{2+}). As a divalent ion chelator, the activity of PAN-811 is nonspecific. Besides Fe^{2+} , PAN-811 can also chelate other divalent metals, such as copper (Cu^{2+}) and Zinc (Zn^{2+}). In cell-free and enzyme-free systems, we found that PAN-811 can chelate Ca^{2+} , a key factor in excitatory neurotoxicity, and can also scavenge free radicals, another important toxic factor in neurodegeneration. PAN-811 has been found to protect cultured cerebral neurons from any tested neurotoxic insults, including glutamate or hypoxia/hypoglycemia (stroke models), hydrogen peroxide or aggregated beta-amyloid (Alzheimer's disease models), anticancer drugs (chemotherapy-induced cognitive impairment model), and in the rd1 mouse-derived retina cells (retinitis pigmentosa-induced blindness model). In an animal stroke model with middle cerebral artery occlusion, PAN-811 delivered *via* i.v. route significantly reduced infarct volume and decreased mortality. For its application in cancer therapy, PAN-811 has gone through preclinical and phase I and II clinical trials, with an acceptable safety profile and known pharmacokinetic outline. We have completed the manufacturing process development of its salt PAN-811·Cl·H₂O (MW 249.72 Da) and have produced large quantities under GMP conditions. We expect to evaluate PAN-811 for neuroprotection in early clinical trial in near future.

IL- 6

Track: Diabetes and Obesity Drug Discovery & Therapy

CHILDHOOD OBESITY AND NUTRITIONAL INTERVENTIONS**Emilio Jirillo**

Department of Basic Medical Sciences, Neuroscience and Sensory Organs, University of Bari, Bari, Italy; E-mail: emilio.jirillo@uniba.it

Obesity is an epidemic in western and westernized countries. High intake of hypercaloric food leads to a progressive accumulation of abdominal fat, which represents a continuous source of inflammatory mediators. Therefore, obesity is characterized by a condition of low grade inflammation which involves vital organs such as the cardiovascular and the central nervous systems. Obesity in children has tremendously increased owing to consumption of junk food and sedentary habits with less physical activity. In this framework, recent studies on childhood obesity have emphasized the immune status on the one hand, and, the intestinal microbiota composition on the other hand. In fact, a link exists between type of adaptive immune response and intestinal microbes in obesity. In fact, microbiota composition in obese people, even including children, supports an inflammatory profile characterized by high concentrations of circulating proinflammatory cytokines. In particular, interleukin (IL)-17, an inflammatory cytokine, is predominantly released in blood with lower concentration of IL-10, an antiinflammatory cytokine. This inflammatory profile is complicated by the association of asthma and childhood obesity also according to data of our own group. Moreover, our recent studies conducted on normal weight children have evidenced that the IL-10/IL-17 salivary ratio changes according to the type of diet and modifications of BMI. In other words, children who followed a Mediterranean type diet over a period of one year exhibited lower BMI than children who ate junk food in the same period of time. Lower BMI corresponded to lower salivary amounts of IL-17 and higher levels of IL-10. Conversely, higher BMI correlated to lower levels of IL-10 and higher concentrations of IL-17. Lower physical activity corresponded to higher BMI and *vice versa*. Therefore, consumption of junk food leads to a progressive condition of low grade inflammation, increase in BMI and, finally, frank overweight/obesity status. Major nutraceuticals, such as polyphenols, will be illustrated for their ability to reduce inflammation and prevent obesity and related complications since childhood.

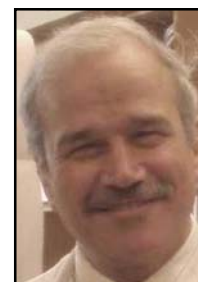
**IL- 125**

Track: Cardiovascular Drug Discovery & Therapy

ROLE OF MICROVASCULAR ENDOTHELIAL TYPE B ENDOTHELIN RECEPTOR DURING PREGNANCY. IMPLICATIONS IN HYPERTENSIVE PREGNANCY AND PREECLAMPSIA**Raouf A. Khalil**

Harvard Medical School Brigham and Women's Hospital Division of Vascular Surgery 75 Francis Street Boston, Massachusetts 02115, USA; E-mail: raouf_khalil@hms.harvard.edu

Normal pregnancy is associated with decreased vascular resistance and increased release of vasodilators. Endothelin-1 (ET-1) causes vasoconstriction *via* endothelin receptor type A (ET_AR), but could activate ET_BR in the endothelium and release vasodilator substances. However, the role of ET_BR in the regulation of vascular function during pregnancy and the vascular mediators involved are unclear. Also, preeclampsia is a pregnancy-related disorder characterized by hypertension (HTN) with unclear mechanism. Studies have shown endothelial dysfunction and increased ET-1 levels in hypertensive pregnancy (HTN-Preg), but the role of endothelial ET_BR in the changes in blood pressure (BP) and vascular function in HTN-Preg is unclear. To examine the role of ET_BR during normal pregnancy and to test if downregulation of endothelial ET_BR expression/activity plays a role in HTN-Preg, BP was measured in Norm-Preg rats and rat model of HTN-Preg produced by reduction of uteroplacental perfusion pressure (RUPP), and mesenteric microvessels were isolated for measuring



diameter, $[Ca^{2+}]_i$, and ET_A R and ET_B R levels. BP, ET-1 and KCl-induced vasoconstriction and $[Ca^{2+}]_i$ were greater in RUPP than Norm-Preg rats. Endothelium-removal or microvessel treatment with ET_B R antagonist BQ-788 enhanced ET-1 vasoconstriction and $[Ca^{2+}]_i$ in Norm-Preg, but not RUPP, suggesting reduced vasodilator ET_B R in HTN-Preg. ET-1+ ET_A R antagonist BQ-123, and ET_B R agonists sarafotoxin 6c (S6c) and IRL-1620 caused less vasorelaxation and nitrate/nitrite production in RUPP than Norm-Preg. The NOS inhibitor L-NAME reduced S6c- and IRL-1620-induced relaxation in Norm-Preg but not RUPP, supporting that ET_B R-mediated NO pathway is compromised in RUPP. RT-PCR, Western blots and immunohistochemistry revealed reduced endothelial ET_B R expression in RUPP. Infusion of BQ-788 increased BP in Norm-Preg, and infusion of IRL-1620 reduced BP and ET-1 vasoconstriction and $[Ca^{2+}]_i$ and enhanced ET_B R-mediated vasorelaxation in RUPP. Thus enhanced ET_B R-mediated microvascular relaxation may contribute to the decreased vasoconstriction and vascular resistance during normal pregnancy. Downregulation of microvascular vasodilator ET_B R is a central mechanism in HTN-Preg, and increasing ET_B R activity could be a target in managing preeclampsia.

IL- 251

Track: Neurodegenerative Diseases

IMAGING AGENTS FOR DIAGNOSIS OF NEURODEGENERATIVE DISEASES

Jeff Kuret

Department of Molecular and Cellular Biochemistry, The Ohio State University College of Medicine, 1060 Carmack Rd, Columbus, OH 43210, USA; E-mail: kuret.3@osu.edu

Currently approved whole-brain imaging methods for assessing dementia target the aggregated forms of the β -amyloid ($A\beta$) peptide that accumulate in senile plaques. Development of radiotracers that are selective for lesions composed of other proteinaceous aggregates could complement the established $A\beta$ imaging signature in several ways. First, their ability to detect specific lesions at sites of predilection before the onset of dementia could provide the means to detect disease at very early stages. Second, selective imaging agents also may aid the diagnosis of dementing illnesses that lack $A\beta$ lesions and that are difficult to distinguish based solely on clinical presentation, including Lewy Body Dementia and certain forms of frontotemporal lobar degeneration. Finally, unlike $A\beta$, aggregates composed of other protein protomers correlates better with neurodegeneration and cognitive decline in AD, providing a potential surrogate marker for disease. Because of the well-established relationship between disease progression and spatial distribution of neurofibrillary pathology, tau-based imaging could help establish Braak stage in living patients. Here I will summarize progress in developing lesion-selective imaging agents, with emphasis on molecular descriptors of binding selectivity. The results suggest a route for optimizing the binding affinity of radiotracers while maintaining favorable pharmacokinetic properties.

SL- 126

Track: Innovative Drug Discovery and Nanotechnology

GENOMICS WITH GRAPHENE NANOTECHNOLOGY

Jean-Pierre Leburton

G. Stillman Professor of Electrical and Computer Engineering, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; E-mail: jleburto@illinois.edu

In the recent years there has been a tremendous interest in using solid-state membranes with nanopores as a new tool for DNA and RNA characterization and possibly sequencing. Among solid-state porous membranes the use of mono-layer graphene is particularly attractive because of its electric versatility, physical robustness and good electronic properties.

In this talk, we will present a scenario that integrates biology with graphene-based field-effect transistor for probing the electrical activity of DNA molecules during their translocation through a graphene membrane nanopore, thereby providing a mean to manipulate them, and potentially identify by electronic technique their molecular sequences

IL- 301

Track: Pharmaceutical Biotechnology

A COMPREHENSIVE APPROACH TO UNDERSTAND GLYCOSYLATION OF MONOCLONAL ANTIBODIES

Jun Qian, David Mahon, Babita Parekh, Qinwei Zhou and Tun Liu

BioAnalytical Sciences, Eli Lilly & Co., Branchburg, NJ, USA; E-mail: tun.liu@lilly.com

Glycosylation is a complex process of post-translational modification and provides functional and structural support for the protein modified. All therapeutic monoclonal antibodies (mAb) are glycosylated at the conserved Fc site and some are also glycosylated at Fab site. We have taken a comprehensive approach to depict the glycosylation pattern, glycan structure, and levels of glycosylation of our pipeline products (IgG1). Glycans on a monoclonal antibody were either released by PNGase F or analyzed directly. The released glycans were subsequently tagged with a fluorescent probe and profiled by normal phase HPLC. Individual glycans were analyzed by mass spectrometry in combination with sequential glycosidase digestion for glycan sequence and linkage information. Glycoforms were identified with the aid of FabRicator to demonstrate Fab and Fc glycosylation patterns. A glycosylation levels were determined and found to vary among different cell lines. Furthermore, core fucosylation of the glycans at the Fc site was shown to impact ADCC activity of mAbs. Sialylation of the glycans, on the other hand, was demonstrated to be one of the major contributing factors to the charge heterogeneity of the monoclonal antibody products.



IL-7

Track: Regenerative Medicine

MECHANICALLY TUNABLE AND DEGRADABLE HYDROGELS FOR REGENERATIVE MEDICINE

Tao L. Lowe

Department of Pharmaceutical Sciences, Biomedical Engineering Program, University of Tennessee Health Science Center, Memphis, TN 38163, USA; E-mail: tlowe4@uthsc.edu

The mechanical and degradation properties of hydrogel scaffolds play important roles in providing appropriate environment mimicking nature conditions for tissue regeneration. Different tissues such as bone, cartilage, nerve and heart have different mechanical properties. The hydrogels for regenerating an injured tissue need to have the mechanical properties similar to the tissue to be repaired to provide proper load-bearing and mechanical signal stimuli for the tissue development. The degradation of the hydrogels with time facilitates more space for the growth of new tissue and also increases the mass transport through pores formed through degradation for the diffusion of nutrients in and metabolic wastes out. In this talk, I will discuss the design and development of polysaccharide-based hydrogels with tunable mechanical and degradation properties through controlling the composition, polysaccharide molecular weight and crosslinking density of the hydrogels for cartilage repair and dental pulp stem cell growth.



1L-227

Track: Hot Topics in Medicinal Chemistry

FRAGMENT BASED DESIGN OF NON-ATP COMPETITIVE KINASE INHIBITORS THROUGH REPLACE**Campbell McInnes**

Medicinal Chemistry, South Carolina College of Pharmacy, University of South Carolina, CLS 109, 715 Sumter St., Columbia, SC, 29208, USA

Protein-protein interactions involved in kinase regulation and substrate recognition have significant potential in drug discovery due to their unique features and therefore allow selectivity and potency of inhibition by avoiding the catalytic site. Since these interfaces typically involve shallow clefts and more diffuse interactions, they are more challenging than the ATP binding site. We have developed and validated REPLACE as a general strategy for protein-protein interactions in the development of non-ATP competitive inhibitors of protein kinase oncology targets including in the first instance, cyclin dependent kinases through the substrate recruitment site. Replacement of N and C-terminal determinants of a potent octapeptide has been accomplished through application of REPLACE to generate more drug-like compounds with on target cellular activity [1-4]. Further validation of REPLACE was achieved through application to the Polo-Box domain of PLK1 to generate selective compounds that have cellular phenotypes consistent with inhibition [5,6] and which are active against a mutant form of the kinase resistant to clinically used ATP competitive inhibitors. Blocking sub-cellular localization of PLK1 is therefore a promising strategy for next generation PLK1 inhibitory compounds. Overall we have demonstrated that REPLACE is an effective approach to the generation of non-ATP competitive kinase inhibitors for the development of anti-tumor therapeutics.

1L-20

Track: Diabetes and Obesity Drug Discovery and Therapy

THE USE OF BARIATRIC SURGERY IN PATIENTS WITH DIABETES AND OBESITY**Jeffrey I. Mechanick**

*Internal Medicine – Endocrinology, Icahn School of Medicine at Mount Sinai, USA;
E-mail: jeffreymechanick@gmail.com*



The global prevalence of diabetes and obesity is increasing at epidemic proportions due to a complex interaction of multiple drivers that remain poorly understood. Interventions within an effective complications-centric preventive care paradigm are limited but comprise three broad categories: structured lifestyle, pharmacotherapy, and procedures. Bariatric surgery is currently a reasonable option for a durable beneficial response in high-risk patients with both diabetes and obesity. However, the performance of this modality (represented by a portfolio of various procedures) depends on proper patient and procedure selection, safe implementation, and appropriate comprehensive preoperative nutritional and metabolic management. The mechanisms of action, evidence base, and clinical guidelines supporting these statements will be provided.

Keywords: Obesity, diabetes, bariatric surgery, nutrition, metabolic complications.

IL- 170

Track: Drug Discovery in Preclinical Research

SMALL-MOLECULE “DRUG-LIKE” TSH RECEPTOR AGONISTS AND ANTAGONISTS**Susanne Neumann**

National Institutes of Health, NIDDK, Building 50, Room 4128, Bethesda, MD 20892, USA; E-mail: SusanneN@intra.nidk.nih.gov



Thyroid-stimulating hormone (TSH) activates the TSH receptor (TSHR) thereby stimulating the function of thyroid follicular cells (thyrocytes) leading to biosynthesis and secretion of thyroid hormones. Recombinant human TSH (rhTSH, Thyrogen[®]) has been used in the follow-up of patients with thyroid cancer to increase the sensitivity for detection of recurrence or metastasis. rhTSH is difficult to produce and must be administered by injection. A small molecule TSHR agonist could replace costly rhTSH and produce the same beneficial effects but with greater ease of oral administration. We developed a small molecule ligand that is a full agonist at TSHR. This agonist elevated serum thyroxine and stimulated thyroidal radioiodide uptake in mice after its absorption from the gastrointestinal tract following oral administration which is important for its clinical potential.

Graves' disease (GD) is caused by persistent, unregulated stimulation of thyroid cells by thyroid-stimulating antibodies (TSABs) that activate the TSHR. An antagonist that targets the TSHR directly may have therapeutic potential to block TSABs in Graves' hyperthyroidism. We identified the first small molecule TSHR antagonists, which inhibit TSH- and TSAB-stimulated signaling. To be used as therapy for GD, it would be important that an antagonist inhibits TSHR activation by the majority of TSABs. The TSHR antagonist inhibited cAMP production by average 61% by all thirty GD sera tested. Furthermore, the antagonist inhibited TSAB-induced up-regulation of the expression of thyroperoxidase mRNA in primary cultures of human thyrocytes by 65%. We demonstrated *in vivo* that an improved and selective antagonist decreased endogenous serum free T4 levels and lowered the TSAB-induced increase of free T4. The antagonist lowered also mRNAs for sodium-iodide transporter and thyroperoxidase.

Another potential clinical application of a TSHR antagonist beyond treatment of the hyperthyroidism of GD could be alleviating the symptoms of Graves' ophthalmopathy (GO), an inflammation of orbital tissue which is associated with GD and leads to enlargement of extra-ocular muscles and orbital adipose tissue. Excessive production of hyaluronic acid and new fat cell development are underlying these changes. We showed that the TSHR antagonist reduced TSH- and TSAB-stimulated cAMP production and hyaluronic acid production in primary cultures of Graves' orbital fibroblasts.

Our data provide proof-of-principle for effectiveness of small molecule agonists and antagonists for TSHR. We suggest that the small molecule agonist may be a drug candidate to be used in patients with thyroid cancer. The small molecule antagonists are lead compounds for the development of orally active drugs that target the TSHR to treat GD and GO.

IL-67

Track: Cancer Targeted Drug Delivery

PROSTATE CANCER-SPECIFIC DESTRUCTION OF ANDROGEN RECEPTOR WITH DEUBIQUITINASE INHIBITORS**Teresita Reiner, Alicia de las Pozas, Ricardo Parrondo, Deanna Palenzuela and Carlos Perez-Stable**

Miami VA and University of Miami Miller School of Medicine, Miami, FL 33125, USA;
E-mail: c.perezstable@med.miami.edu

Prostate cancer (PCa) is a leading cause of death in men, especially when unresponsive to androgen deprivation therapy, known as castration-resistant PCa (CRPC). Enzalutamide (Enz) is a new approved drug that inhibits androgen receptor

(AR) activity and increases overall survival. However, most responding patients develop resistance to Enz, indicating that new therapies are required to block CRPC. We discuss the alternative strategy of utilizing new agents that decrease AR expression in order to overcome resistance to AR inhibitors such as Enz, and more effectively treat CRPC.

We discovered that betulinic acid (BA), a plant-derived small molecule that selectively targets cancer cells for apoptosis, inhibits multiple deubiquitinases (DUBs). DUBs increase the stability of key proteins by removing poly-ubiquitin (Ub) and therefore, making them less susceptible to degradation by the ubiquitin-proteasome system (UPS). Our results are summarized as follows: 1) BA is a powerful inducer of apoptosis in TRAMP PCa *in vivo* and multiple human PCa cell lines *in vitro*; 2) BA lowers AR protein in TRAMP PCa but not in normal prostates; 3) BA inhibits DUB activity in PCa cells, leading to the accumulation of poly-Ub proteins, and the UPS-mediated degradation of multiple proliferation/pro-survival proteins, including AR; and 4) BA has minimal effect on apoptosis or poly-Ub accumulation in normal mouse tissues and does not inhibit DUB activity or increase apoptosis in multiple human non-cancer cell models. Despite BA having an effect on multiple signaling pathways (AKT, ERK, JNK), the increase in the degradation of AR and in apoptotic cell death in PCa cells is dependent on inhibition of DUBs and on an active UPS pathway.

In addition to reducing AR protein stability, our results indicate that BA also reduces AR mRNA. One possible reason for the BA-mediated decrease in AR mRNA is an increase in Ub-histone 2A, a known epigenetic transcriptional repressor. Since AR is the most important factor for the emergence of CRPC, the ability of BA to reduce AR at both the protein and RNA levels makes it a very attractive agent for the treatment of PCa. These functions of BA are also reproduced by another DUB inhibitor, WP1130, suggesting that DUB inhibitors as a class can efficiently target AR.

A candidate DUB that is inhibited by BA and regulate AR is Ub-specific protease (USP) 10. Our results show that in LNCaP PCa cells, shRNA knockdown of USP10 reduces AR whereas overexpression of USP10 increases AR protein. In normal human prostate tissue, USP10 is strongly expressed in the cytoplasm and nucleus of epithelial cells whereas in PCa tissues, there is greater variability in USP10 expression and less nuclear localization with increasing Gleason grades. Further work is required to identify additional AR-regulatory DUBs inhibited by BA or WP1130 and whether these DUBs are co-expressed with AR. High expression of AR-regulatory DUBs in CRPC compared to hormone naive PCa could provide a clue as to why AR is elevated in CRPC, i.e., protection from UPS-mediated degradation by AR-regulatory DUBs.

It is clear that combinations of chemotherapeutic agents will be required for the effective treatment of CRPC. Our results show that treatment of Enz resistant LNCaP PCa cells with the BA + Enz combination further decreases AR protein and increases apoptotic cell death compared to BA alone. In addition, we obtained similar results with the WP1130 + Enz combination, suggesting that Enz enhances multi-DUB inhibitor-mediated AR degradation. These results suggest that the BA + Enz combination may provide a novel treatment strategy for Enz resistant PCa by enhancing AR degradation and increasing cell death. Overall, we conclude that the identification and inhibition of AR-regulatory DUBs will positively impact development of effective non-toxic clinically selective strategies for the prevention and treatment of PCa.

IL-165

Track: Inflammation and Immunology

NITROGEN AND OXYGEN INTERMEDIATES AND ANTIBODY MEDIATED PLATELET CYTOTOXICITY AS PROGNOSTIC MARKERS FOR DENGUE SEVERITY

Sunil Premawansa, M.S. Mapalagamage, M.N.S. Bandara, Premawansa G. Mark Loeb, A.D. De Silva and S.M. Handunnetti

Department of Zoology, University of Colombo, College House 94, University of Colombo, Cumaratunga Munidasa Mw, Colombo 03, Sri Lanka; E-mail: suviprema@gmail.com

Serum Reactive Nitrogen Species (RNS), Reactive Oxygen Species (ROS) and platelet cytotoxicity are thought to play an important role in pathogenesis of severe dengue infection, among several other immune mechanisms. Principal objectives of these studies were to seek whether RNS, ROS, Platelet cytotoxicity and serum LDH levels could be used as prognostic indicators for disease severity and to study their associations with dengue severity. Two blood samples [on admission (A) and on discharge (D)] were collected from dengue patients admitted to North Colombo Teaching

Hospital. Dengue positivity of patients was confirmed by IgM ELISA and RT-PCR. Griess assay and ABTS assay were used to measure serum nitrite and antioxidant capacity (AOC; an indirect measure of ROS). Serum LDH levels and level of LDH released due to *in vitro* patient's sera induced platelet destruction upon incubation of platelets with sera (with and without complement) were measured using a platelet cytotoxicity assay. Results showed that the serum nitrite levels in DHF patients on admission (DHF-A) were significantly higher than in DF-on admission (DF-A) sera and Healthy controls. High serum nitrite levels thus indicate increased production of NO (Nitric oxide) associated with dengue severity. Serum AOC levels in DHF patients on admission were also significantly higher compared to DF on admission and healthy controls. Increase of AOC in DHF patients may also be due to more ROS production associated with dengue severity.

In the *in vitro* cytotoxicity assays, in the presence and the absence of complement, significantly higher platelet cytotoxicity levels (induced by patient's serum) were observed in DHF-A sera compared to all other study groups; DHF-D, DF-A, DF-D and HC ($p < 0.001$). Platelet cytotoxicity levels of each group were significantly elevated with complement, confirming that the platelet cytotoxicity is due to complement mediated antibody action. Increased destruction of platelets with DHF-A patient's sera may be due to the presence of more anti-dengue antibodies in them and this can be associated with dengue severity. Further, platelet cytotoxicity levels in different study groups were significantly correlated with the respective serum LDH values suggesting that serum LDH levels may partly represent the destruction of platelets *in vivo*. These results must be further validated with larger patient samples as to suggest the use of serum nitrite, AOC, platelet cytotoxicity, serum LDH as prognostic indicators of dengue severity. Use of serum nitrite and ROC as prognostic markers for severity may be appropriate as they are promptly produced in dengue infections through innate immune mechanisms.

IL-298

Track: Drug Delivery and Targeting

PHOTOTRIGGERABLE LIPID ASSEMBLIES AND THEIR APPLICATIONS FOR DUAL DRUG DELIVERY

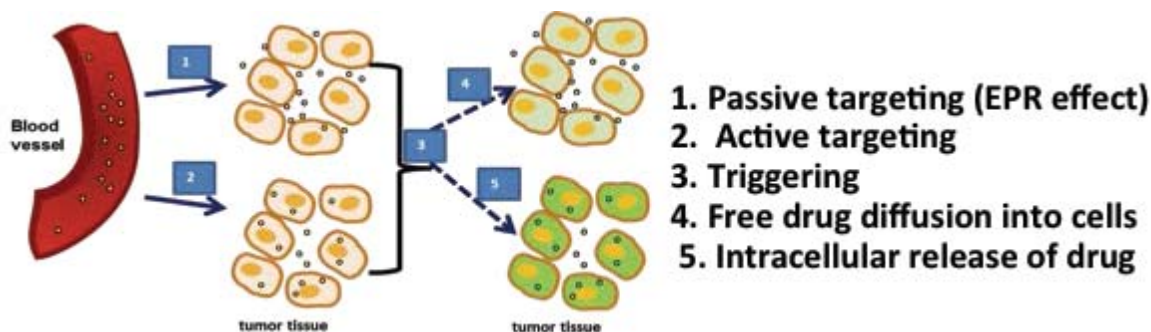
Anu Puri

*Membrane Structure and Function Section, Center for Cancer Research, National Cancer Institute, Frederick, Basic Research Library, CCR, NCI-Frederick, NIH, USA;
E-mail: puria@mail.nih.gov*

We are developing tunable lipid-based nanoparticles bearing on-demand drug release properties to improve delivery of cancer therapeutics. For light-triggered applications, we have designed liposomes from a photopolymerizable diacetylenic phospholipid (DC_{8,9}PC) [1, 2], based on its unique partitioning in the liposome membrane. Our formulations also include a photoagent of choice to promote light-triggered drug release.

Detailed analyses of packing properties of DC_{8,9}PC suggest that this photoreactive lipid preferentially clusters in gel phase matrix lipid (such as DPPC), critical for light-triggered release [3, 4]. We hypothesize that the permeation of photo-activated compounds occurs through pockets in the liposome membrane of enhanced fluidity and have dubbed them "Pocket" liposomes. DC_{8,9}PC is known to photo-crosslink upon exposure to UV (254 nm) light source. However, visible light triggered mechanisms of drug release from these liposomes do not follow the classic photo-crosslinking process and occur *via* distinct pathways that involve reactive oxygen species [5]. The results on the design and characterization of "Pocket" liposomes containing anticancer agents (doxorubicin and/or a PDT drug HPPH) [6] and their *in vitro* and *in vivo* biological activity including tumor regression data will be presented. The liposomal nanomedicine platform described here has the potential for dual drug delivery towards treatment of certain types of cancers.





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IL-13

Track: Diabetes and Obesity Drug Discovery and Therapy

ROLE OF HIF1 α /NF κ B PATHWAY IN OBESITY

Matteo A. Russo

Department of Experimental Medicine, University of Rome, Sapienza, Viale Regina Elena, 324, 00161, Rome, Italy; E-mail: matteoantonio.russo@uniroma1.it

In obesity adipocyte are hypertrophic, showing cell volume increase due to an abnormal accumulation of lipid droplets. This *false* hypertrophy establish in the tissue a progressively insufficient perfusion giving rise to a hypoxic microenvironment and consequent activation of the HIF1 α /NF κ B pathway, cell damage, chronic inflammation, repair and fibrosis in the obese adipose tissue.



Hypoxia and inflammation in obese adipose tissue appear strictly interconnected with important consequences at clinical and therapeutic level. Acute hypoxia mostly leads to cell necrosis, while in chronic hypoxia adipocytes are able to survive adapting their phenotype through the expression of a number of genes, including proinflammatory receptors for alarmins. These receptors are activated by alarmins which are released by necrotic adipocytes and generate signals for transcription factors such as NF κ B, STAT3, AP1, *etc.* which control hundreds of genes for innate immunity and damage repair. Clinical consequences of chronic inflammatory reparative response activation include cell and tissue remodeling, damage in the adipose tissue, fibrosis and systemic involvement of distant organs and tissues.

Herewith, the reciprocal relationships between hypoxia and inflammation in obese adipose tissue will be shortly reviewed to underline its importance in the pathogenesis of obesity and in identifying potential therapeutic targets.

Hypoxia produces *necrosis* of cells in the region more distant from tissue vessels and an HIF-driven *adaptation* of cells in the region along the hypoxic gradient close to the vessels. *Necrosis*: the O₂ shortage causes inhibition of ATP production, rapid fall of the energy charge, and loss of ionic gradients with the alteration of cytosolic calcium homeostasis. The increase of cytosolic Ca⁺⁺ concentration above 10⁻⁶ M, dramatically activates the peroxidative metabolism, an irreversible contraction and degradation of the cytoskeleton and the activation of Ca⁺⁺-dependent cytosolic proteases (calpains) and DNA-ases, all leading to a rapid and irreversible cell degradation. Plasmamembrane rupture is responsible for the release of intracellularly segregated molecules, many of which are called *alarmins* for their ability to signals the cell damage to specific receptors on adjacent cells. *Adaptation* of cells surviving to the hypoxia occurs through the activation of HIF1 α and the expression of a number of genes, such as VEGF, Glut1 and HKII, TERT, and stemness genes which increase the stem cell compartment. However, very importantly in our case, is the expression of endogenous alarmin receptors, such as RAGE, P2X7, Toll-like receptors, NOD-like receptors, *etc.* which allow the

IRR directly in adipocytes and precursors. In fact, alarmins released by necrotic adipocytes (HMGB1, ATP/ADP, membrane debris, nucleic acids, *etc.*) bind their receptors and activate NFkB with the expression of IRR genes, including adipokines, mediators and other proinflammatory cytokines, increasing the number of leukocytes in the affected adipose tissue.

A number of evidences will be presented suggesting that in obesity hypoxia and inflammation are sequentially related at the molecular, cellular, and clinical levels. These evidences include HIF1a activation, reprogramming stem cells, alarmin receptor increased expression, NFkB activation, proinflammatory genes expression in hypoxia-activated adipocytes and their precursors, and the activation of profibrotic TGF-b pathway. Finally, treatments with HIF1a and NFkB-inhibitor and NSAID are under study for their anti-adipogenic effects.

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IL-266

Track: Neurodegenerative Disorders

DIETARY RESTRICTION TO PREVENT AD PATHOGENESIS

Kumar Sambamurti, Pannerselvam Chinnakannu, Miguel A. Pappolla, Narayan R. Bhat, Debomoy K. Lahiri, Nigel H. Greig and Vasudeva Padmaraju

Medical University of South Carolina, Charleston, SC, USA; E-mail: sambak@musc.edu

Homocysteine and cholesterol have both been identified as major risk factors for Alzheimer's disease (AD) and age-related macular degeneration (AMD). Interestingly, the amyloid beta protein (Ab) of 42 aa (A β 42) is deposited in extracellular lesions in both these diseases. Critical to the understanding of AD is the recognition that A β accumulation does not take place in most individuals although everyone expresses high levels of A β . We previously reported that high homocysteine can lead to misprocessing of the A β precursor (APP) and increase A β in cell cultures and mouse models. In contrast, we have obtained convincing evidence that restriction of just one amino acid, methionine, signals in cells to reduce shedding of APP. We then treated transgenic APP or MAP-Tau mice with a diet consisting of just 25% of normal intake of methionine for a period of 3, 6 and 16 months starting at 1 month. This diet represents a 75% methionine restriction in the diet. It has been previously reported that 75% methionine restriction in the diet of both rats and mice lead to an increase in life expectancy and a improvement in glucose tolerance. Importantly, the control diet used contains a mix of roughage; carbohydrates, fats and amino acids that are balanced to resemble the normal rodent diet for optimal growth and are also synthetic to resemble the restricted diet. The methionine-restricted (MR) diet is matched for both calorie and nitrogen content to prevent confounding changes in calorie and total protein. Our data indicates that methionine restriction reduces soluble Ab42 levels in mouse brain but did not affect levels of APP or insoluble Ab42. The studies also show that methionine restriction can improve the levels of oscillatory potentials without changing other aspects of scotopic electroretinograms. In summary, a simple dietary restriction paradigm reduces Ab levels without inhibiting essential proteolytic activities. This treatment strategy may be applied safely in combination with existing treatment protocols to help prevent AD.



IL-133

Track: In-Silico Drug Design and In-Silico screening

USEFUL DESCRIPTORS IN BEYOND RULE-OF-FIVE DRUG DESIGN SPACE?

Marina Shalaeva

Department of Medicinal Chemistry, Pfizer R&D, USA; E-mail: marina.shalaeva@pfizer.com

The rule-of-5 properties successfully led drug design for over 20 years. Exploration of peptides, macrocycles, ADCs, polyketides and other large molecules as therapeutic agents requires better understanding of the molecular features critical for drug-like properties in that chemical space often called beyond rule of 5 (BRO5). We have explored the role and the physical meaning of parameters derived from various types of chromatographic interactions. The chromatographic interactions, kinetic in nature, could be considered as surrogates for different environments encountered by the drug molecules in the body and, therefore, could provide useful insight into change in the molecular conformations and properties depending on the matrix. The chromatographic parameters (such as ElogD, EPSA, PLRP-S), and corresponding computational models, in relation to hydrophobicity, polarity, HBD, HBA, ionization are investigated and a few examples demonstrate the application of these parameters as useful descriptors in BRO5 drug design space.

Keywords: Beyond-rule-of-5 drugs, ElogD, molecular conformations.

IL-262

Track: Hot Topics in Medicinal Chemistry

DEVELOPING STRATEGIES TO BUILD COMBINED DIRUTHENIUM-PHARMACEUTICAL METALLODRUGS TARGETING CANCER THERAPY

Denise de Oliveira Silva

Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, São Paulo, São Paulo, Brazil; E-mail: deosilva@iq.usp.br

Developing novel anticancer drugs is urgent required since the number of cancer cases and new cancer types are predicted to increase significantly in the next decades. The contribution of metal compounds to oncology is well-known due to the clinical relevance of platinum cancer chemotherapy. A diversity of other metal compounds exerting antitumor activities has been reported, with significant progress achieved for ruthenium-based drugs that reached clinical phases. Our group has worked on the approach of binding clinically used organic pharmaceuticals to diruthenium cores to target cancer cells. In particular, we have explored the effects of binding non-steroidal anti-inflammatory drugs to ruthenium dimetal cores. The novel metallodrugs were investigated for distinct cancer types (glioma, myeloid leukemia, colon cancer, and larynx and bladder tumor) with the best activity found for glioma cancer models. In fact, in comparison with the organic drugs, the combined ruthenium-pharmaceutical metallodrugs showed synergistic effects that enhanced the antiproliferative activity in rat glioma models *in vitro*. The metallodrugs were also found to exert activity *in vitro* in human glioma cell lines, and *in vivo* (rats). This presentation will give an overview of our strategies to build the combined diruthenium-pharmaceutical metallodrugs, and will also show the most recent findings associated to their role in targeting cancer therapy. (Financial support from FAPESP, CNPq and CAPES is gratefully acknowledged).

Keywords: Ruthenium metallodrugs, pharmaceuticals, anticancer drugs, cancer.

IL-271

Track: Neurodegenerative Disorders

PRENATAL VITAMIN A DEFICIENCY FACILITATES ALZHEIMER'S DISEASE PATHOGENESIS**Weihong Song**

Townsend Family Laboratories, Department of Psychiatry, The University of British Columbia, 2255 Wesbrook Mall, Vancouver, BC V6T 1Z3, Canada; E-mail: weihong@mail.ubc.ca

Deposition of amyloid β protein ($A\beta$) to form neuritic plaques in the brain is the pathological hallmark of Alzheimer's disease (AD). Mild or marginal vitamin A deficiency (MVAD) is a serious and widespread public health problem in pregnant women and children in developing countries. There has been increasing evidence for the involvement of vitamin A in AD pathogenesis. However, the role of vitamin A in the development of AD is not well defined. Our studies in an elderly population have shown that vitamin A deficiency (VAD) could enhance the risk to develop AD, and retinoic acid receptors (RARs) plays an important role in the amyloid β precursor protein (APP) metabolic pathway. To further examine vitamin A's effect on AD pathogenesis, we established a prenatal MVAD model in APP/PS1 double-transgenic AD model mice. Our study showed that MVAD significantly increases $A\beta$ production by inhibiting ADAM10-mediated α -secretase cleavage and increasing β -secretase (BACE1) cleavage of APP. MVAD significantly increased neuritic plaque formation and aggravated spatial learning and memory deficits. Our findings provide a mechanistic explanation for the role of MVAD in AD pathogenesis and demonstrate the importance of retinoic acid signaling as a target for AD therapy.

IL-97

Track: Process Chemistry and Drug Manufacturing

LARGE-SCALE CONTINUOUS MANUFACTURE OF NANO-COCRYSTAL DRUGS AND THERAPEUTICS OR HOW DEFENCE RESEARCH MAY CONTRIBUTE TO PROTECT HEALTH**Denis Spitzer and Marc Comet**

NS3E (Nanomatériaux pour les Systèmes Sous Sollicitations Extrêmes), UMR3208, ISL-CNRS-UNISTRA, France; E-mail: denis.spitzer@isl.eu

Numerous examples of cross-fertilization between defence and medicine are found. In 1847, Ascanio Sobrero discovered nitroglycerin, which has been one of the most used explosives. This nitric ester with vasodilatation properties, was proposed in 1878 by William Murrell to treat angina pectoris. Pentaerythritol tetranitrate (PETN), another explosive, is also used for healing heart conditions.



NS3E laboratory developed the Spray Flash Evaporation (SFE) for preparing energetic organic nanoparticles at industrial scale [1]. The energetic solution is kept in a pressurized tank separated from a vacuum chamber by a hollow cone nozzle, used both to heat and spray the liquid. The instantaneous evaporation of the solvent originates from the combination of the abrupt pressure drop and the high energy stored by the overheated solvent prior to nebulisation. The flash evaporation leads to small crystallites with narrow size distribution. The nanoparticles may be composed of single compounds, mixtures of several substances or cocrystals (Fig. 1). The idea to transport these findings to the medicine became evident. In this domain, cocrystals are of critical importance as they enhance bioavailability and up-take by the human body of Active Pharmaceutical Ingredients (API). Up to now, most used techniques are of batch nature and not able to give access in big amounts to nanosized crystals or cocrystals of therapeutic interest. The SFE permits the continuous manufacturing of nano-sized cocrystals, in large amounts with a kinetic complying with the pharmaceutical industry's requirements. The efficiency of SFE was shown by the manufacturing of nano-cocrystals based on caffeine/oxalic acid (2/1) and caffeine/glutaric acid (1/1), with a mean

particle size of 60 and 100 nanometers respectively. SFE currently used to produce nano-cocrystals, offers other promising prospects at the interplay between medicine and pyrotechnics that will be highlighted in this conference.

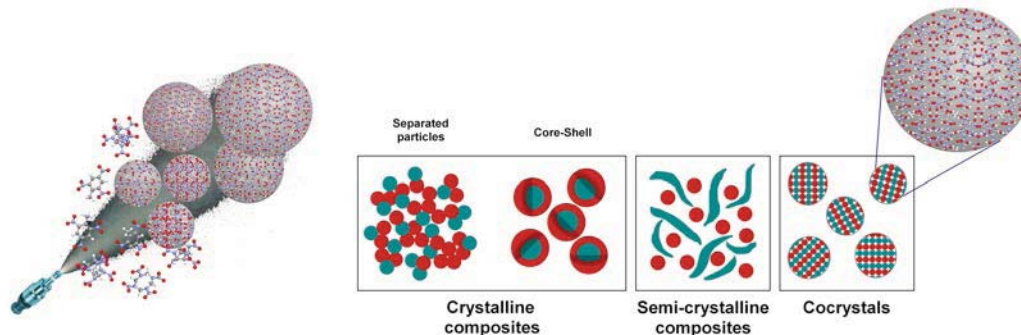


Fig. (1). Process scheme, and types of organic mixtures obtained by Spray Flash Evaporation.

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IL-164

Track: Drug Discovery in Preclinical Research

EXPANDING CHEMICAL SPACE BEYOND DRUGABILITY-RULES: THE SEARCH FOR MECHANISTICALLY DISTINCT PROTEASOME INHIBITORS

Jetze J. Tepe

Chemistry Department, Michigan State University, USA; E-mail: tepe@chemistry.msu.edu

Natural products isolated from plant, animal or fermentation have long been the main source for compounds used in the chemotherapeutic intervention of cancer. However, in the later part of the 20th century, the advances of combinatorial chemistry have taken center stage in the drug discovery process and natural product synthesis took a temporary backseat for these new chemical processes. Combinatorial techniques and compound repurposing have resulted into large libraries in a very cost-efficient manner that can be screened for their biological activities against a desired target. Although cost-effective, these libraries suffer from a lack of diversity with respect to the structural complexity, stereochemistry and chemical space. In addition, the enforcement of restrictions of structural complexity and “drugability rules”, further narrows the chemical space and thus limits the discovery of novel drug-target interactions.



The goal of our program is to discover mechanistically distinct drug-target interactions by generating small libraries with high levels of structural diversity. Our approach is to simulate the structural complexity found in natural products and translate this into structurally diverse abridged scaffolds. Phenotypic screening of these abridged scaffold libraries followed by target identification resulted into two mechanistically distinct classes of proteasome inhibitors. We will discuss how this approach lead to the discovery of two classes of proteasome inhibitors that regulate the the proteasome *via* a mechanism that is unlike any other proteasome inhibitor and how this translates in reduction of tumor growth and overcoming acquired resistance.

IL-130

Track: Drug Discovery in Preclinical Research

TARGET BASED VS PHENOTYPIC SCREENING APPROACHES IN DRUG DISCOVERY**Tove Tuntland**

Genomics Institute of Novartis Research Foundation, Novartis Institute of Biomedical Research (NIBR), San Diego, CA, USA; E-mail: ttuntlan@gnf.org

Target-based drug discovery can effectively develop novel treatments for a validated target, but the process of target validation is complex and associated with high degree of uncertainty [1]. As an alternative to the target based approach, phenotypic screening is making a comeback in drug discovery. Such assays characterize phenotypic events related to disease modification and do not require prior understanding of the mechanism of action [2]. At GNF/Novartis, scientists have used both target based and phenotypic screening approaches to successfully identify novel drugs. Target based methods lead to the discovery of ceritinib (Zykadia™, formerly LDK378), a highly potent and selective anaplastic lymphoma kinase (ALK) inhibitor which was recently approved by the FDA for the treatment of patients with ALK-positive metastatic non-small cell lung cancer who were previously treated with crizotinib [3, 4]. Phenotypic screening led to the discovery of cipargamin (formerly KAE609), the first new antimalarial drug candidate with a completely novel mechanism of action to reach phase 2 clinical development in over 20 years. When tested clinically in adults with uncomplicated *P. vivax* or *P. falciparum* malaria, cipargamin was shown to clear parasitemia after 3 days of repeated dosing [5, 6]. The approaches used to discover and develop the two novel drugs ceritinib and cipargamin will be discussed in this seminar.

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IL-38

Track: Traditional Chinese Medicine

CHEMICAL, PHARMACOLOGICAL AND QUALITY CONTROL STUDIES OF TRADITIONAL CHINESE MEDICINE (TCM) AND ETHNIC MEDICINE**Wei Wang**

TCM and Ethnomedicine Innovation & Development Laboratory, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, China; E-mail: wangwei402@hotmail.com

24 new compounds were isolated from *Kadsura heteroclita*, an anti-arthritis Tujia ethnomedicine called “Xue Tong”. 8 new xanthone glycosides included three potent neuroprotective compounds were isolated from *Swertia punicea*, named as “Gan Yan Cao” in the folk of Southwest China. Six compounds from n-BuOH extract of this plant displayed herbicidal activity against dicot *Lactuca sativa* (lettuce) and monocot *Agrostis stolonifera* (bentgrass). Four antifungal coumarins were obtained from TCM plant *Angelica dahurica* showed potential biopesticide activity. Moreover, marine shelled TCM *Concha Arcae* quality control studies in 2015 Chinese pharmacopoeia, *Typhane Pollen* and *Magnoliae Flos* quality



control studies in European Pharmacopoeia were performed, as well as qualitative and quantitative determination of Liuwei Dihuang preparations by multi-marker components and UHPLC dual-wavelengths fingerprint mode.

IL-323

Track: Innovative Drug Discovery and Nanotechnology

ADVANCES IN NANOTECHNOLOGY FOR DRUG DELIVERY: A SUMMARY OF 15 YEARS OF RESEARCH AND COMMERCIALIZATION

Thomas J. Webster

Department of Chemical Engineering, Northeastern University, Boston, MA 02115, USA;

E-mail: websterthomas02@gmail.com

Nanotechnology (or the use of materials with one dimension less than 100 nm) has been revolutionizing the field of drug delivery for several decades due to the ability of nanomaterials to circulate longer in the blood stream, penetrating cells and tissues, simultaneously detecting and treating disease, and targeting select cellular receptors. However, so far, nanotechnology has proven not to be the panacea for eliminating cancer, cardiovascular diseases, osteoporosis, neural diseases, and many other diseases. Issues such as toxicity, efficacy, drug loading, cost, and lengthy FDA approval times have still proven to be significant obstacles. This presentation will summarize recent advances in developing improved drug delivery vehicles for faster approval by focusing on not changing chemistries, but altering surface energy of existing FDA approved chemistries at the nanoscale. Such approaches have led to improved interactions with mammalian cells (such as bone, cartilage, vascular, neural, bladder, *etc.* cells) and decreased interactions with immune cells (such as monocytes, macrophages, *etc.*) to minimize nanoparticle clearance. Studies focusing not just on traditional biodegradable polymers but also metal oxides will be covered. Combined nanomaterial drug delivery-medical devices will also be covered where fast approval times have been achieved to treat infections, bone defects, and cartilage defects. Approaches which have combined treatment with diagnosis (*i.e.*, theranostics) will also be emphasized for orthopedic, neural, and cancer treatment. Lastly, a new approach to medicine focused on controlling picoscale events will also be introduced where one can control electron interactions within a material to improve cellular functions leading to greater disease detection and treatment. In summary, this presentation will cover what has been learned over the past several decades of translating nanotechnology to improve disease detection and treatment while emphasizing future developments that we should expect for the field to grow (such as picotechnology).

IL-294

Track: Drug Delivery and Targeting

SPECIFIC DELIVERY OF DRUGS TO MITOCHONDRIAL TARGETS: MANIPULATING MITOCHONDRIAL FUNCTIONS

Volkmar Weissig

Midwestern University College of Pharmacy Glendale, Glendale, Arizona, USA;

E-mail: vweiss@midwestern.edu

The efficiency and efficacy of drug action depends largely on how well an unaided drug molecule is able to reach its intracellular target or even its target inside organelles such as mitochondria. Subsequently, the specific delivery of a drug to its site of action inside cells will dramatically improve its action. Mitochondria play a key role in apoptosis and several clinically used as well as experimental drugs are known to trigger apoptosis by directly interacting with target site at or inside mitochondria.

A random observation at the laboratory bench has helped pave the way towards the development of organelle-targeted pharmaceutical nanocarriers. A fortuitous discovery in the mid-1990s involving the self-assembly of a molecule, known



to accumulate inside mitochondria, has led to the development of subcellular nanocarriers suited for the selective delivery of biological active molecules to mitochondria inside living mammalian cells. In this presentation, applications for mitochondria-specific drug and DNA delivery will be described, the current state-of-the-art of mitochondrial drug targeting technology will be reviewed and its future perspective shall be discussed.

IL- 128

Track: Regenerative Medicine

CONTROLLED RELEASED SCAFFOLDING BIOMATERIAL FOR BONE TISSUE ENGINEERING: A NOVEL THERAPEUTIC STRATEGY FOR CLEFTS PATIENTS

Adam Qingsong Ye

Office 218, D1 Building, College of Medicine and Dentistry James Cook University (Cairns), P.O. Box 6811, Cairns QLD 4870, Australia; E-mail: adamqingsong.ye@jcu.edu.au



Cleft lip and palate is the most common congenital craniofacial defects, which can affect the normal development of a child's appearance and speech. Conventional treatments for cleft palate involve bone grafting with patient's own bone or artificial bone substitute. This can cause an inflammatory response known as foreign body reaction (FBR), resulting in degradation of bone grafts. In order to prevent the FBR and the multi-surgical procedures on the cleft patient, a novel strategy to repair bony defects through stem cells and tissue engineering has been studied. The strategy consists of two stages: the first stage is the discovery of the signalling pathways for the proliferation and renewal of adult stem cells and Notch 1 signalling has been identified; the second stage is design of double layer microsphere to be used as vehicle of the two groups of growth factors: primary factors--stem cell homing and proliferation inducers and secondary factors--bone regeneration inducers. Two-step electrospray method was used to fabricated the double release core-shell microsphere system, this method avoids organic solvents and effectively preserves the biological activity of the growth factors towards stem cells, as such provides a novel therapeutic modality for bone regeneration in treating clefts patients. In trend for the future therapy of cleft palates will move from bone seeking to bone seeding.

IL-8

Track: Medical Imaging

QUANTITATIVE MULTIMODAL MULTI-PARAMETRIC IMAGING: ITS INTEGRATION AND APPLICATIONS

Yun Zhou

The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA

Positron emission tomography (PET) is a main molecular imaging modality used to *in vivo* measure biochemical and physiological activities at molecular levels in human beings and laboratory animals. Dynamic PET is now considered as a standard quantitative functional imaging technique to measure physiological and biochemical parameters such as blood flow/perfusion, metabolism, receptor density, and drug occupancy in the laboratory animals and living subjects. The validated tracer kinetic modeling methods for quantification of dynamic PET image data are adapted by single-photon emission computer assisted tomography (SPECT), dynamic contrast-enhanced MRI/CT. The talk will start with basic principles and main techniques of PET kinetic modeling including compartment model, graphical analysis, and parameter estimation algorithms. Quantitative dynamic whole-body FDG PET-CT approach and its applications in oncology are recently proposed. The developed methods and results obtained from an ongoing international multi-site

dynamic whole-body FDG PET-CT project led by Dr. Zhou and his colleagues will be highlighted. Due to inherent spatial and temporal tumor/cancer heterogeneity, enormous efforts have made in using multimodal multi-parametric imaging technique to fully characterize the tumor growth and response to treatments. It is also widely accepted to use multimodal multi-tracer imaging technique to detect and monitor neuro network and neurotransmitter interaction responding to treatments or stimulations, and to determine optimal dose of CNS drug for various mental illness. As the advances in multimodal imaging instruments such as PET-CT, PET-MRI, and PET-CT-MRI, its integration and optimization in data acquisition and quantification are the very active topics for scientists and clinicians. The challenges and opportunities in the integration and optimization of multimodal multi-parametric imaging with constraint in radiation dose and cost will be discussed in the presentation.

SESSION LECTURES

SL- 31

Track: CNS Drug Discovery and Therapy

MODULATORY EFFECT OF CELASTROL ON TH1/TH2 CYTOKINES PROFILE, TLR2 AND CD3+ T-LYMPHOCYTE EXPRESSION IN A RELAPSING-REMITTING MODEL OF MULTIPLE SCLEROSIS IN RATS

Amany A. Abdin

Department of Pharmacology, Faculty of Medicine, Tanta University, Egypt;
E-mail: amanyahr@hotmail.com



Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of brain and spinal cord that has an increasing incidence worldwide and classically presents in a relapsing-remitting form. This study was designed to induce a relapsing-remitting model of experimental autoimmune encephalomyelitis (EAE) to investigate the possible modulatory effect of celastrol on Th1/Th2 cytokines profile, immunohistochemical expression of TLR2, and CD3+T-lymphocytic count. Eighteen female Sprague Dawley rats were divided to 3 groups; where group I served as normal control, group II as EAE+vehicle, and group III as EAE treated by celastrol (1 mg/kg/day, i.p.) started at 10th day till 42nd day post-immunization. The clinical score of rats in group II (EAE+vehicle) was relapsed after the re-challenge at the 35th day post-immunization and exhibited significant positive association with serum TNF- α , NF-kB expression and nitrites levels in brain and spinal cord, and CD3+ T-lymphocytic count in brain tissues while serum IL-10 showed significant negative association. Treatment by celastrol caused amelioration of the clinical score and inhibited the relapse. It caused significant shift in cytokines profile from Th1 by decrease in TNF- α towards Th2 pattern by increase in IL-10. Moreover, celastrol treatment resulted in significant reduction in NF-kB expression, nitrites levels, as well as immunohistochemical expression of TLR2 and CD3+ T-lymphocytic count. The beneficial effect of celastrol was further confirmed histopathologically by reduction in H&E score. These results provide a promising pre-clinical evidence about use of celastrol in treatment of multiple sclerosis that must be accessed in further clinical studies.

Keywords: CD3+ T-lymphocyte, celastrol, experimental autoimmune encephalomyelitis, multiple sclerosis, Th1/Th2 cytokines, toll like receptors.

SL- 61

Track: Combinatorial Chemistry

NOVEL PLATINUM COMPLEXES [Pt(N-N)Cl₄], AS SUGGESTED ANTICANCER DRUGS

Anita Abedi, Nasser Safari, Vahid Amani and Amir-Hassan Zarnani

Chemistry Department, North Tehran Branch, Islamic Azad University, Tehran, Iran;
E-mail: anitaabedi@yahoo.com

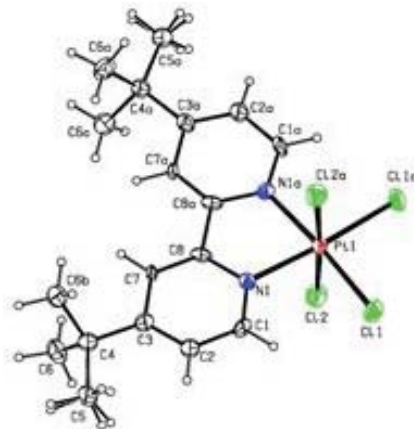


Platinum-based anticancer drugs are known as some of the most efficient chemotherapeutic agents, to treat a variety of solid tumors, especially those of the testes, ovaries, esophageal, head, neck and bladder carcinomas [1]. Although Cisplatin is used as the most effective antitumor drug, the severe toxic side effects and drug resistance returned world attention into new generation of platinum compounds, such as platinum(IV) complexes, which illustrates a different spectrum of activity with some advantages over Pt(II) drugs, including less reactivity towards biomolecules, efficiency in cisplatin-resistant tumors and kinetic inertness [2].

Herein, we report a series of platinum(IV) complexes, with the general formula [Pt(N-N)Cl₄], where N-N is 4,4'-dimethyl-2,2'-bipyridine, 4,4'-di-tert-butyl-2,2'-bipyridine, 5,5'-dimethyl-2,2'-bipyridine, 6-methyl-2,2'-bipyridine, 5-methyl-1,10-phenanthroline, 4,4'-bithiazole, 2,2'-dipyridylamine and two 3-phenyl-pyridine. All complexes were fully characterized and their crystal structure was determined. Furthermore they were used for *in vitro* cytotoxicity evaluation

against control normal cells hAECs and NIH- 3T3; and several cell lines , HT-29 PC-3, DU-145, SKOV-3, Caov-4, Caco-2, MDA-MB-231, MCF7 and T47D, with using of MTT assay.

Interestingly, One compound was illustrated the most potent drug due to its high selective cytotoxic activity. Therefore, its cytotoxicity was further tested and compared with Cisplatin. We also used spectrophotometric and viscometry methods to determine whether our desired compound exerted its cytotoxic impacts through binding to DNA.



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SL- 172

Track: High-Throughput Screening & Laboratory Automation

DEVELOPMENT OF NOVEL CLASS OF M1 ERAP AMINOPEPTIDASE INHIBITORS FOR TREATING AUTOIMMUNE DISEASE

Nitish Agrawal

The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane, QLD, Australia; E-mail: n.agrawal1@uq.edu.au

Arthritis is the major cause of disability and chronic pain globally. One in three people has a musculoskeletal condition such as back pain, with 2% of people gradually develop spondyloarthropathy such as Ankylosing spondylitis (AS) disease, inflammatory disease that affects primarily the spine and joints. About 10% of AS cases also develop either Inflammatory Bowel disease (IBD), form of intestinal inflammation or Psoriasis, a skin disease. A series of genome-wide studies have demonstrated significant associations of M1 aminopeptidases [1-3] with these diseases. Functional data from ERAP1, ERAP2 genes suggests that the mutations which protect from AS, IBD, Psoriasis result in loss of function of its encoded protein, raising the possibility that ERAP1, ERAP2 inhibition could represent a novel class of therapeutic drug treatment [4] for these diseases.

Aim: This work focuses on development of fluorogenic method for ERAP1 aminopeptidase enzyme-based targeted high-throughput screening (HTS) of inhibitors.

Methods. The HTS assay was developed and validated using leucine-7-amido-4-methylcoumarin (L-AMC) as primary fluorogenic substrate. To demonstrate its reliability for HTS assay, the coefficient of variation (CV %) and the Z' factor were chosen as validation parameters to measure the assay's suitability and robustness for small molecule inhibitors screening.

Results and Discussion: Our signal measurements gave high average Z' factor of 0.84 and <10 % average Coefficient of variation for positive and negative controls. The values suggest that our fluorogenic assay is statistically more robust method than described before, not only for measurement of ERAP1 activity but also for discovery of novel ERAP1 inhibitors.

Conclusion. We have developed and validated a fluorogenic assay for ERAP1 functional investigation, which is sensitive and accurate. This assay is not only suitable for accurately measuring ERAP1 activity, but also useful for high-throughput screening of ERAP1 antagonists. To the best of our knowledge, it is the first reported HTS system targeting ERAP1 M1 aminopeptidase, a key target for treating autoimmune diseases such as AS, Psoriasis and IBD. The method presented here will be potentially useful for targeting other ERAP-mediated cancer and viral diseases.

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SL- 17

Track: CNS Drug Discovery and Therapy

FROM BASIC RESEARCH TO DRUG DEVELOPMENT - THE STORY OF COPAXONE IN THE TREATMENT OF MULTIPLE SCLEROSIS, MECHANISM OF ACTION, AND NEW APPLICATIONS

Rina Aharoni

*Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel;
E-mail: rina.aharoni@weizmann.ac.il*

Multiple sclerosis (MS) is currently recognized as complex diseases involving autoimmune inflammation as well as axonal and neuronal pathology. Novel treatment strategies thus aim to act within the CNS not only by reducing the inflammation but also by inducing neuroprotection and repair processes.

Glatiramer acetate (GA, Copaxone®), an approved drug for the treatment of MS, is the first and so far the only therapeutic agent to have a copolymer as its active ingredient. Using the animal model of MS-experimental autoimmune encephalomyelitis (EAE), the immunomodulatory mechanism of action of GA was elucidated. Furthermore, recent studies revealed neuroprotective consequences of GA treatment in the CNS. These include elevation in neurotrophic factors expressions, and reduction in both myelin and neuroaxonal damages. Furthermore GA induces repair processes such as remyelination and neurogenesis in EAE-inflicted mice.

Based on its immunomodulatory mode of action, additional potential applications of GA are investigated, such as prevention of immune rejection, improvement of stem cells transplantation and amelioration of inflammatory bowel diseases (IBD).



SL- 265*Track: Pharmaceutical Research and Development***BOTTOM-UP APPROACHES FOR POLYMERIC VASCULATURE TISSUE CONSTRUCTS AND CELLULAR MICROENVIRONMENT****Shamsher Ali^{1,2} and Alireza Khademhosseini²**

¹Harvard T. H. Chan School of Public Health, Boston, MA-02115, USA;
E-mail: alishamsher72@yahoo.com

²Harvard-MIT Division of Health Sciences and Technology, Wyss Institute for Biologically Inspired Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA



Microscale technologies offer powerful tools to address some of the challenges in tissue engineering. The ability to generate tissue constructs is of great benefits for diagnostics and drug screening applications. In particular, the ability to generate three dimensional (3D) tissue models that can mimic the native vascularized organ tissue structure and function is important for enabling improved methods of drug research. A potentially powerful approach to engineer the microvasculature is to use cell-laden hydrogels. The ability of this approach to mimic tissues is useful in engineering complex, vascularized organs. We present a bottom-up approach to direct the assembly of cell-laden microgels to generate tissue constructs with tunable microarchitecture and complexity. This assembly process is driven by the tendency of multiphase liquid-liquid systems to minimize the surface area and the resulting surface free energy between the phases. We demonstrate that shape-controlled microgels spontaneously assemble within multiphase reactor systems into predetermined geometric configurations. Furthermore, we characterize the parameters that influence the assembly process, such as external energy input, surface tension, and microgel dimensions. This bottom-up approach for the directed assembly of cell-laden microgels provides a powerful and highly scalable approach to form biomimetic 3D tissue constructs and opens a paradigm for directing the assembly of mesoscale materials.

SL- 208*Track: Hot Topics in Drug Targets***VDACs DEFICIENT MICE: AN ORIGINAL MODEL TO TARGET SELECTIVELY METASTASIS****Keltoum Anflous-Pharayra**

Baylor College of Medicine, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; E-mail: ayapharayra@yahoo.com

Metastasis is the primary cause of death in most cancer patients because of the lack of effective targets. Extracellular matrix (ECM) is a dynamic structure that can influence cell migration and metastasis *via* integrin signaling pathways regulation. Voltage-dependent anion channels (VDACs), major proteins in the outer mitochondrial membrane, play a dynamic role in signal transduction from mitochondria to the cytosol in an isoform specific manner [1]. VDACs also serve as mitochondrial receptors for cytoskeletal elements. Because the cytoskeleton is connected, *via* integrins, to the ECM, VDACs may regulate metastasis. In accordance with this hypothesis, it has been recently reported that silencing VDAC1 expression by SiRNA inhibits cancer cell proliferation and tumor growth *in vivo*. This effect is not only due to impaired metabolic and energy homeostasis, but also to inhibition of cancer cell migration and metastasis formation [2]. In line with this finding, we previously reported altered actin content and structure in VDACs deficient fibroblasts, as seen by confocal microscope (Preliminary Data). We also observed increased attachment properties of VDACs deficient fibroblasts grown on 2D surface as compared to the control fibroblasts. These data point to alterations in the molecules/pathways that regulate cell attachment in VDACs deficient fibroblasts. Therefore, VDACs deficient fibroblasts offer a unique and original tool to explore the signaling pathways that connect mitochondria to cell

attachment/migration with potential applications for cancer therapy. It is a system that offers the possibility to study a highly “druggable” node to target specifically metastasis.

Keywords: Outer mitochondrial membrane, VDACs, metastasis.

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SL- 68

Track: Combinatorial Chemistry

COMBINATORIAL MULTIVALENT DISPLAY USING A SYNTHETIC, PNA-BASED SCAFFOLD TO CHARACTERIZE LIGAND-RECEPTOR INTERACTIONS

Daniel Appella

National Institutes of Health, National Institute of Diabetes Digestive and Kidney Diseases, Bethesda, Maryland 20892, USA; E-mail: appellad@niddk.nih.gov

The interactions between ligands and receptors at the surfaces of cells are typically multivalent. By attaching ligands to scaffolds, multivalent effects of ligand binding to receptors can be investigated. We are developing a new strategy to attach ligands in highly controlled fashion using ligand-modified peptide nucleic acids (L-PNAs). Our multivalent scaffold is highly programmable and very versatile. In addition, our approach allows multivalent combinatorial libraries to be rapidly made and screened for activity. The application of using this scaffold to investigate binding interactions between ligands and different membrane receptors will be presented.



SL- 200

Track: Drug Discovery in Preclinical Research

TARGETING HIV NUCLEOCAPSID PROTEIN VIA A SMALL MOLECULE PLATFORM AND RELATED PRECLINICAL DEVELOPMENT

Daniel Appella

National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, Maryland 20892, USA; E-mail: appellad@niddk.nih.gov

The development of a new strategy to target the nucleocapsid protein of HIV-1, NCp7, using a unique class of small organic molecules will be presented. The protein NCp7 is a relatively small protein that contains 55 amino acids and consists of two zinc-finger motifs that bind to mRNA. There are a number of regulatory roles for NCp7 in the HIV life cycle, but the principle functions are to bind viral RNA, protect it from degradation, and facilitate packaging of genomic RNA into a new viral particle. The zinc-fingers of NCp7 are highly conserved among HIV viral strains, and mutations of the zinc-finger motif renders the virus non-infectious. Therefore, disruption of NCp7 is an effective strategy to prevent HIV replication and inhibit cell-to-cell transmission of the virus. The small molecule, N-(3-amino-3-oxopropyl)-2-mercaptobenzamide, is highly effective at inactivating NCp7. Treatment of HIV-infected white blood cells with this molecule or different prodrug derivatives results in crosslinking of NCp7, mis-processing of the gag polyprotein, and production of non-infectious virus particles. These observations are consistent with a mechanism of action where the molecule promotes ejection of zinc from NCp7. Another facet of this mechanism is that the molecule reacts



intracellularly with acetyl CoA to form an acetyl thioester intermediate. This S-acyl intermediate reacts directly with NCp7 to transfer an acetyl group to the protein, leading to zinc ejection, inactivation of NCp7, and regeneration of the original mercaptobenzamide molecule which can re-enter the acetyl CoA acylation pathway. Therefore, N-(3-amino-3-oxopropyl)-2-mercaptobenzamide acts as a catalytic HIV inactivator where a single molecule has the potential to inhibit multiple NCp7 molecules. In addition to presenting the mechanism of action, recent results on antiviral activity, formulation, and translation of prodrug derivatives to an orally bioavailable drug will be presented.

SL- 213

Track: Biologics

BIOACTIVE PROPERTIES OF TURKISH ENDEMIC *ASTRAGALUS CHRYSOCHLORUS*

Şule Arı, Semra Hasançebi, Neslihan Turgut Kara and Özgür Çakır

Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey;

E-mail: sari@istanbul.edu.tr

Astragalus L. species are old and well known curative plants with immunostimulant, hepatoprotective, antiperspirant, diuretic, and tonic properties. Flavonoids, triterpenoid glycosides, cycloartane saponins and polysaccharides have been isolated from this genus. It has been reported that crude extracts of some *Astragalus* species have anti-viral and cytotoxic activity.



Almost 50% of 450 *Astragalus* species are endemic and they are used primarily for the production of the economically important gum tragacanth and also for curative purposes in Turkey. *A. chrysochlorus* Boiss. & Kotschy is one of Turkish endemic species, listed in the Turkish red list and is traditionally used for wound healing.

We have established efficient *in vitro* cell culture, regeneration and transformation systems of *A. chrysochlorus* to study and produce of its secondary metabolites. First two enzyme genes of the phenylpropanoid pathway (PAL and C4H) were cloned and characterized. Optimized cell cultures of *A. chrysochlorus* have shown high PAL and C4H activities and accumulation of phenolic compounds by biotic elicitation. In addition to antioxidant activity phagocytic, cytotoxic and apoptotic activities of root extracts of *A. chrysochlorus* were shown in neutrophilic, Vero and Hela cells, respectively. Nicotiflorin (Kaempferol 3-O-rutinoside) and a new cycloartane-type triterpenoid glycoside was isolated from *A. chrysochlorus*.

Keywords: *Astragalus chrysochlorus*, biological activities, nicotiflorin, phenylpropanoid pathway.

SL- 155

Track: Cardiovascular Drug Discovery & Therapy

SYNTHETIC HEPARAN SULFATE MIMETICS AS NOVEL ANTICOAGULANTS: *IN VITRO* EVALUATION FOR XENOTRANSPLANTATION

P.K. Benipal, H.Iwase, R.A. Al-Horani, A.Y. Mehta, R. Karuturi, L. Burdorf, X.F. Cheng, D.G. Harris, R.N. Pierson III, S.C. Robson, D.K.C. Cooper, U.R. Desai and A.M. Azimzadeh

University of Maryland Baltimore School of Medicine, Baltimore, MD, USA;
E-mail: aazimzadeh@smail.umaryland.edu



Cross-species transplantation (known as Xenotransplantation) has the potential to solve the shortage of human organs available for transplantation by creating an unlimited source of donor organs. During the past decade, significant advances were made to control immediate xenograft rejection with the generation of genetically modified pigs attenuating antibody binding and complement activation. However, xenogeneic organs are still injured and consumptive coagulopathy develops in recipients receiving a xenograft, identifying inflammation and coagulation cascade activation as important therapeutic targets. This collaboration evaluates the effect of novel heparan sulfate (HS) mimetics and chemoenzymatic heparin analog (CHA) targeting factors Xa, XIa and/or thrombin to alleviate coagulation dysregulation in xenotransplantation.

A library of synthetic HS mimetics was screened (100uM) using a xenogenic clotting assay in which coagulation of 10% human plasma is activated by porcine aortic endothelial cells (pAEC) and assessed by clot formation (score 0-3) and thrombin anti-thrombin (TAT) levels after 45 min. Platelet aggregation was quantified in human blood activated by thrombin or pAEC. Therapeutic dosing range in blood was estimated by thromboelastography and Activated Clotting Time (ACT).

Clotting score was significantly decreased by heparin (4IU/ml, score 0 ± 0.3) and Hirulog (200 ng/ml, 0.03 ± 0.3) compared to no treatment (2.6 ± 0.7 , $p < 0.001$). Eighteen of the 68 HS mimetics inhibited clotting to variable levels, 5 of which, including the CHA, were comparable to heparin and hirulog. Five HS mimetics and CHA inhibited TAT formation by $\geq 99\%$, of which three HS mimetics and CHA also inhibited thrombin- and pAEC-induced platelet aggregation. Likewise, four HS mimetics prevented blood clotting to a similar extent as heparin (1-3 IU/ml) (ACT > 300 sec., reaction time R > 200 min, maximum amplitude MA < 10 mm) at a concentration of 0.2-1mM.

Overall, several HS mimetics and CHA show considerable promise to reduce coagulation dysregulation in xenotransplantation. Future work will evaluate their effect in transgenic human thrombomodulin-induced protein C activation, pAEC-induced clotting under shear stress and *ex vivo* lung perfusion experiments.

SL- 183

Track: Inflammation & Immunology

TARGETING IMMUNE COSTIMULATION USING A NOVEL aCD28 PEGGYLATED FAB AND CD154 BLOCKADE: EFFICACY IN A PRE-CLINICAL TRANSPLANT MODEL

Agnes M. Azimzadeh, Tianshu Zhang, Braileanu Gheorghe, Wenji Sun, Anthony Kronfli, Joshua Miller, Siamak Dahi, Natalia Kubicki, Jade Whoddal, Mehmet C Uluer, Evelyn Sievert, Lars Burdorf, Xiangfei Cheng, Bernard Vanhove and Richard N. Pierson III

Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, USA; E-mail: AAZIMZADEH@smail.umaryland.edu

Background: Current therapy in clinical transplantation relies on potent non-specific immunosuppressive drugs to inhibit rejection. These therapies given chronically over the life of a transplant result in numerous undesirable effects and are relatively ineffective at preventing late graft injury (chronic rejection). Strategies to promote tolerance (permanent graft

acceptance without chronic immunosuppression) would reduce the side-effects associated with immunosuppression, and prevent late immune-mediated graft failure. Modulation of T cell co-stimulatory signals offer great hope to selectively depress/ inhibit pathogenic immune responses directed against a transplanted organ. One well-characterized alloantigen-driven pathogenic pathway is mediated by interaction of CD28 and CTLA-4 on lymphocytes with the B7 family of molecules on antigen presenting cells. Targeting this pathway by blocking B7 molecules, using one of several forms of CTLA4-Ig, interferes with potentially salutary immunomodulatory (tolerogenic) effects associated with CTLA-4/B7 interactions. We posit that selective inhibition of CD28-mediated pathogenic alloimmunity using FR104, a novel α CD28 Fab antagonist, will preserve CTLA-4-dependent immune regulation, either used alone or with blockade of a second costimulatory pathway (CD40/CD154).

Cynomolgus monkey cardiac allograft recipients received α CD28 (FR104, n=7), α CD154 (hu5C8, n=4; IDEC-131, n=7), or combination (n=5). Acute (AR) and chronic (CAV) rejection scores were quantified histologically in protocol biopsies, and in for-cause biopsies performed if graft dysfunction was detected on telemetry. Anti-donor antibodies, CD28 receptor occupancy (RO), blood regulatory T cells (Tregs) were analyzed by flow cytometry.

“Low-dose” FR104 (plasma half-life 10-14 days, n=4) monotherapy at 5 mg/kg incompletely prevented pathogenic immunity (Median graft survival time (MST) 80 days, two ongoing). “High-dose” FR104 monotherapy at 10 mg/kg more consistently prevented pathogenic immunity during treatment (to MST 167d, n=3). CD28 saturation was 100% during therapy, and became undetectable in blood and LN by d150, about 50 days after therapy was stopped. Anti-CD154 monotherapy with hu5c8 (n=4, dosed until day 84) is associated with consistent (4 of 4 grafts) protection from graft failure during treatment, unlike IDEC-131 at identical dosing (2 of 7 surviving to 90d). Low (n=3) or high (n=2) dose FR104 combined with α CD154 (hu5c8) consistently prevented pathogenic immunity during treatment. Graft survival was significantly prolonged – four weeks beyond hu5c8 alone, and 12 weeks longer than with low-dose FR104 alone – confirming additive efficacy to significantly delay pathogenic alloimmunity. CAV was decreased with FR104+hu5C8 (0.5 ± 0.3) compared to hu5C8 (1.6 ± 0.7 p<0.05) and AR tended to be lower (0.8 ± 0.4) on biopsy/explanted functional grafts vs hu5C8 (1.4 ± 0.6 , p=0.07). Blood Treg frequency increased on d7-21 (fold change vs baseline) with FR104 (1.5 ± 0.2), hu5C8 (1.4 ± 0.3), and FR104+hu5C8 (2.3 ± 0.1). Donor-reactive antibodies were generally undetectable until graft failure.

FR104 significantly prolonged cardiac allograft survival in cynomolgus monkeys. Rejection events despite CD28 saturation in peripheral blood with “low dose” treatment, and a trend toward improved outcomes using higher FR104 dosing, suggest that CD28 blockade is dose-dependent, and that receptor coverage in other compartments than peripheral blood may be a better index to guide drug dosing. We show that CD28-independent rejection can occur with low-dose FR104, and appears to be prevented by additional CD154 blockade. These data suggest that non-activating CD28 blockade is likely to be effective in clinical transplantation.

SL- 179

Track: Academic CRO/Industrial Collaborations in Drug Discovery

NEW FRAMEWORKS FOR TRANSLATIONAL RESEARCH AT THE MAYO CLINIC: CENTER FOR CLINICAL & TRANSLATIONAL SCIENCES (CCATS)

Andrew D. Badley

Nephrology Hypertension and Infectious Diseases, The Mayo Clinic, 200 First Street SW, Rochester, MN 55905 (4-1646), USA; E-mail: badley.andrew@mayo.edu



The Mayo Clinic's Center for Clinical and Translational Science (CCaTS) facilitates research activities and programs to improve patient care and community health. A key initiative of the CCaTS is to develop the expertise and capabilities to “translate” clinical findings and basic research discovery from Mayo's three sites into new drug therapies and medical devices therapies. Our approach has been to establish strategic alliances and partnerships with other non-profit institutes to access industrial scale drug discovery & development capabilities or track record of bioengineering and product commercialization and accessing state economic developmental funding sources. This approaches leverages our operational funds to provide documented rapid implementation with measurable ROIs, while avoiding fixed capital investments and sinking assets. We will discuss our working operational models and our strategic alliance and partnerships with the Prebys Center for

drug discovery & development at the Sanford/Burnham Medical Institutes and with the University of Minnesota for product development and commercialization focused in the near term on medical devices.

Dr. Andrew Badley is the Co PI of CCaTS and Medical Director of the Office of Translation to Practice at the Mayo Clinic and the Director of the HIV Immunology Lab with an active program in anti-HIV therapies and in the mechanism of TRAIL. Additionally he is a Professor of Medicine on clinical service, seeing and treating patients. He provides strategic leadership in developing, structuring, governing and adjudicating strategic alliances and partnerships to advance Mayo's translational research portfolio.

SL- 117

Track: Chemistry

BISVANILLIN AND BIS ARYL ETHER SCAFFOLDS FOR ELABORATION OF ANTIATHEROGENIC AGENTS: SYNTHESIS AND BIOLOGICAL ACTIVITIES

Michel Baltas, Florence Bedos-Belval, Corinne Bacqué, Christiane André-Barrès and Chantal Carayon

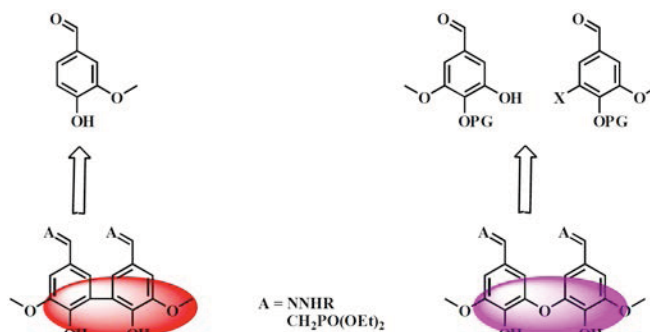
Synthèse et Physicochimie de Molécules d'Intérêt Biologique, UMR 5068, Université Paul Sabatier, 118, route de Narbonne - 31062 TOULOUSE cedex 9, France;
E-mail: baltas@chimie.ups-tlse.fr



Reactive oxygen species (ROS) generated within the vascular wall trigger LDL oxidation, lipid peroxidation and carbonyl stress that are involved in atherogenesis. The generation of drugs sharing both antioxidant cytoprotective and/or carbonyl scavenger properties may represent a new therapeutic challenge in the prevention and treatment of atherosclerosis.

Families of compounds with antioxidant activities have been developed around the phenolic phosphonates or hydrazones derived from cinnamic acid, syringaldehyde, bis-vanillin and bis aryl ether scaffolds. A rapid review of the monomers synthesized and tested, based on cinnamoyl scaffold will be followed by the presentation of two dimeric systems.

The first concerns biaryl derivatives possessing 5-5' bisvanillin scaffold and identical or different substitutions patterns on the aldehydic frames. Synthesis of symmetric and dissymmetric biaryl compounds possessing phosphonate and/or hydrazone functions will be reported.



The second concerns a family of compounds based on bis aryl ether structure. Synthesis of symmetric biaryl ethers possessing phosphonate or hydrazone functions will be reported. One compound of each dimeric family has undergone *in vivo* studies.

Their antioxidant, cytoprotective and radical trapping properties will be presented. In conjunction with theoretical and electrochemical studies on hydrazone monomers a rationale concerning activities of all compounds will be proposed.

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SL- 132

Track: Innovative Drug Discovery and Nanotechnology

NANOPORE-BASED DETECTION OF BIOMARKERS FOR CANCER DIAGNOSTICS

Jiwook Shim, Ann M. Nardulli, Farhad Kosari, George Vasmataz, David A. Ahlquist and Rashid Bashir

Department of Bioengineering, University of Illinois at Urbana, Champaign, Urbana, IL, USA

Epigenetic alterations involving DNA methylation are early and frequently observed events in carcinogenesis. Hypermethylation is reported to be associated with cancers of the prostate, colon, lung, liver, breast head and neck, and further correlated with metastatic potential in many other tumor types. Also, high-throughput methylation analysis has uncovered aberrant DNA methylation in both premalignant and malignant neoplasia. Thus, methylation analysis in DNA can play a critical role in the diagnosis of cancer, especially at an early, pre-cancerous stage. Previous studies have demonstrated the feasibility of detecting cancer by assessing methylation patterns from genomic extracts of body fluids such as plasma, serum, urine, and stool. However, the level of methylated DNA in these fluids is extremely low and the size of the DNA is quite small. As a result, most conventional methylation assays require bisulfite conversion based PCR for large sample volumes, leading DNA degradation thus compromising the detection sensitivity. Consequently, a simple, rapid, and reliable method to detect epigenetic modification of DNA, which uses small samples and eliminates bisulfite treatment and PCR amplification, has potential to revolutionize cancer diagnostics.

Single nanopores were drilled with condensed electron beam using JEOL 2010F (TEM) on the free standing SiN membranes in 10nm thickness with $50 \times 50 \mu\text{m}^2$, supported on Silicon substrate. All custom DNA fragments including methylation patterns for nanopore experiments are synthesized from Integrated DNA Technologies (Coralville, IA). The nanopore measurements were performed in 1M KCl at pH 7.6 or in 0.2 M NaCl at pH 7.6 containing 10mM Tris and 1mM EDTA. The methylated-DNA/MBP complexes were prepared and incubated for 15 minutes at room temperature ($25 \pm 2^\circ\text{C}$) immediately before nanopore experiment. Nanopore current traces were recorded using Axopatch 200B and Digidata 1440A at 10 kHz built-in low pass Bessel filter and 10 μs sampling rates. Instrumental control and data analysis was performed using Clampex 10.2 and Clampfit 10.2.

We demonstrate the capacity of nanopore sensors to detect methylation in 30, 60, and 90 bp double stranded oligos. Hypermethylated DNA (hyMethDNA) can be selectively labeled using MBP as a methylation specific label, and can be detected without the need for any further processes, such as bisulfite conversion, tagging with fluorescent agent, or sequencing. Discrimination of hyMethDNA fully bound with MBP mixed with unmethylated DNA (unMethDNA) revealed through nanopore ionic signatures of current blockage and duration. Such nanopore-based methylation assays have the potential to identify abnormally methylated DNA in clinical tests aimed at diagnosis of diseases such as cancer. This approach could be compatible with small amounts of genomic extracts and direct methylation detection without

fluorescence-labeling and bisulfite-conversion. However, nanopore-based methylation assay should improve efficiency for low sample volume obtained from body fluids.

SL- 95

Track: Cancer Targeted Drug Delivery

MULTIFACETED APPROACH TO CANCER BIOMARKER DISCOVERY IN A CLINICAL SETTING**Darrell R. Borger**

*Biomarker Laboratory, Translational Research Laboratory, Massachusetts General Hospital Cancer Center, GRJ-1008A, 70 Blossom Street, Boston, MA 02114, USA;
E-mail: DBORGER1@mgh.harvard.edu*



Molecular profiling in a clinical setting has provided an important means of cancer biomarker discovery and delivery. One benefit has been through patient stratification to targeted agent trials. Next generation sequencing technologies are further expanding clinical utility. However, a true understanding of tumor heterogeneity and how it impacts response to therapy will require a multifaceted approach that includes multiplexed genotyping approaches in parallel with slide-based techniques.

SL- 39

Track: Cardiovascular Drug Discovery and Therapy

THE MOLECULAR BASIS OF PURINERGIC SIGNAL METABOLISM REGULATING HEMOSTASIS AND ECTOPIC CALCIFICATION**Demetrios T. Braddock**

Yale University, Department of Pathology, New Haven, CT, USA; E-mail: demetrios.braddock@yale.edu

NPP4 is a type I extracellular membrane protein on brain vascular endothelium inducing platelet aggregation *via* the hydrolysis of Ap3A, while NPP1 is a type II extracellular membrane protein principally present on the surface of chondrocytes that regulates tissue mineralization. To understand the metabolism of purinergic signals resulting in the physiologic activities of the two enzymes, we determined the high-resolution crystal structure of human NPP4 and explored the molecular basis of its substrate specificity with NPP1. Both enzymes cleave Ap3A, but only NPP1 can hydrolyze ATP. Comparative structural analysis reveals a tripartite lysine claw in NPP1 that stabilizes the terminal phosphate of ATP, whereas the corresponding region of NPP4 contains features that hinder this binding orientation, thereby inhibiting ATP hydrolysis. Furthermore, we show that NPP1 is unable to induce platelet aggregation at physiologic concentrations reported in human blood, but could stimulate platelet aggregation if localized at low nM concentrations on vascular endothelium, a finding of note when considered in light of reports that NPP1 is present on the capillaries within the brain, but absent on capillaries elsewhere [1]. The combined studies expand our understanding of NPP1 and NPP4 substrate specificity and range, provide a rational mechanism by which recently reported polymorphisms in NPP1 confer stroke resistance to pediatric patients afflicted with sickle cell anemia [2], and point the way to the development of novel therapeutics for the treatment of stroke and ectopic calcification.

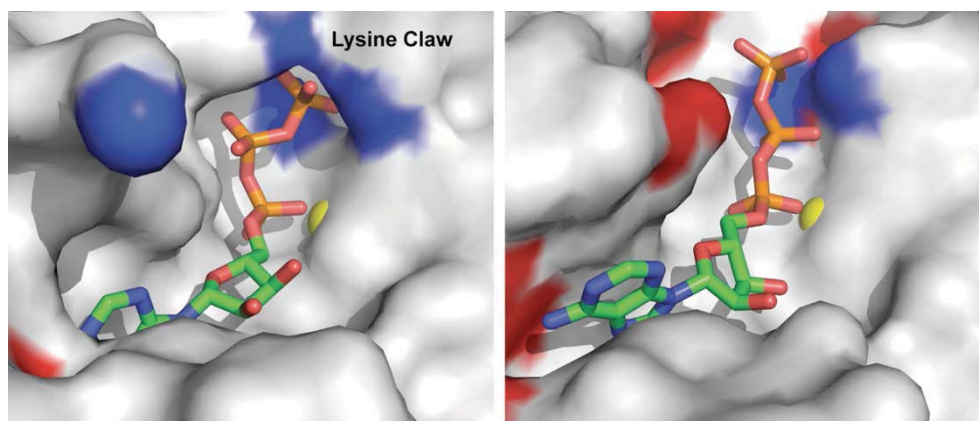


Fig. (1). Models of ATP bound in an AMP-like orientation are shown for NPP1 (left) and NPP4 (right), based on the AMP cocrystal structures for each enzyme. In NPP1, the gamma-phosphate of ATP is simultaneously stabilized by three lysine residues, two of which line the upper edge of the pocket and become ordered only when substrate is present due to electrostatics. As a result of this tri-partite lysine claw, the gamma-phosphate of bound ATP is favorably charge-stabilized and largely shielded from solvent by an induced-fit lid comprised of the long hydrophobic side chains of these two lysines along with an adjacent tyrosine ring. In contrast, NPP4 offers a significantly less favorable gamma-phosphate environment for a similarly bound ATP, with less charge-stabilization, a more open architecture with no lid mechanism, and two nearby aspartate residues for charge-repulsion. As a result, ATP is not likely to bind in this orientation to NPP4 very often.

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SL- 214

Track: Proteomics and Bioinformatics

3D ANALYSIS OF THE BINDING SITES FOR PREDICTING BINDING AFFINITIES IN DRUG DESIGN

Ali Osman Atac, Ozlem Erdas, Ferda Nur Alpaslan and Mehmet Erdem Buyukbingol

Pharmaceutical Chemistry, Ankara University, Faculty of Pharmacy, Turkey;
E-mail: erdem@pharmacy.ankara.edu.tr

Understanding the interaction between drug molecules and proteins is one of the main challenges in drug design. Several tools have been developed recently to decrease the complexity of the process. Artificial intelligence and machine learning methods have promising results in predicting the affinities. Recently, accurate estimations have been performed by extracting the electrostatic potentials from images of the drug-protein binding sites which were generated by autodocking simulator. In this study, a new algorithm has been implemented, which is a modified version of CIFAP [1], to predict binding affinities of CheckPoint1 Kinase Inhibitors.



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SL- 45

Track: Traditional Chinese Medicine

A NATURALLY-DERIVED IMMUNOSUPPRESSIVE DRUG, RAPAMYCIN, DECREASES CIRCULATING TUMOR CELLS IN PATIENTS WITH LYMPHANGIOLEIOMYOMATOSIS

Xiong Cai, Gustavo Pacheco-Rodriguez, Mary Haughey, Suowen Xu, Hai-Ping Wu, Thomas N. Darling and Joel Moss

Institute of Diagnostics in Chinese Medicine, Hunan University of Chinese Medicine, Changsha, Hunan, China; E-mail: caix12@qq.com

Background: Lymphangioleiomyomatosis (LAM), occurring sporadically (S-LAM) or in women with tuberous sclerosis complex (TSC), is a cystic lung disease which appears to result from metastatic dissemination of neoplastic LAM cells bearing inactivating mutations or having loss of heterozygosity (LOH) in the tumor suppressor genes, TSC1 or TSC2, which leads to hyper-activation of mechanistic target of rapamycin (mTOR). Rapamycin, a naturally-derived immunosuppressive agent, has been showed to slow the decline in lung function, reduce chylous effusions, and shrink the size of AMLs. The purpose of this study was to determine the effect of rapamycin on circulating tumor cells (CTCs) in patients with LAM.

Methods: Cells from blood obtained by the OncoQuick density-gradient fractionation and from urine and chylous effusions obtained by centrifugation were incubated with anti-CD45-fluorescein isothiocyanate (FITC) and anti-CD235a-R-Phycoerythrin (PE) antibodies, and anti-CD44v6-FITC and anti-CD9-R-PE antibodies, respectively. Cell samples were sorted based on antibody reactivity and sorted cells were analyzed for TSC2 LOH.

Results: LAM cells with TSC2 LOH were identified in 100% of blood specimens and 75% of urine specimens from patients before therapy; in contrast, over a mean duration of 2.2 ± 0.4 (SEM) years of rapamycin therapy, detection rates of LAM cells were significantly decreased to 25% in blood ($P < 0.001$) and 8% in urine ($P = 0.003$). Following therapy, greater loss of circulating LAM cells was seen in post-menopausal patients ($P = 0.025$).

Conclusion: Patients receiving rapamycin had a progressive loss of circulating tumor cells, which was dependent on time of treatment and menopausal status.

Keywords: Circulating tumor cells, lymphangioleiomyomatosis, loss of heterozygosity, rapamycin.

SL- 10

Track: CNS Drug Discovery and Therapy

ACAT1/SOAT1 AS A THERAPEUTIC TARGET FOR ALZHEIMER'S AND OTHER RELATED NEURODEGENERATIVE DISEASES

Ta Yuan Chang

Department of Biochemistry, Geisel School of Medicine at Dartmouth 7200 Vail Hanover, NH 03755, USA; E-mail: Ta.Yuan.Chang@dartmouth.edu

Acyl-CoA:cholesterol acyltransferase 1 (A1) is a resident ER enzyme that prevents the built up of cholesterol in membranes by converting it to cholesteryl esters. Our laboratory had previously shown that A1 gene knockout or gene knockdown decreases amyloidopathy and rescued cognitive deficits in a mouse model for Alzheimer's disease (AD). Here we show that A1 gene knockout or a specific A1 inhibitor K604 stimulates autophagosome formation and lysosomal proteolysis in cultured microglia and in primary neurons. Autophagy is needed to degrade misfolded proteins/peptides. Our results implicate that blocking A1 may provide a new way to benefit multiple neurodegenerative diseases including AD.



SL- 87*Track: Traditional Chinese Medicine***EFFECTS OF ELECTROACUPUNCTURE ON THE ULTRASTRUCTURE OF ICC AND EXPRESSION OF PROTEIN CX43 IN FD RATS****Mi Liu, Guoshan Zhang, Wei Hu, Shen Xie, Yuer Liu, Mailan Liu and Xiao-Rong Chang***Hunan University of Chinese Medicine, Changsha 410208, Hunan Province, China; E-mail: xrchang1956@163.com*

Acupuncture is a common and effective therapeutic method to treat functional dyspepsia (FD). However, its underlying mechanism remains unclear. This study was aimed to investigate the effects of electroacupuncture on the ultrastructure of interstitial cells of Cajal (ICC) and expression of the gap junction protein connexin 43 (Cx43) in FD rats. Rats were randomized into three groups, a normal group, a model group and an electroacupuncture group. Electroacupuncture was applied at *Zusanli* (ST36) in the electroacupuncture group once daily for 10 days, while electroacupuncture was not applied in the model group. In the electroacupuncture group, ultrastructure of ICC recovered normally in the gastric antrum and small intestine, and the expressions of Cx43 in these tissues were significantly increased, compared with the model group. We conclude that electroacupuncture is effective to alleviate pathological changes of ICC and the reduction of Cx43 in FD rats. These findings suggest that ICC and Cx43 are involved in electroacupuncture treatment in rats with FD to improve their gastrointestinal motility disorders.

SL- 94*Track: Traditional Chinese Medicine***INFLUENCE OF ELECTROACUPUNCTURE PRETREATMENT ON SERUM NO AND NOS AND MYOCARDIAL ULTRASTRUCTURES IN MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY****Liu Zhao¹, Wang Chao², Zhang Jiali¹, Yan Jie¹, Liu Mi¹ and Chang Xiaorong¹**¹*Master Graduate Student of 2011 in Hunan University of TCM, China;*²*The College of Acupuncture and Massage in Hunan University of TCM, Changsha 410208, China;
E-mail: 592436380@qq.com*

Objective: To observe the effect of electroacupuncture pretreatment (EP) on contents of serum NO and NOS and Myocardial Ultrastructures in Myocardial Ischemia and Reperfusion Injury.

Methods: Forty New Zealand white rabbits were randomly divided into sham-operation (sham), Myocardial Ischemia and Reperfusion Injury (MIRI), Neiguan (PC 6), Lieque (LU 7), and Hegu (LI 4) groups (n = 8/group). MIRI model was established by occlusion of the descending anterior branch of the left coronary artery for 30 min and reperfusion for 60 min. EP was applied to the bilateral PC 6, LU 7 and LI 4 for 5 days (20 min/day). The content of serum NO, NOS was determined by ELISA method. Myocardial Ultrastructures were observed by electron microscope.

Results: Compared with sham, contents of serum NO and NOS of MIRI group decreased mildly ($P < 0.05$), myocardial structures and the run of myocardial fiber were normal in sham. In comparison with the model group, serum NO and NOS of PC 6 group upregulated considerably ($P < 0.01$) and there were no obvious dissolution and necrosis of myocardium found and a small amount of mitochondria were oedema in PC 6 group. No significant differences were found between LU 7 and MIRI groups, between LI 4 and model groups and between LU 7 and LI 4 groups in the abovementioned 3 indexes ($P > 0.05$).

Conclusion: EP of PC 6 can upregulate the contents of serum NO and NOS and reduce the degree of myocardial ischemia and reperfusion injury effectively, showing a good protective effect on myocardium.

Fund Projects: This project has been supported by the National Science Foundation (81102661, 81072868).

Keywords: Electroacupuncture pretreatment, myocardial ischemia and reperfusion injury, neiguan (PC 6), NO, NOS.

SL- 22

Track: Medical Imaging

MULTIPARAMETRIC PET VALUES IN THE PREDICTION OF ALZHEIMER'S DISEASE PROGRESS

Xueqi Chen, Rongfu Wang, Rui Gao, Haoyin Cao, Dean F. Wong, Yun Zhou

Johns Hopkins University School of Medicine, Baltimore, MD, USA

Objective: To evaluate the predictive potential of glucose metabolism, amyloid deposition measured by PET, CSF biomarkers and cognitive measurements in monitoring the progression from mild cognitive impairment (MCI) to Alzheimer's disease (AD) and cognitively normal (NC) to MCI in a longitudinal study.

Methods: We investigated 82 Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects (48 MCI and 34 NC) for up to 96 months (median= 72 months). CSF β -amyloid (ABETA), phosphorylated tau (PTAU), and total tau (TAU) were collected. Mini-Mental State Exam (MMSE) scores and Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) were used as cognitive measurements. All preprocessed PET images (^{18}F -FDG, ^{18}F -AV45, ^{11}C -PiB) were spatially normalized to MNI space using MRI and SPM8. 35 regions of interest (ROI) were manually drawing in a high resolution MRI template from VBM8. Standard uptake values ratios (SUVR) to cerebellum were calculated. Predictive value of progression was achieved using ROC analysis and logistic regression models.

Results: In MCI group, the diagnostic potential could be verified by AUC values for regions in FDG and PiB scans, which is comparable to the CSF Tau (AUC=0.751). None of regions in AV45 scans showed significant difference between converters and non-converters. In NC group, parietal (AUC=0.804) in FDG scans was significantly different between converters and non-converters; while CSF ABETA AUC was 0.873. In subjects with FDG scans, the predictive model showed that 1) ADAS-cog, MMSE and SUVR of posterior cingulate; 2) ADAS-cog, SUVR of posterior precuneus and parietal were the best predictors for conversion from MCI to AD and NC to MCI, respectively. In subjects with PiB scans, this model of predictions consisted of MMSE and SUVR of medial temporal.

Conclusion: ^{18}F -AV45 and FDG scans were sensitive to detect early stage of disease progression (NC group), and ^{11}C -PiB scans act effectively in distinguishing MCI converters from non-converters. Cognitive markers combined with PET imaging appear to be best predictors for conversion.

SL- 209

Track: Academic CRO/Industrial Collaborations in Drug Discovery

DEVELOPMENT OF A CLOUD-BASED HISTOLOGY DATABASE FOR COLLABORATIVE CANCER RESEARCH

Ke Cheng

*HistoWiz, Inc., Advanced Biotechnology Park, 760 Parkside Avenue, Brooklyn, NY 11226, USA;
E-mail: ke@histowiz.com*

Introduction: HistoWiz is an innovative histology outsourcing company that processes cancer research tissue samples and digitizes the image results for researchers in academia and industry. Currently there is no centralized Whole Slide Imaging (WSI) repository for histopathology data, making collaboration difficult and bioinformatics-driven discovery impossible. HistoWiz is uniquely positioned to bring cutting-edge web technologies and practices to histopathology, enabling researchers to maximize the impact of their work. In this SBIR, HistoWiz proposes to build a cancer WSI

database on the Cloud, and in parallel to develop a histopathological image tagging web application. These tools will allow for a novel approach to synergistically share, search, and compare histopathology research data.

Significance: Recent developments of WSI technology has revealed a lack of scalable IT infrastructure for histopathological digital image data, which has impeded sharable WSI database generation, data mining, and global collaboration. Histology-based research remains insular. Our cancer WSI database will facilitate the collaboration between scientists and clinicians, and will also enable advances in discovery by allowing searching and comparison of cancer histopathology data across different laboratories around the world. In sum, HistoWiz will *fight cancer cooperatively instead of individually* by using a crowd-sourcing model.

The Product: WizBase™ is the first centralized commercial Cloud-based WSI database for cancer histopathology with an image-tagging web application to facilitate histology-driven data mining. WizBase™ can be regarded as the scientific hybrid of a microscopy product with the viewing power of Google Earth combined with the search and crowd-sourcing of Flickr.

We have developed a robust IT infrastructure allowing for viewing, tagging and searching of histology images. We have built HistoWiz Viewer, the first mobile-compatible online viewer on the Cloud using HTML-based Deep Zoom technology, which reduces loading times by limiting downloads only the region being viewed and only at the resolution displayed, allowing users to instantly view their virtual slides on any internet-connected device without the need to download the virtual slides or install software or plug-ins. It also uses a responsive design to render images perfectly for the client's screen size and resolution, whether they are on a desktop, tablet, or mobile device. Users can also generate. HistoWiz Viewer has additional collaboration and analysis tools such as generating secure URLs for the zoomable slides and sharing the link with colleagues. Furthermore, we have establish ontology parameters for the classification and tagging of digital pathology cancer images, and slide metadata are captured during online order submission. Finally, through defined field and free-text tagging of the slide metadata, users can search for slides meeting specific criteria. Searches based on similarity to a particular subject slide metadata allows ranking slide relevancy.

Long-Term Goals: In order to amass a compendium of cancer histopathologic entities, HistoWiz aspires to expand this database from mouse research material to patient samples

We plan to expand this histology knowledgebase to include all mouse and human cancers categorized using our image-tagging web application. We also plan to enhance the collaboration features by allowing scientists and pathologists around the world to register and annotate images. After registration, scientists can search the directory to find pathologists with domain expertise while pathologists can find interesting slides to debate and analyze. We will better evaluate the commercialization strategy of WizBase in offering subscription-based access to pharmaceutical and biotechnology companies.

SL- 306

Track: Drug Delivery and Targeting

CO-DELIVERY OF ETOPOSIDE AND VORINOSTAT USING POLY(ETHYLENE GLYCOL) BASED BIODEGRADABLE NANOGELS

Aruna Chhikara, Parveen Kumar, Madhu Chopra

Department of Chemistry, Dyal Singh College (University of Delhi), Lodhi Road, New Delhi, India; E-mail: arunachhikara@gmail.com

Drug delivery is the method of administering a pharmaceutical compound with the help of carrier to achieve a therapeutic effect. In recent years, we have used disulfide cross-linked poly(ethylene glycol) based biodegradable nanogels for the delivery of Gallic acid and Ellagic acid. We herein present the Co-delivery of Etoposide and Vorinostat from POEOMA [Poly(oligo(ethylene oxide) monomethyl ether methacrylate)] nanogels. The use of single chemotherapeutic drug has some limitations in anti-tumor treatment, such as development of drug resistance, high toxicity and limited regime of clinical use. The combination of two or more therapeutic drugs can overcome these limitations. The translation of specific drug ratio which is previously selected *in vitro*, to the clinical setting is complicated because each drug has different pharmacokinetics and biodistribution. Nanocarriers can deliver the drug combination *in vivo* so that



the effective drug ratio is maintained after systemic administration and is ultimately exposed to tumors. The present work describes the synthesis of nanogel by AGET ATRP reaction in an inverse miniemulsion. The drugs were loaded into the nanogels by physical encapsulation method. Drug loading of etoposide and vorinostat was determined by HPLC. Loading efficiency and loading level of etoposide was found to be 49.42, 55.73, 64.13% and 14.82, 11.14, 6.41% respectively and of vorinostat was found to be 28.26, 28.48, 24.39% and 2.82, 2.84, 2.43% respectively for 10:3:1, 10:2:1, 10:1:1 of Gel:Etop:Vor. Size distribution characterization and morphology of the nanogels was determined by DLS and TEM respectively and were found to be almost spherical and their mean diameter was in the nanometre range. Fourier Transform Infrared (FTIR) spectra of etoposide, vorinostat, blank nanogels, and etoposide-vorinostat-nanogels were recorded. *In vitro* cytotoxicity assay was performed by MTT assay on HeLa cell-lines.

Keywords: Biodegradable nanogels, co-delivery, POEOMA.

SL- 189

Track: Translational Medicine

SYNERGISTIC EFFECT OF HDAC INHIBITOR (PANOBINOSTAT) AND TOPOISOMERASE INHIBITORS ON CERVICAL CANCER CELLS

Madhu Chopra and Lubna Wasim

*Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India;
E-mails: mchopradu@gmail.com, mchopra@acbr.du.ac.in*

Histone deacetylase inhibitors (HDACi) have been extensively studied as potential candidates for treatment of various malignancies. HDACi not only induce growth inhibition, cell cycle arrest and apoptosis of cancer cells, but can also increase the sensitivity of cancer cells to chemotherapeutic drugs. LBH589 (Panobinostat) is a potent pan-deacetylase inhibitor which has demonstrated antiproliferative and cytotoxic activity against hematological malignancies and solid tumors. The aim of this study was to investigate the combined effect of HDACi, Panobinostat and topoisomerase inhibitors (topotecan and etoposide), in human cervical cancer cell line (HeLa). LBH589 showed a time and dose dependent decrease in the HeLa cell viability, slight increase in G0/G1 cell cycle phase and increased the production of ROS in HeLa cells. Next, combination of LBH589 with DNA topoisomerase inhibitors, showed a synergistic effect having CI < 1. LBH589 in combination with topoisomerase inhibitors showed significant increase in cell death shown by cell cycle, % of cells in sub-G1 phase and colony formation assay. Mechanism of cell death was found to be apoptosis as evident by DNA laddering pattern, PARP cleavage and also shown through modulation of proteins associated with apoptosis such as p21, Bax, Bcl-2 and caspases.

SL- 320

Track: Neurodegenerative Disorders

ROLE OF NEPRILYSIN IN ALZHEIMER'S DISEASE

Nipun Chopra, Kwangsik Nho, Apoorva Bharthur, Justin Long, Kumar Sambamurti , Nigel H. Greig, Andrew J Saykin and Debomoy K Lahiri

¹*Laboratory of Molecular Neurogenetics, Departments of Psychiatry and Radiology, Neuroscience Research Center, Indiana University School of Medicine, 320 West 15th Street, Indianapolis, IN-46202, USA; E-mail: chopran@iupui.edu*

²*Medical University South Carolina, Charleston, SC- 29425, USA*

³*National Institute on Aging, NIH, Baltimore, MD 21224, USA*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and characterized mainly by the presence of brain neuritic plaques and neurofibrillary tangles. Amyloid-beta (A β) peptide-loaded neuritic plaque is one of the major

neuropathological hallmarks of AD. The generation of the toxic A β fragment results from the cleavage of the large A β precursor protein (APP) by β -site APP cleaving enzyme (BACE)-1 and the γ -secretase complex. A β can be cleared by various A β -degrading enzymes, such as Neprilysin (NEP) and Insulin degrading enzyme (IDE). NEP degrades both oligomeric and monomeric A β peptide and plays a critical role in AD pathology. NEP is a single transmembrane ectozyme and differentially expressed in different tissues and cell types. Microarray expression data from the AD neuroimaging initiative (ADNI) consortium suggests that peripheral gene expressions of two of four transcript variants of NEP are increased in AD samples compared to healthy control. The expression in early mild cognitive impairment (MCI) or late MCI subjects is not significantly changed. Gene expression of APP, BACE-1 and IDE were not changed. In order to test whether NEP expression is under post-transcriptional control, we inserted the NEP 3'UTR mRNA downstream of a dual reporter vector and functionally characterized its expression in neuronal and non-neuronal cells. Notably, NEP-3'-UTR construct increased luciferase activity compared to control vector in human glioblastoma (U373), differentiated and undifferentiated neuroblastoma (SK-N-SH) and HeLa cells. Therefore, it is possible that there are positive regulators of NEP expression. MicroRNA (miRNA) species are known regulators of post-transcriptional expression that target the 3'UTR. Currently, there are no known miRNA regulators of NEP. By using bioinformatics algorithms, we identified putative miRNA targetsitesinthe NEP 3'UTR. One of these miRNAs – miR-181 – does not change expression of NEP protein, using western blotting. Finally, we showed that NEP is expressed in primary human fetal brain cultures as well as differentially expressed in rat organs, which could be further investigated to understand the neurobiological role of NEP in AD.

This work is supported by grants from Alzheimer's Association and NIH to Dr. D.K. Lahiri.

SL- 88

Track: Cancer Targeted Drug Delivery

OVERVIEW OF THE CURRENT AND FUTURE ROLE OF IMMUNE CHECKPOINT BLOCKADE IN ONCOLOGY

James Cleary

Dana-Farber Cancer Institute, USA; E-mail: jcleary@partners.org

Recently, immunotherapies directed against the immune checkpoint molecules CTLA-4 and PD-1 have shown great promise in the treatment of metastatic melanoma. Ipilimumab, a fully humanized antibody directed against CTLA-4, was FDA approved for the treatment of melanoma after two randomized phase 3 clinical trials showed a significant survival benefit. Monoclonal antibodies directed against PD-1, pembrolizumab and nivolumab, have also shown promising results in metastatic melanoma and are now FDA approved. Importantly, in melanoma the majority of responses to both CTLA-4 and PD-1 directed-therapies appear to be durable and last for greater than one year. The success of immune checkpoint blockade in melanoma has sparked intense clinical investigation of these drugs in numerous malignancies. These clinical trials have shown that PD-1 directed therapy has activity in non-small cell lung cancer, renal carcinoma, triple negative breast cancer, gastric cancer, bladder cancer and Hodgkin's lymphoma. This talk will review the progress made with immune checkpoint inhibitors and will explore potential future horizons for these drugs.



Keywords: Immunotherapy, PD-1, cancer.

SL- 115

Track: Cancer Targeted Drug Delivery

SYNTHESIS OF COVALENT BIOCHEMOTHERAPEUTICS WITH PROPERTIES OF SELECTIVELY “TARGETED” ANTI-NEOPLASTIC CYTOTOXICITY

Cody P. Coyne

Department of Basic Sciences, College of Veterinary Medicine at Wise Center, Mississippi State University, USA; E-mail: coyne@cvm.msstate.edu



Covalent biochemotherapeutics designed to possess properties of selective “targeted” delivery have significant merit due to their potential capability to promote progressive intracellular chemotherapeutic accumulation; and impose minimal chemotherapeutic exposure to healthy tissues and organ systems. Chemotherapeutics most extensively employed in organic chemistry reactions schemes for the synthesis of covalent immunochemotherapeutics or other biochemotherapeutics with similar characteristics have been the anthracyclines, but have also included bleomycin, calicheamicin, chlorambucil, dexamethasone, fludarabine (Coyne 2015), gemcitabine (Coyne 2011, 2012), methotrexate, maytansinoids, monomethyl auristatin E, paclitaxel and vinca alkaloids. A limited spectrum of organic chemistry reaction schemes have been utilized to covalently bond chemotherapeutics and other pharmaceuticals to synthetic macromolecules, immunoglobulin (*e.g.* IgG, Fab₂, F(ab')₂) receptor ligands (*e.g.* EGF), glycoproteins, polysaccharides and lectins that can facilitate selective “targeted” delivery within populations of neoplastic cell types. Immunoglobulin affords the attribute of potentially being able to promote anti-neoplastic activity through both an anti-trophic effect (*e.g.* anti-EGFR) and induction of multiple immune responses (*e.g.* antibody dependent cell cytotoxicity; complement cytotoxicity; opsonization). Although highly integrated and prolonged methods have been described for synthesizing and purifying covalent biochemotherapeutics, alternative methods and techniques have been developed that are efficient, rapid-in-duration, generate homogenous end-products with minimal side reactions, and have modest requirements for advanced instrumentation.

SL- 156

Track: Innovative Drug Discovery and Nanotechnology

NANOPARTICLE BASED SENSORS WITH BIOLOGICAL APPLICATIONS

Georgeta Crivat

University of Maryland, College Park, MD 20742, USA; E-mail: Georgeta.Crivat@iavalley.edu

In this presentation, I will report the development of new composite materials and their use in fluorescence FRET-based assays for biological applications. Quantum dots (QDs) are excellent probes in exploiting sensitive FRET interactions due to their unique spectroscopic properties such as broad absorption and narrow emission, photo-stability *etc.* Gold nanoparticles (AuNPs) are also versatile nanoparticles with well-defined spectroscopic characteristics and broadly used in nanomedicine due to their biocompatibility, easy attachment chemistry and lack of toxicity. However, the applicability of NPs in general is often limited by their poor solubility properties. In this talk, I will describe alternative methods to increase the water solubility of QDs and AuNPs for improved applicability as bioreporters; to bypass problems of NP solubility and aggregation, multiple covalent and hydrophobic interactions between QD nanocrystals, AuNPs and polystyrene microsphere (PS) can be exploited. Further, I will discuss the use of these nanocomposite materials for detection of various biochemical phenomena. In particular, I will discuss the development of intracellular fluorescent probes with ability to detect activity of intracellular proteins of importance in study of chronic illness such as diabetes.

SL- 303

Track: Innovative Drug Discovery and Nanotechnology

IMMUNOLOGICAL PROPERTIES OF ENGINEERED NANOMATERIALS AND CHALLENGES WITH THEIR PRECLINICAL CHARACTERIZATION

Marina A. Dobrovolskaia

Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, MD 21702, USA; E-mail: marina@mail.nih.gov

Nanomedicine is rapidly growing field. Many nanoformulations have been granted regulatory approval for use in medical applications, while many more are in various phases of preclinical and clinical development. This presentation will review data regarding nanoparticle-mediated immunological and hematological compatibility, which I will use to highlight common challenges in preclinical characterization of nanoparticles. Case studies demonstrating how manipulation of nanoparticle physicochemical properties can influence their interaction with components of the immune system will be discussed. The presentation I will focus on endotoxin detection and quantification, pyrogenicity testing nanoparticle depyrogenation, sterility and sterilization, nanoparticle interference with traditional immunological tests, and applicability of traditional *in vivo* immune function tests to engineered nanomaterials. I will also present case studies demonstrating the significance of comprehensive physicochemical characterization of engineered nanomaterials prior to their toxicological evaluation as well as correlation between toxicological *in vitro* assays and relevant *in vivo* tests.

Funded by NCI Contract No. HHSN261200800001E.

SL- 302

Track: Drug Delivery and Targeting

THREE DIMENSIONAL CELL CULTURE MODELS OF THE SOLID TUMOR MICROENVIRONMENT FOR THE TESTING OF PHARMACEUTICAL NANOCARRIERS

Gerard G.M. D'Souza

*Department of Pharmaceutical Sciences, MCPHS University, School of Pharmacy-Boston, USA;
E-mail: gerard.dsouza@mcphe.edu*

Pharmaceutical nanocarriers designed for drug delivery to solid tumors are most commonly tested first in cell culture models and then in small animal (usually murine) xenograft tumor models. Simple monolayer culture models are relatively cheap and readily suited to high throughput testing methodology while animal models are better representation of the physiological and structural barriers that need to be overcome by drug delivery strategies. It is unfortunately common for approaches that show significantly improved effects in cell culture testing to show little to no improvement in effect when tested *in vivo*. *In vitro* testing in a model that more closely resembles the physiological environment of a solid tumor but is still amenable to high throughput testing methodology could serve as a more powerful primary selection tool for drug delivery strategies to improve tumor therapy. Our efforts to develop such an *in vitro* model have focused on the use of tumor spheroids. Using low cost materials and simple protocols amenable to high throughput testing methodologies, we are able to reliably generate large numbers of tumor spheroids. Using these simple three dimensional cultures we are able to test parameters typically measured in monolayer cell culture like cell uptake, subcellular disposition and antiproliferative effect as well as parameters that are usually only measured *in vivo* like tumor penetration and accumulation. Most importantly we found that formulations that showed significantly improved effects in the *in vitro* model were most likely to show significantly improved effects in an *in vivo* model. Efforts are currently underway to add additional levels of physiologically relevant structure to the spheroid model in an attempt to create a true *in vitro* solid tumor.

SL- 240

Track: Anti-Infectives

COMPARISON OF *IN VIVO* AND *IN VITRO* TESTS OF RESISTANCE IN *PLASMODIUM FALCIPARUM* POSITIVE PATIENTS TREATED WITH ARTEMISININ BASED COMBINATION THERAPY IN NORTHWESTERN NIGERIA

Mukhtar Dauda

Department of Microbiology, Bayero University Kano, Nigeria;
E-mail: mukhtardauda03@gmail.com



Comparative studies on *in vitro* and *in vivo* drug response of *Plasmodium falciparum* isolates were carried out among malaria positive resident of Kano and Katsina states of Nigeria, to determine the effectiveness of *in vitro* antimalarial drug sensitivity test as a substitute for therapeutic response analysis. The *in vitro* test was carried out using schizont growth inhibition assay which was evaluated by comparing its results with the therapeutic response determined by 28 days follow-up of the *Plasmodium falciparum* positive patient treated with different Artemisinin combination therapy (Artemether-lumefantrine, Dihydroartemisinin - piperaquine, Artesunate Amodiaquine). Out of 652 patients enrolled, 227 (34.8%) completed the 28 days follow-up, and 120 isolates yielded an interpretable *in vitro* test. A total of 100 of 120 patients (83.3%) had adequate clinical and parasitological response. The geometric mean 50% inhibitory concentrations (IC_{50}) of the isolates obtained from these patients were 2.03nM, 3.65nM and 4.68nM for Artemether-lumefantrine (AL), Dihydroartemisinin-piperaquine (DHP) and Artesunate amodiaquine respectively (AA) (*in vitro* and *in vivo* sensitive). Treatment failure was observed in 20 (16.7%) of 120 patients whose IC_{50} values were 2.11 nM, 3.77 nM and 4.80 nM for AL, DHP and AA respectively. Moreover all the isolates of the patients responding with treatment failure yielded a discordant result (*i.e. in vivo* resistance and *in vitro* sensitive). Thus, the result of this study indicates poor agreement between the *in vitro* and *in vivo* test (Kappa value = 0) with regards to treatment failure. The *in vitro* assay cannot therefore be used as a substitute for *in vivo* therapeutic test for drug efficacy.

SL- 319

Track: Innovative Drug Discovery and Nanotechnology

RELEASE-ACTIVE ANTIBODIES ARE INNOVATIVE ANTIBODIES-BASED DRUGS: NEW WAY TO QUALITY ASSESSMENT

Elena S. Don, Vyacheslav V. Grechenko, Suzanna E. Pokhil, Alena A. Borshcheva, Darya V. Barykina, Marina V. Nikiforova, Evgeny A. Gorbunov and Sergey A. Tarasov

OOO NPF Materia Medica Holding, 47-1, Trifonovskaya Str., Moscow, 129272, Russia;
E-mail: gavrilovaes@materiamedica.ru



Such therapeutic agents as antibodies which have utility across a range of medical areas have undergone different modifications to overcome the difficulties accompanied by their application. Use of release-active forms of antibodies (RA Abs) launched by Prof. Epstein is one of these promising approaches. Technology of RA Abs preparation consists in multiple circles of consecutive decrease in antibodies initial concentration to desired dilution and physical treatment on each technological step. Such technology attributes to these antibodies-derived products the unique ability to modify the interaction between the antibodies and corresponding endogenous antigen instead of its neutralization. Although several such medicines are already presented on pharmaceutical market for years and their efficacy and safety were shown in variety of preclinical and clinical studies, the more comprehensive development of their quality control techniques is still challenging but essential task. To date there is no possibility to detect RA Abs directly because of their ultra-high diluted nature. So for the assessment of RA Abs activity ELISA approach allowing exhibit the influence on pharmacological target's conformational

characteristics was chosen and developed. The finding is going to be an important step in further consolidation of these unique medicines combining high efficacy and safety on pharmaceutical market.

SL- 138

Track: Innovative Drug Discovery and Nanotechnology

BIOMOLECULE ANALYSIS AND DNA SEQUENCING WITH SOLID-STATE NANOPORES

Marija Drndic

Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA 19104, USA

Nanopores have been proposed as an excellent candidate technology for inexpensive and high speed DNA sequencing. Among the most advantageous attributes of such devices is the ability to localize a sensing element to a small region, comparable in size to DNA's individual nucleotide base, while simultaneously allowing DNA to thread through that sensitive region such that the order of nucleotides can be read sequentially. In doing so, nanopores may sequence very long DNA read-lengths, thus avoiding computationally intensive sequence-assembly. The main hurdle to this approach is achieving a sensing method that is discerning enough to distinguish individual nucleotides at a high enough read-rate so that each base can be sensed in time. Here, I will discuss two separate methods to achieve this goal.

First, I will discuss a graphene nanoribbon- nanopore (GNR-NP) type device. Here, I will examine the potential advantages of a GNR-NP transistor for sequencing, including superior signal-to-noise, superior bandwidth sampling, and ease of parallelization. Details of manufacturing technique, including transmission electron beam ablation lithography, damage limiting writing, and integration into microfluidic chambers, will be established.

Second, I will discuss progress toward direct sequencing *via* the ionic current fluctuations induced by individual nucleotides as they pass through a nanopore. Here, I will address three issues: bringing the thickness of the nanopore to the size of the distance between nucleotides; creating high-bandwidth, integrated amplifiers that can read signals as fast as the DNA transverses the nanopore; and reducing the capacitive noise of the nanopore membranes so that nucleotide discrimination can be maintained at high-bandwidth.

SL- 178

Track: High-Throughput Screening & Laboratory Automation

IDENTIFYING NOVEL DIFFERENTIATION AGENTS FOR NEUROBLASTOMA THERAPY

Liqin Du, Zhenze Zhao, Xiuye Ma, Yidong Chen, Alexander Pertsemidis, Susan Mooberry, Matthew Hart, Tzu-Hung Hsiao and Gregory Lin

Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, USA

Differentiation therapy plays an important role in treating neuroblastoma, one of the most aggressive pediatric cancers. However, only a limited number of differentiation agents is available to treat neuroblastoma, and resistance to current available differentiation agents is common. This highlights the urgent needs to develop new and more effective differentiation agents. My research goal is to discover novel differentiation agents from various anti-cancer drug sources using a functional high-content screening approach that was recently developed in our group. This approach is based on quantification of the morphological differentiation marker of neuroblastoma cell - neurite outgrowth. By exploiting this screening approach, we have identified a group of novel differentiation-inducing microRNA mimics, synthetic oligonucleotides used to raise intracellular levels of microRNAs. These microRNA mimics induce the differentiation of neuroblastoma cells that are both sensitive and resistant to current differentiation agents. Given the demonstrated promise of microRNA-based therapeutic agents in cancer treatment, the identified differentiation-inducing microRNA mimics hold the promise to treat neuroblastomas that are resistant to current differentiation agents. Besides the work on

microRNAs, we are currently expanding the discovery of novel differentiation agents to other drug sources, including natural products and synthetic small molecule compounds.

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Financial assistance by the Collaborative Research Grant (AP/3/2012/CG/29) of the University of Colombo, Sri Lanka.

SL- 212

Track: Drug Discovery in Preclinical Research

DISCOVERY, OPTIMIZATION, AND CHARACTERIZATION OF NOVEL CHLORCYCLIZINE DERIVATIVES FOR THE TREATMENT OF HEPATITIS C VIRUS INFECTION

Shanshan He, Jingbo Xiao, Andrés E. Dulcey, Adam Rolt, Zongyi Hu, Xin Hu, Amy Q. Wang, Xin Xu, Noel Southall, Marc Ferrer, Wei Zheng, T. Jake Liang and Juan J. Marugan

National Institutes of Health, National Center for Translational Sciences 9800 Medical Center Drive Rockville, MD 20892-3370, USA; E-mail: dulceyan@mail.nih.gov

Recently we reported the discovery of the antiviral activity of chlorcyclizine, an over-the-counter antihistamine piperazine drug, having both *in vitro* and *in vivo* activity against the hepatitis C virus. Here we describe our structure-activity relationship (SAR) efforts that resulted in the optimization of novel chlorcyclizine derivatives as anti-HCV agents. Several compounds exhibited EC₅₀ values below 10 nM against HCV infection and cytotoxicity selective indices above 2000, as well as showing improved *in vivo* pharmacokinetic properties. The optimized molecules can serve as lead preclinical candidates for the treatment of hepatitis C and as probes to study HCV pathogenesis and host-virus interaction.



SL- 232

Track: Hot Topics in Medicinal Chemistry

TARGETING ARGININE METHYLTRANSFERASES: IDENTIFICATION AND OPTIMIZATION OF FIRST-IN-CLASS PRMT5 INHIBITOR EPZ015666 (GSK3235025)

Kenneth W. Duncan

Molecular Discovery, Epizyme, 400 Technology Square, Cambridge, MA 02139, USA

We describe the identification and characterization of EPZ015666 (GSK3235025), a potent, selective and orally available inhibitor of Protein Arginine Methyltransferase-5 (PRMT5). This novel inhibitor is SAM-uncompetitive, peptide-competitive and interacts with the PRMT5:MEP50 complex through a unique inhibition mode not previously observed for any SAM-dependent enzyme. Treatment with EPZ015666 on Mantle Cell Lymphoma (MCL) cells leads to inhibition of PRMT5 mediated methylation and cell killing. Robust activity was also observed upon oral dosing of EPZ015666 in multiple MCL xenografts.

SL- 136*Track: Drug Discovery in Preclinical Research***DISCOVERY OF FIRST-IN-CLASS DRUGS: TARGET- AND SYSTEMS-BASED APPROACHES****Joerg Eder***Novartis Pharma AG, Fabrikstrasse-16.4.72, 4056 BASEL, Switzerland;**E-mail : joerg.eder@novartis.com*

An analysis of the origins of first-in-class drugs approved from 1999 to 2014 shows that the majority were discovered through target-based approaches. Of those drugs discovered in the absence of a target hypothesis (about 30%) most were found by a chemocentric approach and only few come from true phenotypic screening. The implications for drug discovery strategies will be discussed, including viewing phenotypic screening as a novel discipline rather than as a neoclassical approach.

**Keywords:** First-in-class drugs.**SL- 55***Track: Diabetes and Obesity Drug Discovery & Therapy***BETAINE SUPPLEMENTATION IMPROVES SYSTEMIC METABOLISM AND GLYCEMIA IN HFD-FED MICE****Asma Ejaz, Elizabeth Li, Allison Goldfine, Carles Lerin, Miranda Liang, Robert Gerszten and Mary-Elizabeth Patti***Research, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115, USA;**E-mail: ejaz.asma@gmail.com*

Betaine (N,N,N-trimethylglycine) is a methylated derivative of glycine and an important dietary nutrient with several key physiological functions, including serving as an organic osmolyte, as a methyl group donor, and as a precursor for glutathione biosynthesis. Our prior metabolomics analysis of human plasma demonstrated that betaine levels are correlated with insulin sensitivity ($r=0.55$, $p<0.05$). To determine whether betaine might improve systemic metabolism, we supplemented betaine (1% w/v in water) prior to initiation of high-fat diet (45% HFD). Betaine was well tolerated, and after 20 weeks, plasma levels were 29% higher in supplemented mice. Betaine supplementation increased energy expenditure despite no effects on food or water consumption, body weight, or body composition. Betaine supplementation reduced hepatic triglyceride and cholesterol by 49 and 39%, respectively. We stretched our studies to a larger cohort of mice across a spectrum of fat intake (10%, 45%, and 60%) in order to assess the impact of betaine on glucose metabolism. Body weight was significantly reduced in betaine-treated mice fed either the 10 or 60% fat diet, despite no change in food or water intake. Fasting glucose tended to be reduced with betaine supplementation in mice fed a 60% HFD (208 vs 162 mg/dL, $p=0.08$). Strikingly, there was a marked effect of betaine to reduce plasma insulin levels in mice fed a 60% HFD, with a 56% reduction in insulin in the fasting state, 56% in the refed state, and 28% reduction after an IP glucose load ($p<0.01$ for all).



Together, our data demonstrate that betaine supplementation improves systemic energy metabolism. We hypothesize that improvements in hepatic lipid metabolism may contribute mechanistically to improvements in fasting glucose, fasting insulin, and refed insulin.

Research supported by 3ARP, Ajinomoto Inc.

SL-325

Track: Academic CRO/Industrial Collaborations in Drug Discovery

NANO-LAYERED MATERIALS FOR DRUG DELIVERY AND TRAPPING HARMFUL ANIONS IN TUMOR

Amin A. El-Meligi

National Research Centre, Centre of Excellence for Advanced Science, Nanomaterials and Renewable Energy Group, Physical Chemistry Department, Cairo, Egypt; E-mail: ael_meligi10@hotmail.com



A tumor or neoplasm is an abnormal growth of tissue in which cells proliferate more rapidly than in the tissue from which they came. The need for the development of novel cancer therapies and drug delivery strategies that provide specific targeting of tumor cells has been continually at the forefront of medical science. The nanostructure of the layered materials of MPS_3 , where M represents a divalent transition metal cation ($\text{M} = \text{Mn, Fe, etc.}$) are made up of two dimensional arrays of $\text{P}_2\text{S}_6^{2-}$ bridging legends coordinated to the M^{2+} forming layered compound. The interlayer gap is about 6.4 Å. These materials could intercalate different compounds in the interlayer gap, such as pyridine, pyridinium, 2-methyl pyridine (2-picoline) and 3-methyl pyridine (3-picoline) and N,N'-dimethyl-4,4'-bipyridinium (paraquat). The paraquat is used as a quaternary ammonium 'herbicide. It could be encapsulated by the layered materials, MnPS_3 and FePS_3 to control its poisonous effect. The results indicated that the intercalated compounds could be retained in the interlayer gap and could be released under certain conditions. As known, chemotherapy is one of treatments of tumor but the major pitfall in chemotherapy is the failure to accumulate and retain a therapeutically relevant drug concentration at the tumor site. Therefore, The drug concentration can be accumulated in the interlayer gap of the MPS_3 by intercalation process. For the first time, these materials will be tested as a carrier for the anticancer drugs into tumor and as a trapper for harmful and carcinogenic compounds in the tumor, such as nitrous oxide (NO), singlet oxygen ($^1\text{O}_2$) superoxide radical anion ($\text{O}_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). These compounds and anions have been identified as the most prevalent reactive intermediates responsible for photochemical cell damage

SL- 219

Track: Proteomics and Bioinformatics

KRUPPEL LIKE FACTORS AND HEAT SHOCK PROTEIN 27: NEW POTENTIAL MARKERS OF LARYNX AND LUNG CARCINOMAS

Tannous Rita, Fadous Khalifé Marie-Claire, Aftimos Georges, Paris Francois and Hadchity Elie

Chemistry and Biochemistry, Faculty of Sciences - Lebanese University, Lebanon;
E-mail: eliehadchity@hotmail.com



Larynx and lung carcinomas are among the prevalent human cancer worldwide and no molecular markers are presently used for predicting prognosis in those tumors. Kruppel-like factors (KLF) are transcription factor with diverse physiological functions, and possess opposing roles in different human cancers. Human Heat shock protein 27 (Hsp27) is an antiapoptotic protein characterized for its tumorigenic and metastatic properties, and now referenced as a major therapeutic target in many types of cancers. Some members of the kLF family and Hsp27 are overexpressed in many cancers and their overexpression is often related to the progression and tumor aggressiveness, metastatic potential and short patient survival.

Immunohistochemical analysis of KLF members and Hsp27 was performed on a tumor sample of larynx carcinoma tissues of different tumor stages and lung carcinoma (small and non-small cell carcinomas). Our results demonstrate a significative difference of expression profile between tumor tissue and surrounding normal epithelium. A significative gradual difference for each protein was also observed between tumor stages (I/II/III/IV) for larynx tumors and tumor

types for lung carcinoma. Positive and negative correlations were observed between the expression of each protein studied and various clinicopathologic parameters.

These results suggest that some KLF members and Hsp27 may be considered as a possible biomarker of larynx and lung cancers. Thus, identifying novel biomarkers will be very useful to improve clinical diagnosis and patient stratification.

Keywords: Hsp27, KLF, larynx carcinoma, lung carcinoma.

SL- 197

Track: Academic CRO/Industrial Collaborations in Drug Discovery

EXPLORING NEW IDEAS FOR THE ACADEMIC INDUSTRY INTERFACE

Stephen Freedman

Gladstone Center for Translational Research (GCTR), Gladstone Institute, USA;

E-mail: stephenbarryfreedman@yahoo.com

Translational activities within the Gladstone Institutes occur within the Gladstone Center for Translational Research (GCTR), and encompasses several distinct activities, including strategic management of a Translational Asset List, strategic development of key initiatives, and corporate and venture business development activities. Our internal drug development expertise has over 60 years of drug development experience in Pharma and Biotech with access to a broad external team of consultants that allows us to identify creative and flexible solutions to scientific partnerships and create win-win situations for all parties.

Our strategic focus:

- Scientifically identify those programs within Gladstone that offer translatable assets, including platform technologies, that can lead to potential therapeutic development. Develop tools around these translatable assets.
 - Engage key external organizations in the pharma, biotech, venture, Foundation and funding agency sectors that offer significant translational opportunities.
 - Create innovative and creative business models that allow immediate translational funding, technical support and long term potential growth for Gladstone, leading to funding diversification, and novel opportunities for Gladstone scientists.
-

SL- 139

Track: In-Silico Drug Design and In-Silico Screening

INTEGRATION OF *IN SILICO* PREDICTION IN DRUG DISCOVERY PROCESS

Hua Gao

Department of Computational Chemistry, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA, USA;

E-mail: hgao@amgen.com

This presentation will provide a review of the status of *in silico* ADMET modeling and its importance in drug design and will discuss the application of *in silico* predictions in various stages of drug discovery process. Representative examples of *in silico* predictions of various *in vitro* and *in vivo* ADMET endpoints will be provided.

Keywords: ADME, Computational, *In silico*, QSAR.

SL- 29

Track: Medical Imaging

ASSOCIATIONS BETWEEN THE ALPHA-SYNUCLEIN SPLICED VARIANTS AND MONOAMINERGIC DEGENERATION IN PARKINSON'S DISEASE

Rui Gao, Guangjian Zhang, Xueqi Chen, Aimin Yang, Ming Zhang and Yun Zhou

Department of Nuclear Medicine, 1st Affiliated Hospital of Xian Jiaotong University, Xian, Shaanxi, China

Background and Objectives: To evaluate the associations between α -synuclein gene (SNCA) splice variants and striatal vesicular monoamine transporter 2 (VMAT2) densities in Parkinson's disease (PD).

Methods: Five SNCA transcript isoforms counts assayed in a high-precision NanoString gene expression assay, VMAT2 activities measured using ^{18}F -AV133 PET, and structural MRI for 22 PD patients and 4 controls were collected from the Parkinson's Progression Markers Initiative study (PPMI) project. SPM8 was used for PET alignment, MRI to PET coregistration, and spatial normalization. Regions of interest (ROI) were defined on the standard MNI space, and occipital cortex was used as reference tissue to calculate standardized uptake value (SUVr) images.

Results: Whereas the transcripts with a long 3'UTR region were not significantly correlated with striatal ^{18}F -AV133 uptake, the short SNCA-007 transcript comprises exons 1-4 and SNCA-E4E6 (transcripts that skip exon 5) was related with VMAT2 densities of the left putamen ($r=0.76$ and 0.83 , $P<0.05$). The SNCA-E3E4 (transcripts with boundaries of exon 3 and exon 4) was related with SUVrs of left caudate and ventral striatum ($r=0.72$ and 0.84 , $P<0.05$).

Conclusion: This study provided evidence of correlations between striatal monoaminergic degenerations and SNCA isoforms in PD, suggesting a role for the splice variants in PD development.

SL- 185

Track: Academic CRO/Industrial Collaborations in Drug Discovery

FRIENDS WITH BENEFITS: WHY PARTNERING WITH AN ACADEMIC CRO MAKES ULTIMATE SENSE

Ralph J. Garippa

RNAi & Gene Editing Core Facility, Sloan-Kettering Institute and MSKCC, Memorial Sloan-Kettering Cancer Center, USA; E-mail: garippar@mskcc.org

The Core laboratories of RNAi, Gene Editing, and Small Molecule Compound Screening flourish in a setting that is nestled between a high-performing academic research institute and a world-class patient care hospital. Such an environment creates unique CRO opportunities not available in more singularly-focused surroundings. On-site collaborative efforts with the Tri-Institutional Therapeutic Drug Initiative (Tri-I TDI), the Early Therapeutics Center (ETC), synthetic chemists, and cancer biologists yield a diversity of choices in portfolio development. At the Memorial Sloan-Kettering Cancer Center, our core laboratories offer cutting-edge technologies and services, seamlessly transitioning from target discovery into tool molecule characterization and early stage drug candidate development, towards the evolution of unique partnering and exit opportunities for the central parties.



SL- 161

Track: In-Silico Drug Design and in-Silico Screening

NOVEL DRUG CANDIDATES FOR TYPE-2 DIABETES MELLITUS (T2DM): FROM BENCH TO BEDSIDE**Mustafa Guzel**

*Istanbul Medipol University, International School of Medicine, Department of Medical Pharmacology (Chair),
E-mail: mguzel@medipol.edu.tr*

Diabetes is a life-long disease of high blood sugar caused by either too little insulin, resistance to insulin, or both. Non-Insulin Dependent Diabetes Mellitus (NIDDM) or so called Type 2 diabetes can often exist without symptoms in its early stages. That's the reason there are 40% of people with Type 2 diabetes are unaware of their disease. If the disease ignored it can cause severe complications including loss of vision, neuropathic complication, kidney dysfunction, stroke or heart problems. Due to its increasing health cost, it is one of the main therapeutic drug targets to improve people's life style. Since the beginning of 2000's Glucokinase activators were one of the hot and attractive targets by drug companies for Type-2 Diabetes Mellitus (T2DM) due to its role in insulin secretion in pancreatic β -cells as well as well several published biological data to support its effect in decreasing blood glucose levels. During the presentation the role of GK in insulin secretion and glucose homeostasis then small drug discovery and development efforts as well as their therapeutic application will be highlighted.

SL- 33

Track: Cancer Targeted Drug Delivery

VASCULAR TARGETING SYSTEM - A PROPRIETARY GENE THERAPY PLATFORM TECHNOLOGY**Dror Harats, Yael Cohen, Erez Feige and Eyal Breitbart**

VBL Therapeutics, 6 Jonathan Netanyahu St, Or Yehuda, 7312700, Israel; E-mail: dror@vblrx.com

VBL has developed an innovative platform technology designated the Vascular Targeting System (VTS™ which enables targeted and specific administration of gene therapy to angiogenic sites, to either promote or destroy newly formed blood vessels. The platform comprises three components; adenovirus 5, VBL's proprietary genetically modified promoter, PPE-1-3X and a transgene of choice.



VBL's lead VTS drug VB-111, is a gene-based biologic for the treatment of solid cancers. We investigated a super enhancer element of an exclusive role in the PPE-1 promoter's specificity. We mapped key elements in this regulatory sequence, and generated a modified promoter as next-generation anti- angiogenic biological agent. Given as an IV infusion, VB-111 targets endothelial cells in the tumor vasculature, acting as a "biological knife". Moreover, VB-111 based viral vector therapy may induce an immunotherapeutic anti-cancer effect. VB-111 was found to be safe and well tolerated in over 170 patients, and was administrated in combination with bevacizumab (BEV) in phase I/II in patients with recurrent Glioblastoma (rGBM). Interim analysis of VB-111 in combination with BEV demonstrated a statistically significant improvement in overall survival, with median overall survival of 414 days, compared to 235 days in patients on VB-111 followed by BEV alone.

SL- 280

Track: Nutraceutical Drug Discovery & Therapy

EVALUATION OF SECONDARY METABOLITES, MICRO-MACRO ELEMENTS AND ANTIMICROBIAL ACTIVITY OF CINNAMOMUM TAMALA LEAVES

Waseem Hassan and Syeda Nida Zainab Kazmi

Institute of Chemical Sciences, University of Peshawar, Peshawar -25120, Khyber Pakhtunkhwa, Pakistan;

E-mail: waseem_anw@yahoo.com

This study was devised to explore the crude methanolic extract of the *Cinnamomum tamala* leaves for its phytochemical constituents and pharmacological activity with special emphasis on antimicrobial potential. We also focused on metallic screening to examine the essential and non essential heavy metal contents. Phytochemical screening of the methanolic extract revealed the presence of tannins, alkaloids, flavonoids and terpenoids only. Antimicrobial potential of the crude extract and its fractions *i.e.* aqueous, n-hexane, dichloromethane, and isobutanol were tested against various human pathogenic bacteria including six gram-negative bacterial strains *i.e.* *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Erwinia carotovora*, *Agrobacterium tumefaciens*; three gram-positive bacterial strains *i.e.* *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus atropheus* and a fungal strain named *Candida albicans*, using agar disc diffusion method. All the fractions as well as crude extract showed significant inhibitory potential against *Bacillus atropheus* amongst which aqueous fraction showed the highest zone of inhibition measuring 38mm. The extracts evaluated showed variable degree of inhibition zones against all tested microbes except dichloromethane, aqueous fraction and crude extract which were found completely inactive against *Salmonella typhi*. Heavy metal analysis was carried out to determine Cd, Mn, Pb, Cr, Sb, Na, K, Ca, Cu and Fe using atomic absorption spectrometry (AAS). The results confirmed the presence of Ca as major metallic content yielding 634.25 mg/kg. The concentration of other heavy metals particularly Fe and Na were higher as compared to other metals while Cd was not detected. The antimicrobial activity shows that this plant has therapeutic potential and should be tested for qualitative and quantitative chemical characterisation.

Keywords: Antibacterial activity, heavy metals, phytochemical.

SL- 46

Track: Cardiovascular Drug Discovery & Therapy

INTACT STABLE ISOTOPE LABELED PLASMA PROTEINS FOR MULTIPLEXED LC-MS/MS QUANTIFICATION

Adam M. Hawkrige

Department of Pharmaceutics, Department of Pharmacotherapy & Outcomes Science, Virginia Commonwealth University School of Pharmacy, Smith Building, Room 644, 410 North 12th Street, P.O. Box 980533, Richmond, VA 23298-0533, USA; E-mail: amhawkrige@vcu.edu



The plasma proteome and glycome hold great promise for disease diagnosis, personalized medicine, and the development of novel therapeutic strategies. Mass spectrometry (LC-MS/MS) continues to play a critical role in the detailed molecular-level characterization of complex glycosylated plasma proteins yet quantitation remains a significant challenge. Several MS-based approaches have been developed for the purpose of globally quantifying glycoproteins including label-free (*e.g.*, XIC, spectral counting) and chemical derivatization (*e.g.*, iTRAQ, TMT, INLIGHT). A significant limitation to these strategies is the inability to assess pre-analytical variability which limits quantitative precision and accuracy. We have developed a novel multiplexed quantitative strategy that expands the use of the SILAC metabolic labeling toward the classic plasma proteins (*e.g.*, apolipoproteins, coagulation factors, proteases, inhibitors, and acute-phase) that we hypothesize will greatly reduce pre-analytical variability associated with plasma collection, fractionation, and preparation. The new approach capitalizes on using a SILAC-labeled liver cancer cell line (HepG2) to produce intact

plasma proteins (*i.e.*, secretome) which we have spiked into human plasma as a global internal standard followed by quantitative characterization by high-performance LC-MS/MS. The performance of these standards in plasma and their potential applications in cardiovascular biomarker and drug discovery will be presented.

SL- 109

Track: Cancer Targeted Drug Delivery

INTRATUMORAL MODULATION THERAPY FOR GLIOBLASTOMA

Hu Xu, Mitchell Cooper, Cleusa de Oliveira, Eugene Wong, Susanne Schmid and Matthew O. Hebb

*Department of Clinical Neurological Sciences, Western University, Canada;
E-mail: mhebb@uwo.ca*



There is a critical need for effective strategies to treat glioblastoma (GBM), the most deadly and common primary brain cancer in adults. GBM cells are vulnerable to perturbations in the electrochemical environment but the only form of electrotherapy currently available is an extracranial system that requires the patient to wear a network of electrodes perpetually on the shaved scalp to produce electric fields. We have been pioneering a new treatment, called intratumoral modulation therapy (IMT), which uses small electrodes implanted directly at the site of the tumor. An IMT device that delivers current within tumor-affected brain regions may exploit the known electrosensitivity of GBM cells while providing anatomically targeted, sustained and titratable therapy with low maintenance, concealed hardware for improved self-perception and quality of life. The outcomes of preclinical studies suggest that IMT

may provide direct anti-cancer benefits, enable development of personalized gene therapies and enhance the effect of existing treatments to improve outcomes for patients with GBM and other systemic and nervous system tumors.

SL- 195

Track: Translational Medicine

A FULL-360-TRANSLATIONAL PHARMACOLOGY CONCEPT – FROM PRE-CLINICAL EXPERIMENTS TO CLINICAL DATA AND BACK

Thomas Herz

4SC AG, Am Klopferspitz 19a, 82152 Planegg-Martinsried, Germany; E-mail: Thomas.Herz@4SC.com

An enormous amount of data is generated during a drug development process, from pre-clinical experiments to study data from clinical trials, all serving the purpose of finding the mode-of-action, and/or the accompanying biomarker for the targeted drug. Usually, the data are multidimensional, originate from different sources, and spanning various disciplines, thus making it difficult to gather and analyze them efficiently. 4SC's translational data integration concept comprises an in-house blend of a database to store pre-clinical as well as clinical data, the statistical software R to analyze the data and a software suite for functional pathway analysis. Altogether, this fuels the validation of mode-of-action hypotheses and planning of pre-clinical experiments. This fully integrated forward- and back-translational approach informs drug development in a far-ranging manner, including the design of clinical study protocols as well as providing a system biology explanation for clinical study results.

A case study will be presented, where pre-clinical experiments with 4SC's leading compound resminostat led to a set of genes serving as pharmacodynamic marker to be used in clinical studies. Subsequent analyses of gene expression data

from clinical trials with resminostat in different indications unexpectedly revealed also prognostic or predictive potential for a specific gene.

SL- 154

Track: Drug Discovery in Preclinical Research

RATIONAL IDENTIFICATION OF ENOXACIN AS A NOVEL V-ATPASE (AND/OR MICRORNA?)-DIRECTED OSTEOCLAST INHIBITOR

Lexie Shannon Holliday

Orthodontics, University of Florida College of Dentistry, USA; E-mail: sholliday@dental.ufl.edu

Binding between vacuolar H⁺-ATPase (V-ATPase) and microfilaments is mediated by an actin binding site in the B-subunit of V-ATPase (a large multisubunit enzyme). A conserved "profilin-like" domain in the B-subunit mediates this actin-binding activity. Subtle mutations in the "profilin-like domain" were shown to eliminate actin binding activity without interfering with the ability of the altered protein to associate with other V-ATPase subunits and form enzymatically functional proton pumps. Elimination of actin binding activity perturbed the capacity of osteoclasts to resorb bone *in vitro*. Based on these data, we reasoned that small molecules that bound the B-subunit and competitively-inhibited its interaction with actin might be useful as antiresorptive agents for the treatment of bone disease. We used a virtual screen to identify small molecules that were predicted to bind the "profilin-like" domain of the B-subunit. Of the candidates we identified, several blocked the interaction between recombinant B-subunit and microfilaments in binding assays. Enoxacin, a second-generation fluoroquinolone antibiotic, was one of the small molecules we identified. Almost simultaneously, enoxacin was identified by other groups in screens for molecules that increased microRNA and RNA interference activities in cells. We found that enoxacin inhibited osteoclast differentiation and bone resorption *in vitro*. A bone targeted bisphosphonate ester of enoxacin, called bis-enoxacin, also blocked binding between B-subunit and microfilaments, and like enoxacin stimulates microRNA activity. We found that bis-enoxacin blocked osteoclast-dependent orthodontic tooth movement and alveolar bone loss triggered by periodontal infection in rat model system. Another group reported enoxacin blocked titanium particle-induced bone resorption also in a rat model. Groups focusing of microRNA-stimulating activities found the enoxacin showed promise for colorectal cancer and Ewing's sarcoma, and very recently reports have shown enoxacin crosses the blood brain barrier and reduces learned helplessness in rats, and has been approved as a candidate drug for the treatment of amyotrophic lateral sclerosis. Finally, enoxacin was reported to be an inhibitor of Candida infections. In summary, recent reports have revealed that an old, widely-used antibiotic, enoxacin, has activities that are of potential therapeutic use. However, identification of next generation therapeutic molecules based on enoxacin will require a clear understanding of the underlying activity or activities by which enoxacin exerts its therapeutic effects toward a particular pathology. (This work was supported by NIDCR R21 DE-19862-01A1).



Keywords: Bone disease, cancer, amyotrophic lateral sclerosis, enoxacin, V-ATPase, microRNA.

SL- 194

Track: Drug Discovery in Preclinical Research

DEVELOPMENT OF A β -ARRESTIN BIASED D2R ANTAGONIST FOR THE TREATMENT OF SCHIZOPHRENIA

Edward Holson

Department of Medicinal Chemistry, Stanley Center for Psychiatric Research, The Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, MA 02142, USA;
E-mail: edholson@broadinstitute.org



All FDA approved antipsychotic drugs are dopamine D2 receptor (D2R) antagonists or partial agonists and the gene encoding D2R has achieved genome-wide significance for association with schizophrenia in GWAS studies. At D2R, antipsychotic drugs antagonize both the G_i coupled cAMP and the β -arrestin pathways. The β -arrestin pathway has been implicated in genetic mouse models (β arr2 KO mice) and in studies of the D2R-DISC1 protein complex as a key driver of the antipsychotic effects observed. The lack of receptor and pathway specificity of these antipsychotic drugs is believed to contribute to many of the observed side-effects, which are very often so severe as to be treatment limiting. In order to mitigate these effects we developed highly specific D2R antagonists with activity biased towards the β -arrestin pathway and demonstrate that these biased molecules retain efficacy and reduce extrapyramidal side effects.

SL- 118

Track: Regenerative Medicine

POTENTIAL THERAPEUTIC EFFECT OF HEMATOPOIETIC STEM CELLS ON CEREBELLAR ATAXIA IN ADULT FEMALE RATS SUBJECTED TO CEREBELLAR DAMAGE BY MONOSODIUM GLUTAMATE

Horeya E. Korayem¹, Mohamed Abdo², Magda M. Naim¹, Soha E. Yones³ and Somaya Hosny¹

¹Histology department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt;
E-mail: somaya@hotmail.com

²Physiology department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

³Clinical Pathology department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt



Background: Research evidence has indicated that monosodium glutamate (MSG) consumption produces certain deleterious effects on the cerebellum of adult rats at high doses which can consequently affect cerebellar function. The use of stem cells in nervous system disorders is a growing field, which in numerous reports has shown promising results in the restoration of neurological function.

Aim: To compare the effect of injection of human umbilical cord blood CD34+ stem cells *versus* CD34- fraction in a rat model of cerebellar damage induced by monosodium glutamate.

Methods: Forty adult female albino rats were equally randomized into 4 groups: group I served as control, group II received MSG, group III received MSG followed by CD34+ stem cell separated from umbilical cord blood of human male fetuses, group IV received MSG followed by the CD34- fraction. At the end of the experiment, all rats were subjected to assessment of motor function, histological and immunohistochemical techniques as well as a polymerase chain reaction analysis of male-specific Sry gene.

Results: Group II showed a significant decrease in the mean number of Purkinje cells and cells of the molecular layer. Nissl's granules and length of dendrites of Purkinje cells were markedly decreased. Marked increase of GFAP immunoexpression in astrocytes was also detected. Group III stem cells showed improvement in motor function after 4

weeks of treatment. The CD34- group (IV) showed more increase in the number of cells in the molecular, granular and Purkinje cell layers as well as an increase in Nissl's granules and Purkinje cell dendrite length compared to CD34+ stem cell group (III). There was also a significant decrease in optical density of GFAP immunoreexpression of the CD 34- group compared to both MSG and CD34+ groups. The Sry gene was not detected in either of the CD34+ and CD34- groups implying that the improvement happened without homing of stem cells in the cerebellum.

Conclusion: Both CD34 -ve and CD34+ve stem cells improved cerebellar structure and function against damage induced by monosodium glutamate, however CD34- stem cells showed more improvement than CD34+ stem cells.

SL- 246

Track: Neurodegenerative Disorders

A BRAIN-PERMEABLE ANTIOXIDANT TARGETING A β AMYLOID PATHOLOGY

Xudong Huang, Ying Shi, Mohammed Abid, Charles R. Vanderburg, Nigel H. Greig, Debomoy K. Lahiri, Alpaslan Dedeoglu and Jack T. Rogers

¹*Neurochemistry Laboratory, Departments of Psychiatry and Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA;
E-mail: huang.xudong@mgh.harvard.edu*



The clinical benefits of current antioxidants for redox stress-mediated amyloid diseases such as Alzheimer's disease (AD) and Down's syndrome (DS) are limited due to their poor target specificity. Our long-term objective is thus to design target-specific antioxidants and better understand their pharmacological mechanisms for AD, DS, and other human diseases, based on an emerging "pharmacophore conjugation" concept for drug development. The specific hypothesis for this study is that amyloid-targeting antioxidants assume targeted interdictions against cerebral A β amyloid pathology.

We have rationally designed, synthesized, and characterized a novel bifunctional antioxidant- XH2A with chemically conjugated amyloid binding (benzothiazole) and antioxidant (lipoate) moieties. *In silico*, cellular, and animal studies on XH2A indicated that: (i) XH2A molecule has only one NH group (<5 H-bond donors), two N and one O atoms (<10 H-bond acceptors), MW=414 (<500), agreeing well with the general Lipinski's Rule of Five; (ii) XH2A interacts with A β 1-40 peptide computationally and has an affinity binding constant of $K_D=4.43 \mu\text{M}$ toward monomeric A β 1-40 peptide as determined by the SPR (surface plasmon resonance) technique; (iii) XH2A has no neurotoxicity at low micromolar concentrations, and it interdicted amyloid precursor protein (APP) translation (*via* its 5'UTR, $\text{IC}_{50}=50 \text{ nM}$) in human SH-SY5Y cells and attenuated cerebral A β amyloid pathology in PS1/APP doubly transgenic mice without inducing apparent animal toxicity and behavior disturbances; (iv) BBB penetration studies indicated that XH2A brain concentration can reach 16 nM (*via* oral route, a single gavage dose of 25 mg/kg) in mouse brain in just 2 hours. This is approximately 32% of its $\text{IC}_{50}=50 \text{ nM}$, and its brain to plasma concentration ratio is around 1:2.

We conclude that XH2A interdicts A β amyloidosis both *in vitro* and *in vivo*. Thus, XH2A could be a potential brain-permeable antioxidant targeting A β amyloidosis- one of the salient pathological features for AD and DS.

Supported by grants from NIA/NIH, Ellison Medical Foundation, Alzheimer's Association.

SL- 59

Track: Traditional Chinese Medicine

TOXICOLOGICAL FINDING IN BEAGLE DOGS AFTER A SINGLE OR 4 WEEKS ORAL GAVAGE OF TRIPTOLIDE

Jinqian Liang, Yuwen Qiu, Xinlu Fu and Zhiying Huang

#132, Waihuandonglu, Guangzhou Higher Education Mega Center, Guangzhou 510006, P.R. China;

E-mail: hzhiying@mail.sysu.edu.cn

Introduction: Triptolide, the major active component of *Tripterygium wilfordii* Hook f. (TWHF), has a wide range of pharmacological activities. However, the toxicities of triptolide limit its clinical application. In this study, we intended to provide a preliminary evaluation of the toxicity of TP after a single or 4 weeks repeat-dose in beagle dogs.

Method: All of the animal experimental procedures in this study were performed in accordance with the protocol approved by the Animal Ethical and Welfare Committee of Sun Yat-sen University and conducted in compliance with the GLP regulation issued by CFDA.

Single-Dose Study of TP: Three dogs were individually dosed with oral gavage on day 1 with 0.253, 0.380, 0.570mg/kg TP. Body weights were recorded prior to TP administration and on days 3, 8, and 15 after TP administration. ECG was recorded prior to TP administration, approximately 5 hours post-dosed, and on days 2, 8, and 15. Hematology and serum chemistry samples were collected as with ECG examination. Necropsy was conducted on day 15.

Repeat-Dose Studies of TP: 24 dogs were randomly assigned into 4 groups (3 animals/sex/group): 1 vehicle control and 3 treated groups (20, 40, 80 $\mu\text{g/kg/day}$). Dogs were administered with oral gavage of TP or vehicle daily for 4 weeks and some animals recovered for 2 weeks. Food consumption was recorded daily. Body weight, physical, ECG, ophthalmologic examination, body temperature, hematology and serum chemistry, urine analysis and pathological examination were performed.

Result: Toxicological Finding of Single-Dose TP in Dogs: A single dose with oral gavage of TP was tolerated in dogs at doses up to 253 $\mu\text{g/kg}$, but at 380 and 570 $\mu\text{g/kg}$ TP mortality occurred 2 days post-dosed. Abnormal clinical signs included vomiting, pale gums and watery stool. White blood cell count was markedly increased 2 days after 253 $\mu\text{g/kg}$ TP. Changes in serum chemistry including increases in serum alanine aminotransferase and aspartate aminotransferase. Histopathologic findings were associated with these elevations in the liver enzymes and consisted of plasminic loose and severe edema of liver cell. ECG examination 5 hours post-dosed exhibited accelerated heart rate for all dose groups. Histopathologic examination related to testis at 253 $\mu\text{g/kg}$ dose group included degeneration, necrosis and vacuolation of spermatogenic cells and disarranged cellular layer of seminiferous tubules. At 380 and 570 $\mu\text{g/kg}$ TP doses where clinically significant toxicity and mortality occurred, multiple sites of gastrointestinal hemorrhage and congestion were found.

Toxicological Finding of Repeat-Dose TP in Dogs: For repeat-dose study (4 weeks) in dogs, daily clinical observation revealed decreased food consumption and loose stools after treatment, occurrence rate and severity of which were in a dose-dependent manner. Decreased body weights were observed at 40 and 80 $\mu\text{g/kg}$ TP doses compared with control. The majority of adverse findings were concordant with single-dose, including gastrointestinal, bone marrow/hematologic, lymphoid organ (spleen and thymus), urinary system and testis toxicities. Findings pertinent to the gastrointestinal reaction included decreased appetite, gastrointestinal hemorrhage and congestion. Decreased reticulocytes; increased white blood cell count, monocytes, fibrinogen; prolonged prothrombin time and activated partial thromboplastin time were observed for hematologic examination. Lymphoid organ toxicities were composed of decreased thymus weights and thymus index, and microscopically adipocyte hyperplasia and diminished parenchyma thickness of thymus, and atrophy of spleen and thymus. Similar to the single-dose study, repeat-dose study of TP showed decreased spermatogenic cells, which demonstrated minimal to severe testicular atrophy. Testes weights at 80 $\mu\text{g/kg}$ dose group were significantly lower than control group after a 2-week recovery period. All findings (except for testicular toxicity) were partially or completely reversed after a two-week recovery period, which demonstrated that most of the toxic effects of TP are reversible after a sufficient interval of time.

Conclusion: The single-dose study showed that minimal lethal dose (MLD) of TP in dogs was higher than 253 $\mu\text{g/kg}$ and lower than 380 $\mu\text{g/kg}$. The primary toxicological finding in single-dose study included effects on gastrointestinal tract, liver, and testis. The results of triptolide on beagle dogs following 4 weeks treatment showed that the toxicities in dogs included gastrointestinal tract, hepatic, hematopoietic/immune system and genitourinary system damage. At 20 $\mu\text{g/kg}$ dose group, no

apparent toxic effect was observed except for spermatogenesis suppression. These studies suggest that the toxicity of TP is closely related to its dose. The toxicity profile in dogs provides references for further clinical use.

SL- 230

Track: Anti-Infectives

NEW STRATEGIES FOR FIGHTING MALARIA

Hidayat Hussain

Department of Chemistry, Universität Paderborn, Warburger Straße 100, 33098 Paderborn, Germany;
E-mail: Hidayat110@gmail.com



More than 40% of the population in the world lives in areas infected by malaria. Malaria is a disease that affects hundreds of millions of people, causes more than a million deaths per year, and is in continual increase. *Plasmodium falciparum*, the causative agent of the malignant form of malaria, has high adaptability by mutation and is resistant to various types of antimalarial drugs, a serious setback to antimalarial programs, since it precludes the use of cheap and previously effective drugs like chloroquine. New families of active compounds are needed as well as poly chemotherapy associating molecules with independent mechanism of action, in order to decrease the risk of resistance. In a continuation of our biology-oriented synthesis, a series of new phenazines (Fig. 1; eq-1), quinoline-5,8-dione (Fig. 1; eq-2), and hydroxynaphthoquinone derivatives (Fig. 1; eq-2) [1-3] were synthesized. The synthesized compounds were evaluated for *in vitro* antimalarial activity against *Plasmodium falciparum*, especially against chloroquine-resistant strains. Some of the tested compounds were most prominent in growth inhibition and *in vivo* protection against cerebral malaria was observed. These may prove to be useful forerunners in the design of novel anti-plasmodial pharmacologic agents. Details of this work will be presented.

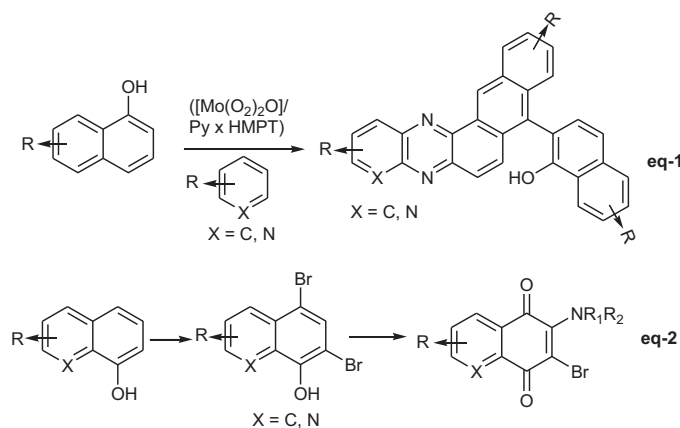


Fig. (1).

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SL- 90

Track: CNS Drug Discovery and Therapy

LOGICAL REFINEMENT OF THERAPEUTIC TARGETS FOR GLIOBLASTOMA BASED ON MULTIDISCIPLINARY PROTEOMIC APPROACHES**Yasuo Iwade**

Department of Neurological Surgery, Chiba University, Graduate School of Medicine, Japan;
E-mail: iwadey@faculty.chiba-u.jp



Many cancers contain heterogeneous cell populations which makes the therapeutic efficacies of various anticancer agents ambiguous. Especially, since glioblastoma (GBM) is a highly heterogeneous tumor with multiple signaling pathways activated, most of molecular targeting drugs failed in clinical trials. To identify appropriate therapeutic targets, we have used multidisciplinary proteomic approaches including the two-dimensional gel electrophoresis (2DE) combined with matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), the serological identification of antigens by recombinant cDNA expression cloning (SEREX), and an antibody microarray. These approaches revealed the pluripotent cell-related proteins and the mesenchymal cell markers to be unregulated in addition to many signaling molecules. Tumor cells usually acquire pluripotency under hypoxia or metabolic aberrations to adapt and survive the microenvironment. We have examined global DNA methylation status of clinical GBM samples in relation to the expression of hypoxia inducible factor-1 α (HIF-1 α), and found that hypoxia is closely related with global DNA hypomethylation which is an epigenetic background of embryonal stem cells. Hypoxia is known to recruit mesenchymal cells, and also activates various signaling pathways to induce epithelial-mesenchymal transition that generates invasive and apoptosis-resistant phenotypes. Thus, most of the aberrantly-expressed proteins found by multidisciplinary proteomics are the result of hypoxic microenvironments. Hypoxia is an appropriate therapeutic target for GBM to make a less invasive phenotype and to make temozolomide and radiotherapy more efficacious, which may be achieved by the vascular normalization effects of bevacizumab. Overexpressed proteins cannot simply be efficacious therapeutic targets because of the redundant protein network, rather the common background for multiple aberrant proteins would be an appropriate therapeutic target.

SL- 177

Track: Inflammation & Immunology

IN VIVO AND IN VITRO IMMUNOSTIMULATORY ACTIVITY OF SRI LANKAN WILD TYPE CARICA PAPAYA L. MATURE LEAF CONCENTRATE IN RATS**Chanika D. Jayasinghe and Preethi Udagama**

Department of Zoology, Faculty of Science, University of Colombo, P.O.Box 1490, Cumaratunga Munidasa Mawatha, Colombo 3, Srilanka; E-mails: chanika81@yahoo.com

Herbal immunomodulators are opening new vistas in modern medicine by positively modulating the immune system against enduring diseases. In the present study, immunomodulatory efficacy of mature leaf concentrate of *C. papaya* (MLCC) of Sri Lankan wild type variety was investigated to fill an existing knowledge gap.

Wistar rats (N=6/ group) were gavaged with 3 doses (low: 0.18, mid: 0.36 and high: 0.72 ml/100g body weight) of the MLCC, and distilled water as the control once daily for 3 consecutive days. Selected nonfunctional and functional immunological parameters were determined post treatment using standard methodology. Moreover, serum antioxidant capacity, liver and kidney functional parameters were determined to assess acute toxicity of the MLCC.

The MLCC elicited significant immunostimulatory activity in both nonfunctional and functional immunological assays. Rat platelet counts were significantly ($P<0.05$) increased by all three doses at post treatment. The mid and high doses of MLCC significantly ($P<0.05$) enhanced total WBC, lymphocyte, monocyte and bone marrow cell counts. Conversely, no

significant ($P > 0.05$) difference was observed for splenocyte counts between the test and control groups. The highest MLCC dose tested, significantly ($P < 0.05$) reduced pro inflammatory cytokines, IL-6 and TNF α levels of rats. The functional assay based on neutral red dye uptake was significantly ($P < 0.05$) increased by all three doses compared to the control. The phagocytic activity of rat peritoneal macrophages was significantly enhanced by the MLCC at 62.5, 125 and 250 $\mu\text{g/ml}$ concentrations in the NBT reduction assay. However, serum antioxidant capacity, liver (ALT and AST) and kidney (Urea and Creatinine) functional parameters remained unaltered with the highest dose of the MLCC compared to the control.

The present study established that the mature leaf concentrate (MLCC) of *Carica papaya* Sri Lankan wild type variant when administered orally is safe, non toxic, and possesses *in vivo* and *in vitro* immunostimulatory properties in the rat model.

ACKNOWLEDGEMENTS

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Keywords: Immunomodulation, Immunostimulation, *Carica papaya*, mature leaf concentrate.

SL- 328

Track: Drug Delivery and Targeting

LIGHT TRIGGERED NANOPARTICLE THERANOSTICS FOR CANCER

Amit Joshi

Department of Radiology and Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, USA;
E-mail: ajoshi@mcw.edu



Nanoparticles enable spatial control over drug delivery by exploiting their size dependent tumor accumulation and clearance routes. Light triggering to create either photo-thermal ablation, hyperthermia for synergistic radiation and chemotherapy response, or delivery of cytotoxic drugs can help overcome the therapy barriers posed by tumor heterogeneity and drug resistance often encountered with conventional treatment. Light triggering also provides additional temporal control for therapy delivery. The extended propagation of light in near-infrared wavelengths can be exploited both for imaging and therapy in tissue up to a depth of multiple centimeters, without ionization hazards. Gold based nanoparticles with size and geometry dependent tunable surface plasmon resonance have emerged as attractive photo-thermal therapy delivery agents and have demonstrated near 100% efficacy in tumor eradication. In this contribution, we will describe the applications and structural modification of silica-gold nanostructures for combined optical and MR image guided photo thermal therapy in mouse models, and discuss the extensions of light triggering technology to drug release from other nanoparticle variants.

SL- 160*Track: Bioactive Lipids***NANOENCAOSULATION OF COENZYME Q10 BY A NEW EMULSIFICATION PROCESS USED IN COSMETIC****Messaouda Kaci, Elmira Arab-Tehrany, Emilie Velot, Gabriel Dostert, Guillaume Gillet, Isabelle Lavisse and Stéphane Desobry***Laboratoire d'Ingénierie des Biomolécules (LIBio), Université de lorraine, 2 avenue de la Forêt de Haye - TSA 40602, 54518 - Vandœuvre-lès-Nancy Cedex, France; E-mail: messaouda.kaci@univ-lorraine.fr*

Emulsifier-free-emulsions developed with new emulsification process by high frequency ultrasound (HFU) was used for CoQ10 vectorization and compared with CoQ10 emulsions containing natural emulsifier (rapeseed lecithin) manufactured with same process and with low frequency ultrasounds coupled with high pressure emulsification (LFU+HPH). Physico-chemical characterization by measurement of average particles size used nanoparticle tracking analysis, zêta potential, surface tension and rheological properties. Bioavailability and cytotoxicity was tested by LDH, MTT and Hoechst with human cells. LFU+HPH emulsions containing lecithin show lower particle size due to cavitation phenomena, unlike HFU emulsions, where droplets are larger due to absence of violent cavitation. Negative high surface charge was obtained due to lecithin for emulsion with emulsifier and electrostatic stabilization by hydroxide accumulation at oil/water interface for emulsifier-free-emulsions. Low surface tension was registered for lecithin emulsions unlike emulsifier-free-emulsions. For bioavailability and cytotoxicity, LDH test show nontoxicity of all systems. MTT and Hoechst analyses show a good cell proliferation, higher for COQ10 and especially for emulsifier-free-system. HFU process gives stable emulsion without emulsifier useful for the vectorization and release of hydrophobic active ingredient. These new vectors can be used in various fields like as cosmetic, tissue engineering and pharmaceutic.

SL- 111*Track: Chemistry***ENZYMATIC SYNTHESIS OF PROTEIN-MIMIC POLYSACCHARIDES AS CANDIDATE FOR NEW BIOMEDICAL MATERIALS****Jun-ichi Kadokawa***Department of Chemistry, Biotechnology, and Chemical Engineering, Kagoshima University, Korimoto, Kagoshima, Japan; E-mail: kadokawa@eng.kagoshima-u.ac.jp*

Polysaccharides are widely distributed in nature and act as important *in vivo* functions as vital materials. They can be expected as biomedical materials comparable to proteins, but researches on their practical applications have been still devoted even in recent years. Therefore, the researches on polysaccharides are still devoted to provide functional materials. Because the specific property of proteins, which polysaccharides do not exhibit, is amphoteric, in this study, amphoteric polysaccharides are enzymatically synthesized. Enzymatic method is a very powerful tool for the precision synthesis of new polysaccharides with well-defined structures.

For example, phosphorylase, which catalyzes glycosylation using α -D-glucose 1-phosphate (Glc-1-P) to produce α -(1 \rightarrow 4)-glucans, is one of practically used enzyme for the polysaccharide synthesis [1]. Phosphorylase also recognizes α -D-glucosamine and α -D-glucuronic acid 1-phosphates (GlcN-1-P, GlcA-1-P) as analogue substrates of Glc-1-P in glycosylations to give non-natural acidic and basic oligosaccharides [2-4]. The enzymatic synthesis of amphoteric α -glucans having both glucuronic acid and glucosamine residues was thus conducted by the tandem glycosylations [5]. The products showed specific inherent isoelectric points (pIs) and formed large aggregates in water at pH = pI, whereas disassembled at pH shifted from pI. These properties of the present materials are similar as those of proteins.

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SL-191

Track: Academic CRO/Industrial Collaborations in Drug Discovery

WHEN OPEN INNOVATION MEETS REPURPOSING

Edward Kahn

Rediscovery Life Sciences, Cambridge, MA, USA; E-mail: ekahn@rediscoveryls.com

Cures Within Reach has been a long time advocate of the deliberate and heightened use of medical repurposing. Repurposing is a safe, fast and affordable means to deliver more treatments to more patients, particularly for 7000 unsolved diseases, many of them rare. RLS views repurposing as a partial answer to the drying pipeline of Big Pharma for major diseases.

Recently, CWR, a non-profit, has begun to innovate not only by utilizing repurposing as the substantive basis for accelerated discovery and delivery to patients, but also through "process" innovations, including "open innovation", for profit incentives and entities, as well as so-called pay for performance techniques - such as Social Impact Bonds.



OPEN INNOVATION

With a grant from the Robert Wood Johnson Foundation, CWR has developed an open-access, online platform to bring together everyone interested in promoting repurposing research: bio-medical researchers, clinicians, funders of various types, patient advocacy organizations, and patients -- who are patiently awaiting a treatment for their disease. CureAccelerator is currently in beta testing and on track for a debut in the Summer of 2015.

FOR PROFIT MODALITIES

CWR encouraged the formation of Rediscovery Life Sciences, which was formed in the Winter of 2014, to utilize the power of the marketplace to bring validated repurposed Rx concepts to full FDA approval and make such repurposed therapies available with complete reimbursement.

In the past, clinicians could prescribe off label those repurposed drugs that showed good anecdotal evidence for utility in additional indications.

Funded by for-profit investors or strategic corporate partners, RLS can now fund FDA trials proving efficacy and getting such drugs onto the Formulary.

PAY FOR PERFORMANCE - COST SAVINGS AND SOCIAL IMPACT BONDS

CWR's founder, Dr. Bruce Bloom, has long advocated the use of Social Impact Bonds to fund therapeutic repurposed drug development.

CWR is working with a Canadian firm to develop feasibility plans for a UK and/or Canadian Rare Disease SIB.

SIBs have long been limited to social conditions, particularly prison recidivism.

SL- 24

Track: CNS Drug Discovery and Therapy

NOVEL IMMUNOSPECIFIC THERAPY WITH SYNTHETIC MULTI-EPITOPE TARGETING AGENT MODULATE MS –LIKE DISEASE BY INDUCTION OF PERIPHERAL REGULATORY MECHANISMS

N. Kaushansky, R. Zilkha-Falb, L. Cohen and A. Ben-Nun

The Weizmann Institute of Science, Rehovot, Israel; E-mail: Nathali.kaushansky@weizmann.ac.il

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS, associated with complex anti-myelin autoimmunity. Among all approaches proposed for MS therapy, an approach that neutralizes only the pathogenic T-cells reacting against myelin, while leaving the innocent immune cells intact, is the ultimate goal in the immune-specific-therapy for MS. The multiplicity of primary target antigens, alongside the dynamic nature of autoimmunity in MS, whereby the specificity of anti-myelin pathogenic autoreactivities may shift or expand in the same patient with disease progression, impose difficulties in devising immune-specific therapy to MS. To overcome this complexity of pathogenic autoreactivities in MS, we have put forward the concept of concomitant multi-antigen targeting as, a conceivably more effective approach to immunotherapy of MS. We constructed an EAE/MS-related synthetic-human-Target-Autoantigen-Gene, designed to encode in tandem only EAE/MS related epitopes of all known encephalitogenic proteins. The MS-related protein-product (designated Y-MSPc) was immunofunctional and upon tolerogenic administration, it effectively suppressed and reversed EAE induced by a single encephalitogenic protein. Furthermore, Y-MSPc also fully abrogated the development of “complex-EAE” induced by a mixture of five encephalitogenic T-cell lines. Overall, the modulation of EAE by Y-MSPc was associated with induction of tolerogenic CD11c+CD11b+Gr1+-DCs and other immune-regulatory mechanisms.

**SL- 235**

Track: Anti Infectives

ANTIBACTERIAL POLYKETIDE FROM THE MARINE ALGA-DERIVED ENDOPHYTIC STREPTOMYCES SUNDARBANSENSIS: A STUDY ON HYDROXYPYRONE TAUTOMERISM

Mouloud Kecha, Ibtissem Djinni, Andrea Defant and Ines Mancini

Laboratory of Applied Microbiology, Faculty of Nature Science and Life, University A.MIRA , Targa Ouzemmour 06000 Bejaia, Algeria; E-mail: biokecha@yahoo.fr

Polyketide **13** [=2-hydroxy-5-((6-hydroxy-4-oxo-4H-pyran-2-yl)methyl)-2-propylchroman-4-one] and three related known compounds **7**, **9** and **11** were obtained and structurally characterized from *Streptomyces sundarbansensis* strain, an endophytic actinomycete isolated from the Algerian marine brown algae *Fucus* sp. Compound **13** was obtained as the major metabolite from optimized culture conditions, by using Agar state fermentation. Due to tautomeric equilibrium, Compound **13** in CD₃OD solution was able to incorporate five deuterium atoms, as deduced by NMR and ESI-MS/MS analysis. The 2-hydroxy- γ -pyrone form was established for these metabolites based on the comparison of their experimental IR spectra with the DFT calculated ones, for both the corresponding 4-hydroxy- α -pyrone and 2-hydroxy- γ -pyrone forms. During antibacterial evaluation, compound **13** stood out as the most active of the series, showing a selective activity against the Gram positive pathogenic methicillin-resistant *S. aureus* (MRSA, MIC = 6 μ M), with a bacteriostatic effect.

Keywords: Marine *Streptomyces*, antibacterial activity, ensity functional calculations, hydroxypyron tautomerism.

SL- 314

Track: Drug Delivery and Targeting

MITOCHONDRIAL TARGETING OF THERAPEUTICS**Shana Kelley**

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, ON, Toronto, Canada; E-mail: shana.kelley@utoronto.ca



The mitochondria of human cells play a central role in the life and death of the cell due to the diverse processes and proteins - such as energy production and cell death regulators - that it houses. The role of mitochondria in cancer progression and tumorigenesis has been widely acknowledged. A major challenge to the study of mitochondrial processes and the development of mito-targeted therapies is presented by the impermeability of the innermost mitochondrial membrane and its highly negative membrane potential, which exclude most exogenous molecules from the organelle. We have developed a new class of peptide-based mitochondria-targeting vectors that can deliver various cargos to this previously impenetrable organelle. We have used these vectors to understand the chemical requirements for mitochondrial entry, to study oxidative stress in the organelle, and to deliver several different therapeutics. Insights into the unique chemical and biochemical features of this organelle gained from the use of these peptides will be presented.

SL- 247

Track: Hot Topics in Medicinal Chemistry

INHIBITING EGFR DIMERIZATION USING TRIAZOLYL-BRIDGED DIMERIZATION ARM MIMICS**Eileen J. Kennedy**

University of Georgia College of Pharmacy, 250 W. Green Street, Athens, Georgia 30602, USA

The epidermal growth factor receptor (EGFR) is overexpressed in multiple carcinomas and is the focus of a variety of targeted therapies. Here we report the design of peptide-based compounds that mimic the EGFR dimerization arm and inhibit allosteric activation of EGFR. These peptides are modified to contain a triazolyl bridge between the peptide strands to constrain the EGFR dimerization arm β -loop. In this study, we demonstrate that these peptides have significantly improved proteolytic stability over the non-modified peptide sequence, and their inhibitory effects are dependent on the number of the methylene units and orientation of the introduced triazolyl bridge. We identified a peptide, EDA2, which downregulates receptor phosphorylation and dimerization and reduces cell viability. This is the first example of a biologically active triazolyl-bridged peptide targeting the EGFR dimerization interface that effectively downregulates EGFR activation.

SL-276

Track: CNS Drug Discovery and Therapy

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TACRINE DERIVATIVES AND TACRINE-COUMARIN HYBRIDS AS CHOLINESTERASE INHIBITORS

Kamil Kuca, S. Hamulakova, L. Janovec, M. Hrabínova, K. Spilovska, J. Korabecny, P. Kristian, E. Nepovimova, P. Marešova and J. Imrich

Biomedical Research Center, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic; E-mail: kamil.kuca@fnhk.cz

A series of novel tacrine derivatives and tacrine-coumarin heterodimers were designed, synthesized, and biologically evaluated for their potential inhibitory effect on both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Of these compounds, tacrine-coumarin heterodimer and tacrine derivative were found to be the most potent inhibitors of human AChE (hAChE), demonstrating IC₅₀ values of 0.0154 and 0.0263 μ M. Other ligands exhibited the highest levels of inhibitory activity against human BuChE (hBuChE), demonstrating IC₅₀ values that range from 0.228 to 0.328 μ M. Docking studies were performed in order to predict the binding modes of selected compounds with hAChE/hBuChE.



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SL- 275

Track: Pharmaceutical Research & Development

UNTANGLING THE OPPORTUNITIES FROM SOUTH AFRICAN PLANTS

Namrita Lall

University of Pretoria, South Africa, E-mail: Namrita.Lall@up.ac.za

South Africa has a wealthy supply of plants (about 23 500 species of higher plants) together with a high degree of endemism (36.6%) in the indigenous South African flora, of which 4000 plant taxa are ethnomedicinally used and approximately 500 species are used in traditional medicine by an estimated 70% South Africans on a regular basis. South Africa has huge potential in identifying novel compounds to treat many diseases. South African plants for various purposes such as infectious diseases, cancer, skin-hyperpigmentation problems, melasma, periodontal diseases, and for ACNE problem have been scientifically investigated. Steady progress in evaluating potential medicinal plants for product development with potential in human medicine has been made.



A potent antituberculosis compound has been identified which is one of the top 3 candidates to be discovered to combat TB from natural product thus far. The compounds were tested in a system for inhibition of an enzyme mycothiol reductase, which protects against oxidative stress and is found in mycobacteria but not mammals, thus allowing selectivity. The target of the compound seems to be Mycothiol reductase (MyR) indicating novel mode of action. A number of plant- extracts and the chemical compounds with significant toxicity against various cancer cell lines have been identified. Medicinal plant extracts were investigated for treating dental diseases such as toothaches, periodontitis (dental plaque), gingivitis and halitosis. Five plant extracts with very good inhibitory activity against *Actinomyces naeslundii*, *Actinomyces israelii*, *Candida albicans*, *Streptococcus mutans*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis* have been identified which are being considered for inclusion in oral rinse and tooth pastes by the pharmaceutical companies. A significant number of plants with potential inhibitory activity against *Propionibacterium acnes* are undergoing clinical studies. Colonization of this bacteria contribute to the etiology of the disease; 'Acne vulgaris' which is a most common skin disorder.

The skin is the bodies' first defence barrier and protecting it against sun damage is the most important component in maintaining healthy skin. Protection from the sun is becoming increasingly important due to the increase in UV radiation in South Africa. Overexposure to the sun can cause skin aging, wrinkles, age spots and loss of elasticity. Through ongoing research two lead plants shown to exhibit great antioxidant potential have been identified. To further exhibit the potent *in vitro* effect of the two plant actives, *in vivo* clinical trials were performed to determine the sun protection factor (SPF). Both the actives showed good antioxidant capacity in the DPPH assay with a 50% inhibitory concentration (IC₅₀) of 22.01 µg/ml and 1.17 µg/ml for S1 and S2 respectively. Furthermore in accordance with SANS1557 guidelines both S1 and S2 passed clinical trials during SPF testing. During SPF testing the standard achieved a SPF value of 16.5 and the S1 and S2 actives achieved higher results of 17.3 and 18.0 respectively. The reduction in erythema, induced through UV radiation, was great enough for both actives to increase the SPF value of the actives significantly, which is due to the potent antioxidant effect which reduces the formation of free radicals during UV irradiation. Isolated compounds from S1 and S2 have been identified as potent antioxidants in the DPPH assay. Compounds isolated from S1 were identified as 2,4,6-trihydroxydihydrochalcone (C1), 2,6,4-trihydroxy-4-methoxydihydrochalcone (C2), 2,6-dihydroxy,4-methoxydihydrochalcone (C3) and 5,7-dihydroxyflavanone [(2S)-pinocembrin] (C4) with IC₅₀ values of 0.895 µg/ml, 2.0 µg/ml, 8.72 µg/ml and 19.5 µg/ml respectively. Compounds present in S2 were identified as Myricetin, Myricitrin and Gallic acid with IC₅₀ values of 0.95 µg/ml, 1.85 µg/ml and 0.818 µg/ml respectively.

The research results have attracted a number of national and international Cosmeceutical companies who are willing to commercialise selected South African plant extracts and purified compounds emanated from our research. This is of important economic value because at present South African companies import cosmetic actives from overseas. The impact of research and development into local plants will therefore have huge spin-offs for both communities and cosmetic companies.

SL-97

Track: Inflammation and Immunology

NEGATIVE FEEDBACK REGULATION OF INFLAMMATION & NOVEL ANTI-INFLAMMATORY STRATEGY

Jian-Dong Li

Institute for Biomedical Sciences, Georgia State University, USA; E-mail: jdli@gsu.edu

Inducible Negative Feedback Regulation of Inflammation

Inflammation is a hallmark of infectious diseases. Although an appropriate inflammation is beneficial for host defense, if excessive, it is clearly detrimental to health. Thus, inflammation must be tightly regulated. However, how inflammation is tightly controlled remains largely unknown. Inducible negative feedback regulators play an essential role in controlling overactive inflammation. Our *objective* is to understand the molecular mechanisms by which inflammation is tightly regulated in infectious diseases and identify novel therapeutic targets. We and others have shown that CYLD and IRAK-M act as a key inducible negative feedback regulator for inflammation in infectious diseases (*Nat Commun* 2012 and *Nat Commun* 2015).

Novel Anti-inflammatory Strategy by Up-regulating Negative Regulators Through Drug Repositioning

Over the past decades, most anti-inflammatory strategies have focused on directly targeting the positive pathways to suppress inflammation. While these agents often showed reasonable efficacy, they exhibited significant adverse effects, *e.g.*, increased susceptibility to infection, which prevented their further clinical use. Thus, there is an urgent need for developing novel therapeutic strategies without serious side effects by up-regulating the negative regulators of inflammation. We recently found that Roflumilast, an existing drug for asthma, suppressed inflammation by up-regulating CYLD, the negative regulator of inflammation (*Nat Commun* 2013 and *PNAS* 2015). In addition to treating inflammatory diseases, up-regulating CYLD may also lead to promising therapeutic strategies for fibrosis in infectious diseases.

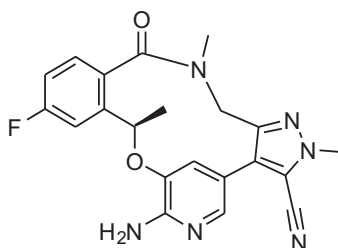
SL- 104

Track: Chemistry

DIRECT ARYLATION AND ITS APPLICATION ON SCALE TO THE SYNTHESIS OF PF-06463922, A MACROCYCLIC INHIBITOR OF *ALK/ROS1***Phuong T. Le**

Pfizer Worldwide Research and Development, Oncology Medicinal Chemistry, 10770 Science Center Drive, San Diego, California, 92121, USA; E-mail: phuong.le@pfizer.com

Crizotinib is a dual *ALK/c-MET* inhibitor approved in over 70 countries to treat ALK positive Non Small Cell Lung Cancer. A sub-set of crizotinib treated patients eventually progress due to drug-induced mutations in the kinase domain of ALK, the most common being the L1196M gatekeeper mutation [1]. A next generation effort began at Pfizer targeting brain penetrant inhibitors of wild-type ALK and relevant resistant ALK mutants. The application of direct arylation to deliver analogs for this next generation program presented efficient access to compounds difficult to prepare via more conventional synthetic approaches leading to a significant expansion of accessible chemical space. This talk will focus on the first utilization of this type of chemistry for the construction of 12 membered macrocycles. The methodology has been successfully implemented to support on-going clinical studies of PF-06463922.

**REFERENCE**

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SL- 256

Track: Neurodegenerative Disorders

ALZHEIMER'S DISEASE VACCINES: BREAKTHROUGHS AND SETBACKS**Cynthia A. Lemere**

Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; E-mail: clemere@partners.org

Alzheimer's Disease (AD) is the most prevalent form of dementia, afflicting 5.2 million Americans and 30 million people or more worldwide (www.alz.org). The number of people with AD is expected to triple by 2050. Currently, there is no cure or prevention. The cerebral accumulation of amyloid- β protein is the one of the earliest changes in AD brain. Inflammation and pathogenic changes in the neuronal protein, tau, follow and are associated with synaptic dysfunction and neuronal loss, all of which result in the clinical symptoms and cognitive decline observed in AD. Recent developments in biomarkers, including CSF levels of A β 42, total tau and p-tau, and PET scans for amyloid and tau, indicate that these pathological changes begin ~15-20 prior to the onset of dementia. Thus, there is a window of opportunity to therapeutically target these changes prior to the synapse and neuron loss that precedes dementia. Active vaccination targeting full-length A β (AN1792) was initiated in 2000 but was halted due to the development of meningoencephalitis in 6% of treated moderate-severe AD subjects. Subsequently, a number of large



passive vaccine trials were initiated using humanized anti-A β antibodies to avoid self-antigen immune reactions. While plaque-lowering effects were observed in many of these trials (*e.g.* Bapineuzumab) in mild-to-moderate AD subjects, cognitive benefits did not reach statistical significance and some transient vascular side effects (ARIA: amyloid-related imaging abnormalities) were seen. Vaccination with an antibody targeting soluble A β , Solanezumab, did not lower plaques but showed cognitive stabilization in mild AD patients. More recently, a fully human A β antibody, BIIB037, was reported to have significant plaque-lowering effects and cognitive stabilization in mild AD patients in a Phase I study. Some ARIA was observed. Current AD vaccine clinical trials are focused on AD prevention and early treatment. These include passive A β vaccine trials in people with familial autosomal-dominantly inherited AD and people who are cognitively intact but have evidence of amyloid accumulation by PET scan, as well as mild AD patients. In addition, Phase I clinical trials are underway for active A β vaccines, which will be helpful for long-term protection of many people if AD can be prevented by a vaccine. Lastly, additional vaccine targets are under investigation including vaccines targeting pyroglutamate-3 A β (a pathogenic form), tau, and Apo E. Importantly, it is becoming clear that AD vaccines appear to be most effective if administered prior to synapse and neuron loss.

SL- 66

Track: Traditional Chinese Medicine

THE METABOLIC AND PHARMACOKINETIC ANALYSIS OF CURCUMINOIDS BASED ON NANOPARTICLE FORMULATIONS BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

Rui Li¹ and Min Ye²

¹*Key Laboratory of Food Biotechnology of Sichuan Province, School of Bioengineering, Xihua University, Jizhou Road No.999, Jinniu District, Chengdu 610039, P.R. China;
E-mail: liruielite@gmail.com*

²*State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China*



Curcuminoids, including curcumin, demethoxycurcumin and bisdemethoxycurcumin are the putative cancer chemopreventive agent with poor bioavailability, which makes the comprehensive metabolic and pharmacokinetic studies difficult and limited. In our research, three curcuminoids loaded nanoparticles formulations were prepared to improve the intestinal absorption and metabolic and pharmacokinetic analysis were conducted by LC/MS method.

The metabolites in rats and tumor-bearing mice after oral administration were investigated. A totally of 37 metabolites were identified in plasma, urine, feces, bile, liver, brain and tumor samples. Reduction, glucuronidation and sulfation were found to be the major metabolic reaction for curcuminoids through intestinal absorption. The glucuronide conjugates, sulfated conjugates and mixed conjugates, together with the related secondary metabolites including tetrahydro-, hexahydro- and octahydro- were unambiguously identified based on their characteristic fragmentations in MSⁿ analysis and UV absorbance. Based on these results, the metabolic pathway of curcuminoids *in vivo* were proposed. Furthermore, a validated LC-MS/MS method was established to determine three curcuminoids in tumor. The pharmacokinetics of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were studied and the pharmacokinetic parameters were calculated. We found that the methoxy group (-OCH₃) on curcuminoids might possess strong tumor-affinity, and the curcumin possess the strongest tumor tissue affinity among three curcuminoids.

SL- 80*Track: Traditional Chinese Medicine***3D QUALITY CONTROL OF ENTIRE MOLECULE COMPLEX OF COMPOUND DANSHEN DRIPPING PILLS****Wei Li***Center for Innovative Research, Tasly Academy, Tasly Holding Group Co., Ltd., Tianjin, China;**E-mail: liwei3@tasly.com*

Traditional Chinese medicine (TCM), as an holistic treatment system historically established through empirical evaluation, mainly exists in China, Japan, and Korea. Medicines, no matter TCM or western medicine, are relevant to priority health needs and public health, therefore high standards of quality, safety, and efficacy of medicines must be ensured.

Normally, for western medicine it is clear that certain molecule produce certain efficacies and adverse effects. And for TCM, it is the entire molecule complex which are of certain efficacies and adverse effects, the relation between certain molecule and efficacy/safety usually is not well known. So The quality control focus on the certain molecule(s) for western medicine, and the entire molecule complex for TCM, which cause the difficulty for the application of the norms and standards of quality evaluation of western medicine to TCM. And obviously it is not accurate to apply the methodology of quality control of western medicine to TCM. Hence the methodology of quality control, especially qualitative and quantitative method for the molecule complex of TCM, should be exemplified to guide the study of quality control of TCM.

Now the tactics of quality control of TCM are introduced by study on Compound Danshen Dripping Pills (CDDP), which developed the three dimensions (3D) quality control method (see Fig. 1), that is on the holistic level, ¹H-NMR fingerprint is applied to holistic qualitative identification; on level of active ingredient complexes, HPLC fingerprint to combination of qualitative identification and quantitative control; on level of QC markers, HPLC quantitation to componential quantitative control.

Through the 3D quality control of entire molecule complex of CDDP, The chemical composition of CDDP, which is the base of efficacy and Safety, is clarified and controlled effectively.

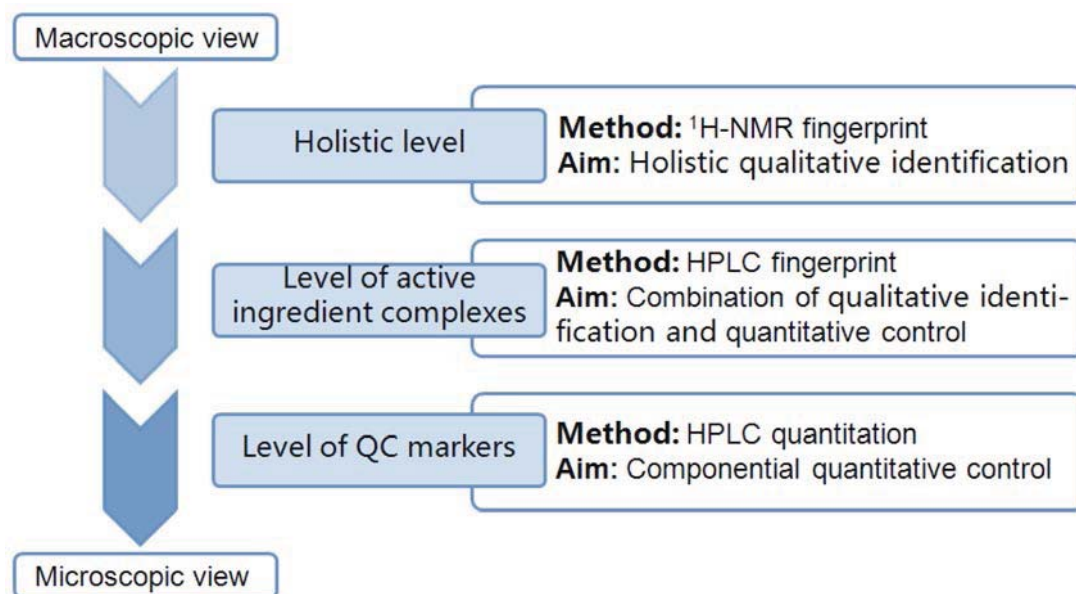


Fig. (1). 3D quality control of entire molecule complex of Compound Danshen Dripping Pills.

SL- 52*Track: Traditional Chinese Medicine***EFFECT OF MOXIBUSTION ON T LYMPHOCYTE SUBSETS OF *HELICOBACTER PYLORI*-ASSOCIATED GASTRITIS RATS****Jing Shen¹, Yan Peng¹, Shou-Xiang Yi¹, Ying-Shuai Feng² and Ya-Ping Lin¹**¹Hunan University of Chinese Medicine, Changsha 410208, China; E-mail: lyp5381161@126.com²Department of Rehabilitation and Physiotherapy, the People's Hospital of Hunan Province, Changsha 410007, Hunan Province, China

Objective: To explore the effect of moxibustion on gastric inflammatory injury and expression of Hp IgG and CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺ of rats with *Helicobacter pylori* (*H. pylori*)-associated gastritis, to reveal the mechanisms underlying the protective effect of moxibustion against gastric inflammatory injury.

Methods: 40 healthy SD rats were randomly divided into 4 groups: blank group (group A), Hp model group (group B), moxibustion groups (group C) and electro-acupuncture group (group D). Gastritis was induced by oral gavage with live *H. pylori*. Content of serum Hp IgG was detected by ELISA. Contents of CD3⁺, CD4⁺, CD8⁺ and CD4⁺/CD8⁺ was detected by flow cytometry.

Results: Compared to group A, the expression of Hp IgG in group B were significantly increased (76.72 ± 11.02 vs 131.91 ± 30.04 , $P < 0.01$), and content of CD3⁺ (17.78 ± 5.00 vs 10.31 ± 3.34 , $P < 0.01$), CD4⁺ (13.79 ± 3.35 vs 8.59 ± 1.93 , $P < 0.01$) and CD4⁺/CD8⁺ decreased (1.93 ± 0.53 vs 1.03 ± 0.35 , $P < 0.01$), content of CD8⁺ increased (3.59 ± 1.02 vs 5.27 ± 1.45 , $P < 0.01$). Compared to group B, the expression of Hp IgG in group C were significantly decreased (131.91 ± 30.04 vs 86.25 ± 18.63 , $P < 0.01$), content of CD3⁺ (10.31 ± 3.34 vs 17.34 ± 5.22 , $P < 0.05$), CD4⁺ (8.59 ± 1.93 vs 12.23 ± 3.19 , $P < 0.05$) and CD4⁺/CD8⁺ (1.03 ± 0.35 vs 1.78 ± 0.58 , $P < 0.05$) increased significantly, content of CD8⁺ decreased (5.27 ± 1.45 vs 3.84 ± 1.00 , $P < 0.05$). No significant difference in group D ($P > 0.05$). Compared to group D, the expression of Hp IgG in group C were significantly decreased (120.25 ± 25.40 vs 86.25 ± 18.63 , $P < 0.01$), CD4⁺/CD8⁺ (1.32 ± 0.37 vs 1.78 ± 0.58 , $P < 0.05$) increased significantly.

Conclusion: The mechanisms of reducing *H. pylori*-induced gastric mucosal inflammatory injury after moxibustion therapy possibly associated with enhancement of body immunity, resistance to microbial pathogens invasion and killing Hp inside, inhibiting the formation of Hp IgG. The effect on Immunity Enhancement of moxibustion is better than that of electro-acupuncture therapy.

Supported by: National Natural Science Foundation of China. No. 81072867.

Keywords: CD3⁺, CD4⁺, CD8⁺, gastritis, Hp, Hp IgG, moxibustion.

SL- 166*Track: Bioactive Lipids***MORPHOLOGICAL AND BIOPHYSICAL PROPERTIES OF NATURAL PHOSPHOLIPIDS-BASED BIOMEMBRANES****Elmira Arab-Tehrany, A. Jacquot, G. Francius, A. Razafitianamaharavo and M. Linder**

Université de Lorraine, Laboratoire Ingénierie des Biomolécules, TSA 40602, Vandoeuvre-lès-Nancy, F-54518, France; E-mail: elmira.arab-tehrany@univ-lorraine.fr

Liposomes are artificial lipid vesicles characterized by a membrane composed of lipids that confers to this structure the ability to encapsulate and protect the active molecules. This particular property allows the liposomes to be used as vectors or biological carriers in food industry, biomedicine, pharmaceuticals (vectorization and drug delivery), and tissue engineering. In this work, we studied the lipid organization and mechanical properties of biomembranes made of marine and plant



phospholipids. Membranes based on phospholipids from rapeseed and salmon were studied under liposome form and as supported lipid bilayer. Dioleoylphosphatidylcholine (DOPC) and dipalmitoylphosphatidylcholine (DPPC) were used as references to determine the lipid organization of marine and plant phospholipid based membranes. Atomic force microscopy imaging and force spectroscopy measurements were performed to investigate the membranes topography at the micrometer scale and to determine their mechanical properties. The mechanical properties of the membranes were correlated to the fatty acid composition, the morphology, the electrophoretic mobility and the membrane fluidity. Thus, soft and homogeneous mechanical properties were evidenced for salmon phospholipids membrane containing various polyunsaturated fatty acids. Besides, phase segregation in rapeseed membrane and more important mechanical properties were emphasized for this type of membranes by contrast to the marine phospholipids based membranes.

SL- 18

Track: Cardiovascular Drug Discovery and Therapy

RE-INTRODUCTION OF BOVINE HEPARIN: REGULATORY AND COMPENDIAL ISSUES

Robert J. Linhardt

Departments of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, Biotech Center 4005, 110 8th Street, Troy, NY 12180-3590, USA; E-mail: linhar@rpi.edu

Heparin is a highly sulfated polysaccharide derived from pig intestinal tissues that commands a worldwide market of ~\$7B. It is widely used as a parenteral anticoagulant in the treatment of cardiovascular diseases. Most (~75%) of this drug, essential for modern medicine, is currently produced outside the United States (US) and from a single animal species, making its supply chain vulnerable to trade sanctions and porcine diseases. In June 2014, a US Food and Drug Administration (FDA) Advisory Committee encouraged the re-introduction of bovine heparin to the US market. The US Pharmacopeia (USP) is beginning to evaluate the need for a revised or new monograph covering bovine heparin. This presentation examines some structure and activity differences between porcine and bovine heparins, and their impact on the potential non-equivalence of these two types of heparin. The structure-activity relationships of porcine and bovine heparins will be discussed. Approaches will also be required to ameliorate the risks associated with bovine spongiform encephalopathy (BSE or “mad-cow” disease). Finally the economic feasibility and impact of re-introduction of bovine heparin to address potential shortages in the US market will be discussed.

SL- 101

Track: Traditional Chinese Medicine

EFFECT OF SHENYUAN ON ANTI-INFLAMMATORY AND HEMODYNAMIC FACTORS IN A PORCINE MODEL OF ACUTE MYOCARDIAL INFARCTION

Wen-yi Liang, Zi-long Zhang, Xin-yuan Zhu, Xue-ru Feng and Mei-lin Liu

Department of Geriatrics, Peking University First Hospital, No. 8, Xishiku Street, Xicheng District, Beijing 100034, China; E-mail: meilinliu@yahoo.com

Aim: To evaluate the potential cardioprotective effect of Shenyuan, which is composed of the bioactive components extracted from the mixture of American Ginseng and Corydalis Tuber.

Materials and Methods: Pigs were divided randomly into five groups: (1) Group S, sham operated; (2) Group C, myocardial infarction (MI) control; (3) Group L, MI + low-dose Shenyuan (240 mg/kg•d); (4) Group M, MI + moderate-dose Shenyuan (320 mg/kg•d); (5) Group H, MI + high-dose Shenyuan (400 mg/kg•d). The experiment was carried out at five time points, *i.e.* pre-MI, post-MI 6 hours, post-MI 2 days, post-MI 7 days and post-MI 14 days.

Results: Expression of T regulatory cell marker Forkhead box P3 (Foxp3) in Group C was lower than that in Group S at post-MI 14 days, but was significantly rescued by the treatment with high dose of Shenyuan in both infarcted and non-infarcted zones of left ventricular. Consistently, high dose of Shenyuan was more potent in up-regulating plasma IL-10 and TGF- β levels than lower dose. Whole blood viscosity at 15s⁻¹ gradually increased after MI, but was markedly attenuated by all doses of Shenyuan at post-MI 14 days. With regard to platelet aggregation rate, an observable increase was found in Group C at post-MI 2d, and treatment of low and high doses of Shenyuan eliminated the changes. Values of peak left ventricular pressure rise (+dp/dt_{max}), left ventricular end-systolic pressure (LVESP), left ventricular mean pressure (LVMP) and aortic systolic pressure (AOSP) in Group C were statistically lower than those in Group S. All doses of Shenyuan ameliorated the attenuation of +dp/dt_{max} and LVMP, with no significant difference compared with Group S.

Conclusion: Shenyuan elicited anti-inflammatory pharmacological actions in myocardium injury and facilitated hemodynamic stabilization after MI onset. High dose of Shenyuan was more potent in a porcine model.

SL- 108

Track: Genomics

IDENTIFICATION OF ASPIRIN RESPONSE RELATED GENE PROFILES IN THE ELDERLY POPULATION WITH CORONARY ARTERY DISEASE

Tengfei Liu, Jingwei Zhang, Yahong Chen, Xueru Feng, Lu wang and Meilin Liu

Department of Geriatrics, Peking University First Hospital, China; E-mail: liutengfei198703@163.com

Background: Aspirin is widely used in the primary and secondary prevention of cardiovascular diseases. Nevertheless, responses to aspirin vary from one patient to another. Recent studies showed that aspirin exposure have influence on the expression of various genes, thus responsible for the variability in aspirin responses. The aim of our study was to identify aspirin response related gene profiles, and investigate the correlation between target genes expression and clinical outcomes in the elderly population with coronary artery disease (CAD).

Methods: A total of 160 aged patients with CAD treated with low-dose aspirin (100mg/d) were enrolled in this study. All enrolled patients were distributed according to quartile of 0.5 mmol/l AA-induced platelet aggregation, and AR was defined as the upper quartile of AA-induced platelet aggregation. Total blood RNA were extracted within 4h and reversely transcribed immediately. Expression of fourteen genes (CLU, CMTM5, CTTN, MPL, TMEM64, SELP, HLA-DQA1, HLA-DRB4, ITGA2B, ITGB3, THBS1, CXCL5, PPBP, SPARC) were measured using real-time quantitative PCR method. Clinical outcomes were defined as the occurrence of cardiovascular events and death during regular aspirin administration.

Results: The quartile cut points of AA-induced platelet aggregation for the 25th, 50th, and 75th percentiles of the enrolled population were 9.48%, 12.16%, and 15.17%, respectively. AR was defined as AA-induced platelet aggregation \geq 15.17%. Expressions of five genes (CTTN, HLA-DQA1, HLA-DRB4, THBS1, ITGB3) were significantly higher in AR group than no-AR group.

SL- 315

Track: Innovative Drug Discovery and Nanotechnology

DEVELOPMENT OF PROTEIN AGENT TARGETING INTEGRINS $\alpha_v\beta_3$ AT A NOVEL SITE BY RATIONAL PROTEIN DESIGN

Ravi Chakra Turaga and Zhi-Ren Liu

Department of Biology, Georgia State University, Atlanta, GA 30303, USA; E-mail: zliu8@gsu.edu

Due to abnormal expression of integrins $\alpha_v\beta_3$ in various disease conditions, this integrin pair has been a focus as targets for drug development. Studies yield a few successful examples. Among them are various antibodies against the integrins, and most recently, Cilengitide, a RGD-based peptidomimetic. Nevertheless, most of current approaches focus on ligand-binding with goal of inhibition of integrin functions. A major draw-back of targeting ligand-binding of integrins is activation of integrin signaling by the developed agent, which largely limit the clinical success of the integrin ligand based antagonist/agonist. We report here development of a new class of therapeutically protein agent (Ref to as ProAgio) by rational protein design using a stable host protein. ProAgio is designed to target integrins $\alpha_v\beta_3$ at a novel site. ProAgio exhibits a strong *in vitro* activity in induction of apoptosis of integrin $\alpha_v\beta_3$ expressing cells. ProAgio induces apoptosis by recruiting and activating caspase 8 to the cytoplasmic domain of the targeted integrins. Tests with tumor xenografts show that ProAgio strongly inhibits tumor growth. Histology analyses indicate that tumor vessels are reduced, while the established vasculatures are not affected. The results confirm targeting of integrin $\alpha_v\beta_3$ as an anti-angiogenic agent. Toxicity analyses demonstrate that ProAgio is not toxic to mouse at very high doses. Our study develops an effective integrin targeting agent *via* a novel mechanism of action. Our approach provides a new platform for development of therapeutics by targeting integrins.

Keywords: Antibacterial activity, heavy metals, phytochemical.

SL- 149

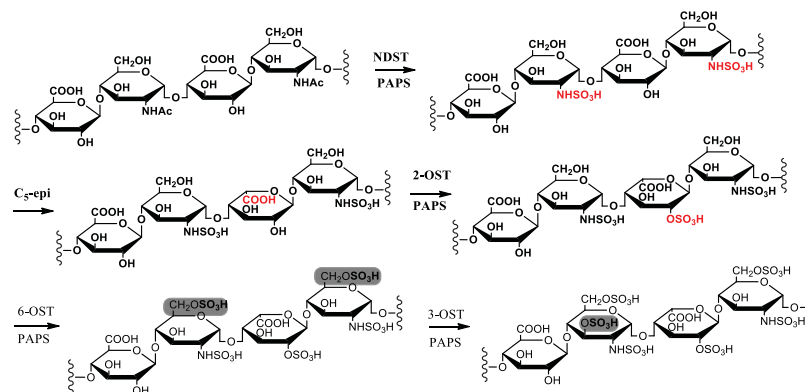
Track: Cardiovascular Drug Discovery & Therapy

CHEMOENZYMATIC SYNTHESIS OF LOW MOLECULAR WEIGHT HEPARINS

Jian Liu

Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA; E-mail: jian_liu@unc.edu

Heparin and low-molecular weight heparins are widely used anticoagulant drugs. Heparin is a highly sulfated polysaccharide, and low-molecular weight heparin is the partially depolymerized heparin. Heparin is a natural product isolated from pig intestine through a long supply chain. Developing an efficient method to synthesize heparin will improve the safety of heparin drugs. A chemoenzymatic method has been developed to prepare synthetic heparin, involving the use of 15 different enzymes, including sulfotransferases, an epimerase and glycosyltransferases. The method has been successfully utilized to synthesize ultra-low molecular weight heparin and low-molecular weight heparins. Furthermore, the anticoagulant activity of one of these synthetic low-molecular weight heparins can be reversed *in vitro* and *in vivo* using protamine, reducing the risk of bleeding side effect. The chemoenzymatic approach provides an opportunity for a cost-effective, modern method to manufacture the next generation of low-molecular weight heparins.



Scheme for the chemoenzymatic synthesis of heparins

SL- 159*Track: Stereoselective Synthesis of Bioactive Compounds***TOTAL SYNTHESIS OF 6-DEOXYPLADIENOLIDE D AND ASSESSMENT OF SPLICING INHIBITORY ACTIVITY IN A MUTANT SF3B1 CANCER CELL LINE**

Kenzo Arai, Silvia Buonamici, Betty Chan, Laura Corson, Atsushi Endo, Baudouin Gerard, Ming-Hong Hao, Craig Karr, Kazunobu Kira, Linda Lee, Xiang Liu, Jason T. Lowe, Tuoping Luo, Lisa A. Marcaurelle, Yoshiharu Mizui, Marta Nevalainen, Morgan Welzel O'Shea, Eun Sun Park, Samantha A. Perino, Sudeep Prajapati, Mingde Shan, Peter G. Smith, Parcharee Tivitmahaisoon, John Yuan Wang, Markus Warmuth, Kuo-Ming Wu, Lihua Yu, Huiming Zhang, Guo Zhu Zheng and Gregg F. Keaney

H3 Biomedicine Inc. 300 Technology Square, Cambridge, MA 02139 USA; Eisai Inc., 4 Corporate Drive, Andover, MA 01810 USA; E-mail: gregg_keaney@h3biomedicine.com

Mutations in several components of the spliceosome have been reported in various hematological (CLL, MDS, *etc.*) and solid tumor (melanoma, pancreatic, *etc.*) malignancies. SF3B1 is a component of the U2 snRNP complex of the spliceosome and is involved in the recognition of splice sites during early spliceosomal assembly. We and others have demonstrated that mutations in SF3B1 result in neomorphic activity and trigger the production of aberrantly spliced transcripts. Thus, the discovery of small molecule modulators of SF3B1 splicing activity may have therapeutic potential in cancers harboring SF3B1 mutations.

Members of the pladienolide family of natural products have been shown to affect RNA splicing through modulation of the SF3b complex. While analogs of 6-deoxypladienolide D have been previously prepared *via* modification of the natural product, this semi-synthetic approach was particularly challenging due to the limited supply of 6-deoxypladienolide D and the synthetic inaccessibility to most regions of the molecule. For these reasons, coupled with the burgeoning interest to identify chemical matter able to modulate splicing in newly-identified mutant SF3B1 cancers, a total synthesis of 6-deoxypladienolide D using versatile and modular fragments was initiated.

This presentation will describe the first total synthesis of the natural product 6-deoxypladienolide D. Two noteworthy attributes are: 1) a late-stage allylic oxidation which proceeds with full chemo-, regio-, and diastereoselectivity and 2) the use of cost-effective starting materials and reagents to enable access to 6-deoxypladienolide D and its analogs for biological evaluation. In addition, we have found that 6-deoxypladienolide D demonstrates: 1) cellular lethality at single-digit nanomolar concentration in Panc 05.04 cells (a mutant SF3B1 cancer cell line), 2) high binding affinity to the SF3b complex, 3) ability to inhibit pre-mRNA splicing, and 4) modulation of aberrantly-spliced transcripts identified in mutant SF3B1 cells.

SL- 145

Track: In-Silico Drug Design and In-Silico Screening

COMPUTATIONAL APPROACHES TO PK-FRIENDLY (DRUG-LIKE) MOLECULES**Franco Lombardo**

Department of Chemical and Molecular Therapeutics, Biogen Idec, Cambridge, MA, United States of America; E-mail: franco.lombardo@biogen.com

This report intends to briefly highlight some of recent models and approaches toward *in silico* ADME and PK prediction and, importantly, thinking from several scientists in the field and the response of the medicinal chemists to the modeling efforts. The major themes of "interpretability" and "structural translation" of findings will be discussed. This presentation is intended to stimulate discussion by the audience

Lastly, a brief list of publicly available, yet curated, structural and physicochemical properties databases, will be presented with ChEMBL and OCHEM (On-line Chemical Modeling Environment) being two major ones.

SL- 32

Track: Cardiovascular Drug Discovery & Therapy

A NOVEL, ACTIVATED PROTEIN C-INDEPENDENT ROLE OF PROTEIN S IN REGULATING THROMBOSIS**Rinku Majumder**

Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, USA; E-mail: rinku_majumder@med.unc.edu

The plasma glycoprotein Protein S (PS) is a critical, negative regulator of blood coagulation. The importance of PS is demonstrated dramatically by the catastrophic purpura fulminans that develops in rare newborns homozygous for PS mutations; heterozygous individuals have an elevated risk for deep vein thrombosis and other life-threatening thrombotic events.

We aim to use PS as a preventive agent for X-linked thrombophilia. Blood coagulation normally protects the integrity of blood vessels. Thrombophilia is an abnormality of blood coagulation (hemostasis) that increases the risk of thrombosis that occurs when a platelet aggregate and/or a fibrin clot forms in an intact blood vessel or in a chamber of the heart. Thrombophilia can cause stroke and heart attack. As an anticoagulant, PS limits thrombosis.

The interaction between coagulation proteins and platelets is both critical to the maintenance of normal hemostasis and the cause of human cardiovascular disease. We discovered that PS plays an important role in hemostasis by inhibiting FIXa, which, in turn, limits activation of FX and thrombin formation with subsequent clotting. We suggest that PS binds to the enzyme FIXa and inhibits FXa generation. Thus, we are determining whether thrombosis caused by an increase in FIXa can be controlled by PS.

Paulo Simioni and co-workers reported a case of thrombophilia associated with a leucine for arginine substitution at position (R338L) of FIX. The clotting activity of FIX from the proband was elevated by approximately a factor of eight, causing a much shorter clotting time. Our preliminary data showed that the clotting time of proband's plasma reverted back to the normal range with externally added PS, increasing from 25.7 seconds to 33 seconds. This result provides an excellent opportunity to prevent thrombosis.

Finally, our work will establish a novel regulatory role of protein S which will enable protein S to be used to treat X-linked thrombophilia.



SL- 116

Track: Protein and Peptide Sciences

UNDERSTANDING BIOLOGICAL ACTIVITY AND SELF-ASSEMBLY OF ANTIMICROBIAL CYCLIC LIPODEPSIPEPTIDE USING A MOLECULAR APPROACH

Jose C. Martins, Matthias De Vleeschouwer, Niels Geudens, Annemieke Madder, Davy Sinnaeve and Tim Van Kersavond

NMR and Structure Analysis Unit, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281 S4, B-9000 Gent, Belgium; E-mail: Jose.Martins@UGent.be



Cyclic Lipodepsipeptides (CLPs) produced by Pseudomonads display a variety of interesting antimicrobial properties yet remain a little understood collection of structurally diverse natural compounds. Establishing structure-function relationships and understanding their interactions with biological membranes at the molecular level are key requirements for the further development of these compounds. The development of a rapid and efficient synthesis route to viscosin CLPs [1] has vastly expanded the chemical space provided from natural variations, enabling to explore the link between structure and function. Over 40 variations have been designed, synthesized and evaluated for biological activity using pseudodesmin A as parent compound. The impact of amino acid composition, stereochemistry [2] and lipid tail length on biological activity allow us to identify several key-modulators of biological activity, which are interpreted in terms of the three dimensional structures obtained using NMR and/or X-ray diffraction [2-4]. The impact of these variations on the self-assembly of viscosin CLPs [3, 4] was also studied and interpreted in terms of an *in silico* model of the self-assembled structure (unpublished). Finally, molecular simulations of CLPs with solvated lipid membranes are evaluated against NMR data obtained from CLPs in the presence of lipid bicelles. All taken together, a molecular picture of these structures, their function and their interaction with membranes is now emerging.

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SL- 81

Track: Cancer Targeted Drug Delivery

NOVEL LIPOSOMES INCLUDING TREHALOSE SURFACTANT INHIBIT THE GROWTH OF TUMOR CELLS ALONG WITH APOPTOSIS

Yoko Matsumoto

Department of Life Sciences, Sojo University, Japan; E-mail: matumoto@life.sojo-u.ac.jp



Novel liposomes composed of L- α -dimyristoylphosphatidylcholine (DMPC) and trehalose surfactant (DMTreCn) were produced by the method of sonication in buffer solution. The thickness of fixed aqueous layer of DMTreCn (n = 14 and 16) was larger than that of DMPC liposomes and increased in a dose-dependent manner. The remarkable inhibitory effects of DMTreCn (n = 14 and 16) on the growth of MOLT-4 cells were obtained without affecting the growth of normal cells. An increase in the accumulation of DMTreC14 and DMTreC16 including fluorescence-labeled lipid (NBDPC) into MOLT-4 cell membranes was observed

without accumulating against normal cells using confocal laser microscopy. Apoptotic DNA for MOLT-4 cells increased after the treatment with DMTrC14 and DMTrC16 as the dose of DMTrCn increased and reached a high apoptotic DNA rate (80-90 %), indicating that DMTrCn induced apoptosis for MOLT-4 cells. DMTrCn induced apoptosis for MOLT-4 cells through the activation of caspase-3, 8, and 9. The mitochondrial transmembrane potential of MOLT-4 cells decreased after the treatment with DMTrCn. It is noteworthy that the remarkable inhibitory effects of DMTrCn on the growth of tumor cells were obtained along with apoptosis without affecting the growth of normal cells.

Keywords: Liposome, antitumor effects, trehalose surfactant, apoptosis.

SL- 74

Track: Cancer Targeted Drug Delivery

SYNTHETIC LETHAL TARGETING OF COLORECTAL CANCER CELLS THROUGH SOD1 INHIBITION

Babu V. Sajesh, Zeldia Lichtensztejn and Kirk J. McManus

*Research Institute of Oncology & Hematology, Department of Biochemistry & Medical Genetics,
University of Manitoba, Winnipeg, Manitoba, R3E0V9, Canada;
E-mail: Kirk.McManus@umanitoba.ca*



Colorectal cancer (CRC) affects both males and females, and is the second leading cause of cancer-related deaths in North America. Novel insight into the disease is highly warranted so that new therapeutic approaches can be developed. Synthetic lethality refers to the lethal combination of 2 independently viable mutations and has been extensively studied in model organisms such as yeast. Recently, synthetic lethality has been applied in cancer contexts and is beginning to show potential as a new therapeutic modality. *RAD54B* is an excellent candidate for therapeutic targeting as it is mutated in CRC and numerous other tumor types. *RAD54B* is an evolutionarily conserved protein that functions in DNA double strand break repair and is essential for chromosome stability. *RAD54B* deficiencies cause chromosome instability, which we believe can be targeted using a synthetic lethal approach. Utilizing cross-species approaches, 80 candidate synthetic lethal interactors of *RAD54B* were subjected to an RNAi-based high-content screen in human cells. Subsequent direct tests validated an interaction between *RAD54B* and superoxide dismutase 1 (SOD1) in both CRC cells and immortalized fibroblasts. Real-time cell analyses showed that chemical inhibitors are able to selectively kill *RAD54B*-deficient cells and demonstrated the decreases in cell numbers observed is due to cellular cytotoxicity rather than cell cycle arrest. Thus, we have identified and validated SOD1 as a novel candidate therapeutic target for the treatment of *RAD54B*-deficient CRCs cells. These data have far reaching implications as *RAD54B* defects have been identified in numerous tumor types including, prostate, ovarian, bladder and breast. Finally, we will discuss evidence that additional colorectal cancer cells with defects in genes encoding functions within the double-strand break repair pathway are also sensitive to SOD1 inhibition.

SL- 47

Track: Pharmacogenomics

ADVANCING MOLECULAR DIAGNOSTICS AND PRECISION MEDICINE USING NON-CODING RNA VARIANTS**Prasun J. Mishra**

National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; E-mail: mishrapj@mail.nih.gov

Referred to as the micromanagers of gene expression, microRNAs (miRNAs) are evolutionarily conserved small noncoding RNAs. Polymorphisms in the microRNA pathway (miR-polymorphisms) can influence gene regulation and are emerging as powerful tools to study the biology of diseases. Advancements in the miRNA field indicate the clear involvement of miRNAs and genetic variations within the miRNA pathway in the progression and prognosis of diseases such as cancer, neurological disorders, cardiovascular disease and Type II diabetes etc. Polymorphisms that may potentially affect miRNA-mediated regulation of cellular processes can be present not only in the target mRNA, but also in the genes involved in miRNA biogenesis and in pri-, pre- and mature-miRNA sequences. A polymorphism in processed miRNAs may affect expression of several genes and have serious consequences, whereas a polymorphism in miRNA target site may be more target and/or pathway specific. Detection of microRNA-polymorphisms can potentially improve diagnosis, treatment and prognosis in patients and has profound implications in the fields of pharmacogenomics and personalized medicine.

SL- 112

Track: Regenerative Medicine

NOVEL THERAPEUTIC STRATEGIES FOR DELIVERING THERAPEUTICS TO BRAIN - CIRCUMVENTING THE EFFLUX AND PERMEABILITY BARRIERS**Ashim K. Mitra**

Division of Pharmaceutical Sciences, University of Missouri-Kansas City, Kansas City, Missouri, USA; E-mail: mitraa@umkc.edu

Various strategies have been employed to improve oral and brain absorptions of protease inhibitors. The main objective of this project is to develop prodrug strategies to circumvent efflux pumps, thereby enhancing oral and brain absorption of protease inhibitors. Lopinavir (LPV) is one of the PIs currently indicated in the treatment of HIV infection. However, oral absorption of LPV is severely limited owing to its low aqueous solubility, intestinal efflux by P-gp and MRP2 and extensive metabolism by CYP3A4 enzymes. Prodrugs have been evaluated for their affinity towards efflux and influx transporters, enzymatic hydrolysis, and oral/brain bioavailability. LPV prodrugs displayed higher affinity towards influx transporters and efficiently circumvented P-gp and MRP2 mediated cellular efflux. Prodrugs generated significantly higher permeability relative to LPV. Moreover, prodrugs produced superior aqueous solubility and metabolic stability and low plasma protein binding. Plasma stability studies demonstrated that the dipeptide prodrug underwent enzymatic hydrolysis in a sequential manner to produce amino acid prodrug. Based on the



results obtained from this project, it is apparent that transporter targeted prodrug modification of LPV might be a viable option to improve oral as well as brain absorption of LPV following oral administration.

Supported by NIH grant AI071199.

SL- 207

Track: Biologics

EFFECT OF *ALTHAEA OFFICINALIS L.* ROOT EXTRACT ON CONCENTRATION OF BLOOD PROTEINS IN MICE

Mehrdad Modaresi

*Department of Physiology, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran;
E-mail: mehrdad_modaresi@hotmail.com*

Marshmallow (*Althaea officinalis L.*) is a herbaceous perennial plant with many therapeutic uses in herbal medicine. In this research, effects of hydro alcoholic extract of marshmallow's root were studied on electrophoresis pattern of blood proteins. Fifty female mice were divided in five groups including control, placebo, and three treatment groups. Hydro alcoholic extract (50, 100, and 200 mg/kg) was injected in peritoneum of mice every other day for twenty days. Blood samples were taken for studying electrophoresis pattern and immunity. Obtained data were analyzed using SPSS program at 5% probability level. According to results, beta globulin concentration was not affected by treatments. Albumin concentration was reduced in 50 and 100 mg/kg groups and the ratio of albumin to globulin was decreased in all experimental groups significantly. Gama globulin concentration of all experimental groups and α -1 globulin concentration of 100 mg/kg were increased significantly. Concentration of α -2 globulin was increased in 50 and 100 mg/kg groups significantly. Results showed that marshmallow's extract affected immune indices and electrophoresis pattern of blood proteins, dose dependently.

Keywords: Blood proteins, marshmallow, immune system, mice.



SL- 310

Track: Drug Delivery and Targeting

TREATMENT STRATEGIES THAT ENHANCE THE EFFICACY AND SELECTIVITY OF MITOCHONDRIA-TARGETED ANTICANCER AGENTS

Josephine S. Modica-Napolitano

*Department of Biology, Merrimack College, North Andover, MA, USA;
E-mail: Josephine.modicanapolitano@merrimack.edu*

Nearly a century has passed since Otto Warburg first observed high rates of aerobic glycolysis in a variety of tumor cell types and suggested that this phenomenon might be due to an impaired mitochondrial respiratory capacity in these cells. Subsequently, much has been written about the putative role of mitochondria in the initiation and/or progression of various forms of cancer, and the possibility of exploiting differences in mitochondrial structure and function between normal and malignant cells as targets for cancer chemotherapy. A number of mitochondria-targeted compounds have shown efficacy in selective cancer cell killing in pre-clinical and early clinical testing, including those that induce mitochondria permeability transition and apoptosis, metabolic inhibitors, and ROS regulators. To date, however, none has exhibited the standards for high selectivity and efficacy and low toxicity necessary to progress beyond phase III clinical trials and be used as a viable, single modality treatment option for human cancers. This talk



explores alternative treatment strategies that have been shown to enhance the efficacy and selectivity of mitochondria-targeted anticancer agents *in vitro* and *in vivo*, and may yet fulfill the clinical promise of exploiting mitochondria as a target for cancer chemotherapy.

SL- 54

Track: Combinatorial Chemistry

NOVEL HIGH DENSITY PEPTIDE ARRAYS WITH COMBINATORIAL DEPOSITION OF AMINO ACID PARTICLES FOR ANTIBODY PROFILING

A. Nesterov-Müller, F.F. Loeffler, L. Hahn, A. Fischer, T. Förtsch, V. Bykovskaya, M. Sedlmayr, B. Ridder, D. Althoun, C. von Bojničić-Kninski, R. Popov, F.R. Bischoff and F. Breitling

Karlsruhe Institute of Technology, IMT, Herrmann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany; E-mail: Alexander.Nesterov-Mueller@KIT.edu

Antibodies increasingly attract the attention of researchers because of their homeostatic, tissue regenerating and regulatory properties. To identify the circulating antibodies, we have developed high-content peptide arrays based on the combinatorial deposition of amino acid particles [1, 2]. This novel combinatorial array synthesis combines several advantages as spot densities up to 1 Million spots per cm² and minimal number of synthesis cycles. Using these peptide arrays we show the possibility of reading out human sera IgM/IgG-antibodies and their amino acid signatures.



Keywords: Hypoxia-ischemia, mast cells, therapeutic hypothermia.

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SL- 171

Track: Inflammation and Immunology

IMMUNE RESPONSES TO CANDIDATE VACCINE ANTIGENS DELIVERED THROUGH NAKED PLASMIDS AND MYCOBACTERIAL VECTORS

AbuSalim Mustafa

Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait; E-mail: abuselim@hsc.edu.kw

Tuberculosis (TB) is a major global health problem and has been declared “a global emergency” by the World Health Organization. The failures of the currently available vaccine against TB, *i.e.* *Mycobacterium bovis* BCG, to impart consistent protection against the TB disease have led to the need for alternative vaccines. The low molecular weight major antigenic proteins, *i.e.* PE35, CFP10 and ESAT6, encoded by *Mycobacterium tuberculosis*-specific region of difference-1 (RD1) are among the antigens considered important to develop new vaccines. To deliver these antigens, two DNA vaccine vectors (pUMVC6 and pUMVC7) and three live mycobacterial species (*M. bovis* BCG, *M. vaccae* and *M. smegmatis*) were used in this study. DNA corresponding to pe35 cfp10 and esat6 genes were amplified using polymerase chain reaction (PCR) from the genomic DNA of *M. tuberculosis* and cloned into pUMVC6 and pUMVC7 to construct the DNA vaccine candidates. Furthermore, the PCR-amplified DNA were cloned into a shuttle plasmid (pDE22), and the recombinant shuttle plasmids (pDE22-pe35, pDE22-cfp10 and pDE22-esat6) were electroporated into

the above stated three mycobacterial species. The recombinant vaccine constructs were used to detect the expression of the cloned proteins *in vitro* using antigen-specific antibodies in western blots. Furthermore, the induction of antigen-specific humoral and cellular immune responses *in vivo* was studied by immunizing mice, guinea-pigs and rabbits. The results showed the *in vitro* expression of PE35 and CFP10 by the recombinant constructs. Furthermore, both humoral (antigen-specific antibodies) and cellular immune responses (antigen-specific cellular proliferation and secretion of protective cytokines by immune cells) were consistently observed only with the recombinant DNA vaccine plasmid and mycobacterial constructs containing pe35 gene. Taken together, our results with five different delivery systems and three animal models suggest the superiority of PE35 antigen to develop a new vaccine against tuberculosis. The work was supported through Kuwait University Research Grants and SRUL02/13.

Keywords: Immune response, mycobacterial vectors, plasmid vectors, tuberculosis, vaccine.

SL- 188

Track: Drug Discovery in Preclinical Research

DESIGN AND SYNTHESIS OF NOVEL δ OPIOID AGONISTS AND THEIR PHARMACOLOGIES

Hiroshi Nagase^{1,3}, Yoshikazu Watanabe², Kohei Hayashida², Daisuke Saito², Toshihiro Takahashi², Junichi Sakai², Eriko Nakata², Takashi Kanda², Shigeto Hiroayama³, Takashi Iwai³, Hideaki Fujii³ and Tomio Yamakawa²

¹International Institute for Integrative Sleep Medicine, University of Tsukuba, Japan;
E-mail: nagase.hiroshi.gt@u.tsukuba.ac.jp

²Discovery Research Laboratories, Nippon Chemiphar Co., Ltd., Japan

³School of Pharmacy, Kitasato University, Japan

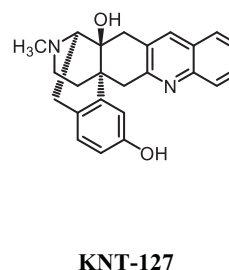
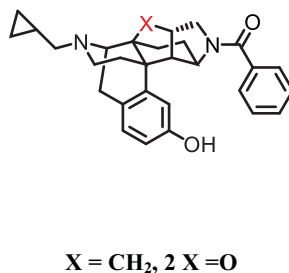
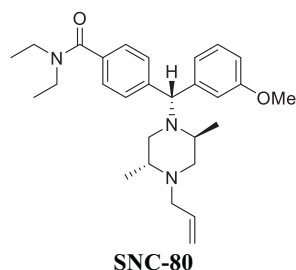


We already reported new types of δ opioid agonists, TAN-67 which had different structure from that of SNC-80. The weak analgesic effect of the compound led us to design a novel δ agonist KNT-127 (cAMP EC₅₀ = 141 nM, δ = 0.80 nM, κ = > 1000 nM, Emax δ = 95%) which showed strong analgesic effect (ED₅₀ = 1 mg/kg) and antidepressant effect (ED₅₀ = 3 mg/kg) in systemic administration.

Furthermore, the agonist showed neither catalepsy nor convulsion at even over 100 mg/kg different from SNC-80 derivatives.

Furthermore, recent our research led us to obtain δ opioid agonists 1 and 2 with novel skeleton which are more potent (1: cAMP EC₅₀ δ = 0.12 nM, μ and κ = 1000 nM) than KNT-127.

We will present the design and synthesis of these agonists and their pharmacologies.



SL- 318

Track: Drug Delivery and Targeting

SINGLE-STEP FORMATION AND CELLULAR RESPONSE OF VESICLES AND DISK-LIKE BICELLES

Wafa Aresh, Ying Liu, Jessica Sine, Derek Thayerc, Anu Puri and Mu-Ping Nieh

Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269, USA;

E-mail: mu-ping.nieh@ims.uconn.edu

The morphology of nanoparticles has been presumed to play an important role in cellular uptake and *in vivo* stability. Through the principle of self-assembly, we are able to construct uniform nanoparticles of two morphologies, *i.e.*, bicellar disks and nanovesicles of similar dimensions in an identical phospholipid mixture using a simple, scalable and reproducible platform. Both nanoparticles have similar bio-stability in serum and cytotoxicity to MDA-MB231 cell line, while the cellular uptake of nanodisks by a human lymphoma cell line, CCRF-CEM at 37°C shows 3~5 folds higher than that of the vesicles, indicating a strong morphological dependence of endocytosis. I will also discuss over four pathways of endocytosis (*i.e.*, clathrin- and caveolae-mediated endocytosis, macropinocytosis and microtubule-mediated transportation) in order to understand the enhanced cellular uptake of bicelles.

**SL- 182**

Track: Drug Discovery in Preclinical Research

DISCOVERY AND DEVELOPMENT OF SMALL MOLECULE MODULATORS OF APOPTOSIS AS NEW ANTICANCER DRUGS

Zaneta Nikolovska-Coleska

Department of Pathology, Medical School, University of Michigan, 4510E MSRB 1, 1150 West Medical Center Drive, Ann Arbor, MI 48109, USA; E-mail: zanetan@med.umich.edu

Human cancers have multiple genetic and epigenetic alterations leading to deregulation of variety of cellular processes and the dysfunction of the programmed cell death (apoptosis) machinery is recognized as one of the cancer hallmarks. Myeloid cell leukemia-1 (Mcl-1) is one of the anti-apoptotic proteins from Bcl-2 family and has a pivotal role in protecting cells from apoptosis. Mcl-1 is overexpressed in a variety of human cancers which become dependent upon this protein for survival and resistance to chemotherapy. Thus targeting Mcl-1 represents effective strategy for the next generation of anticancer therapies that specifically target resistance of cancer cells to apoptosis.

Applying an integrated screening approach through combining high throughput and virtual screenings, several promising lead compounds were identified as Mcl-1 inhibitors. We will present our efforts in development of two different chemical classes as Mcl-1 inhibitors including SAR studies and structural biology information that were crucial for compound optimization. The most potent compounds were further biologically characterized with a series of complementary biochemical, functional and cell based assays using model cell lines and established human cancer cells, demonstrating the on-target effect. Furthermore, development of these compounds allowed exploration of Mcl-1 as a potential therapeutic target for pancreatic cancer and the obtained results demonstrated *in vitro* and *in vivo* activity, as well as radiosensitization in pancreatic cell lines by inducing apoptosis.



SL- 203

Track: Academic CRO/Industrial Collaborations in Drug Discovery

FORMULATION OF FLOATING GASTRORETENTIVE METRONIDAZOLE MICROSPHERES USING CASSAVA STARCH (*MANIHOT ESCULENTA*) AS POLYMER

Oluwatoyin A. Odeku, Aderemi A. Aderogba, Olufunke D. Akin-Ajani and Tolulope O. Ajala

Department of Pharmaceutics & Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria;
E-mails: pejuodeFku@yahoo.com, o.odeku@ui.edu.ng



Floating gastroretentive microspheres have been used to prolong the gastric residence time after oral administration and improve local effect of metronidazole in stomach in the treatment of peptic ulcer caused by *Helicobacter pylori*. In the present study, cassava starch obtained from the tubers of *Manihot esculenta* Crantz, has been used as polymer in combination with sodium alginate for the formulation of floating gastroretentive metronidazole microspheres. Metronidazole microspheres were prepared by ionic gelation method using pregelatinized cassava starch and sodium alginate at different ratios as polymers and calcium chloride (2% w/v) as chelating agent. Sodium bicarbonate (2% w/w) was used as gas releasing agent to impart buoyancy. The microspheres were characterized using particle size, swelling, floating lag time, floating time and drug release properties. The result showed that spherical discrete microspheres with size ranging from 1.52 to 2.40 μm were obtained with floating lag time < 5 min and drug entrapment efficiency of 42 to 60% w/w. Buoyancy was maintained for up to 16h and the microspheres provided controlled release of metronidazole for over 18h. Drug release from the microspheres, swelling and buoyancy depended on the concentration of cassava starch in the polymer blend with formulations containing high concentration of cassava starch showing shorter floating lag time and faster drug release. Thus, buoyancy and rate of drug release appeared to be modulated by the concentration of cassava starch in the polymer blend. The results suggest that pregelatinized cassava could be useful for the formulation of floating gastroretentive metronidazole microspheres.

SL- 89

Track: Hot Topics in Natural Products

ANTICANCER, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME SYNTHESIZED URSOLIC ACID DERIVATIVES

Adebola Omowunmi Oyedeji

Department of Chemistry, Walter Sisulu University, South Africa; E-mail: aoyedeji@wsu.ac.za

Structure Activity Relationship (SAR) on Ursolic acid isolated from *Mimusops caffra* was carried out where three more C3 and C28 modified-analogues of UA were successfully synthesized in good yields; 3-Acetyl-UA-28-methylate (70%), 3-Acetyl-UA-28-benzylate (52%) and 3-Acetyl-UA-28-cinnamate (48%). The biological evaluation of all these compounds as antioxidants, antimicrobial and anticancer. DPPH was used to screen all compounds for their antioxidant property and the derivatives showed higher activity than the parental compounds. The highest percentage inhibition was obtained from 3-Acetyl-UA-28-benzylate (59%) and 3-Acetyl-UA-28-cinnamate (65%) respectively. The antimicrobial activity against five enterococcus strains was evaluated on the compounds and the MIC and MBC of 3-Acetyl-UA indicated that this compound has the potential use as antimicrobial drug. The highest activity was obtained for this compound against the *Enterococcus avium* strain at 0.17mg/mL. The cytotoxicity of all compounds was evaluated on Human embryonic cells (HEK293) and Human hepatocellular carcinoma cells (HepG2) by MTT assay. All the compounds were not toxic to cells since cell functioning was observed in the presence of the drugs and cell mortality did not reach 50%, $\text{IC}_{50} > 300\mu\text{g/mL}$.

SL- 83

Track: CNS Drug Discovery & Therapy

HYPOXIA ISCHEMIA IN THE NEONATAL RAT: THERAPEUTIC HYPOTHERMIA AND MAST CELL STABILIZATION AS ADJUNCT PHARMACOTHERAPY

Shyama D. Patel

*Department of Pediatrics/Newborn Medicine, Weill Cornell Medical College, New York, NY, USA;
E-mail: SHP2010@med.cornell.edu*

Neonatal hypoxia-ischemia (HI) is a major cause of mortality/morbidity; therapeutic hypothermia (TH) is the only available intervention but protection is incomplete. Adjunct therapy is desperately needed to improve neuroprotection. We recently expanded our neonatal rodent model of HI to include TH, as a preclinical platform to screen adjunct pharmacotherapy. Mast cell activation is involved in HI damage; our candidate therapeutic is Cromolyn for mast cell stabilization. We evaluated short- and longterm outcome following HI with TH with and without Cromolyn (TH +C), vs normothermic (N) in the neonatal rat. Histologic analysis demonstrated enhanced protection with Cromolyn at 1 week post-HI

Keywords: Hypoxia-ischemia, mast cells, therapeutic hypothermia.

**SL- 255**

Track: Pharmaceutical Research and Development

IODOTHYRONINE DEIODINASES: DETERMINATION OF THEIR ACTIVITIES BY NOVEL RADIOMETRIC ENZYME ASSAYS

Stanislav Pavelka

Department of Radiometry, Institute of Physiology, Academy of Sciences of the Czech Republic, Czech Republic; E-mail: pavelka@biomed.cas.cz

Iodothyronine deiodinases (IDs) are the key enzymes in the metabolism of thyroid hormones. We developed novel, reliable and robust radiometric methods for extremely sensitive determination of enzyme activities of IDs of types 1, 2 and 3 in microsomal fractions of various rat and human tissues, as well as in homogenates of cultured mammalian cells. The elaborated radiometric enzyme assays were based on the use of high specific-radioactivity ¹²⁵I-labeled iodothyronines as substrates; TLC separation of radioactive products from the unconsumed substrates; film-less autoradiography of radiochromatograms using storage phosphor screens; and quantification of the separated compounds with a BAS-5000 laser scanner (Fujifilm Life Science) equipped with an evaluating software AIDA (Raytest). This methodology enabled us to determine IDs enzyme activities as low as 10 exp -18 katals. We demonstrate applicability of our advanced assays by following the alterations of IDs activities induced in cultured astroglial cells by a series of purinergic agonists, by retinoic acid, and/or combination of these drugs.



SL- 131

Track: Cardiovascular Drug Discovery & Therapy

GINKGOLIDE B REDUCES TLR4 AND INFLAMMATORY PROTEIN EXPRESSION IN GLUCOSE-TREATED HUVECS

Ruomei Qi and Wenjia Sun

Beijing Institute of Geriatrics, Beijing Hospital and key laboratory of Geriatrics Ministry of Health, Beijing, 100730, China; E-mail: ruomeiqi@163.com

Background: Growing evidence indicated that toll-like receptors (TLRs) play a key role in atherosclerotic disease process. High glucose can induce an increase on TLR4 expressions in endothelial cells. However, the mechanism is not completely understood. In the study, we investigated the effect of ginkgolide B (a PAF receptor antagonist) on TLR4, PAF receptor expression and the underline mechanism in high glucose-treated endothelial cells.

Methods: Human umbilical vein endothelial cells were stimulated by high concentration of glucose. TLR4, PAF receptor, the inflammatory protein expression and Akt phosphorylation was analyzed by Western blotting. Transcription factor NF- κ B nuclear translocation was analyzed by immunofluorescence.

Results: Ginkgolide B (PAF receptor inhibitor) dose-dependently decreased TLR4 and PAF receptor expression in high glucose-treated endothelial cells, respectively. Ginkgolide B decreased ICAM-1, VCAM-1, PECAM-1 expression and NF- κ B p65 nuclear translocation. Ginkgolide B abolished MyD88 and TRIM expression induced by high glucose, which are the downstream molecules of TLR4 signaling. Moreover, ginkgolide B significantly suppressed Akt, Erk, p38 MAPK phosphorylation in high glucose-treated cells.

Conclusion: Ginkgolide B can decrease TLR4, PAF receptor and the inflammatory protein expressions in high glucose-treated endothelial cells. The mechanism might be linked to Akt, Erk, and p38 MAPK phosphorylation. Our results provided novel insight that inhibition of TLR4 and PAF receptor by ginkgolide B might be an important strategy for protection of endothelial cells in diabetes.

**SL- 11**

Track: Cardiovascular Drug Discovery & Therapy

CHEMOKINES AS DRUG TARGETS FOR CARDIOVASCULAR DISEASES

Krishna Rajarathnam

University of Texas Medical Branch – Galveston, USA; E-mail: krrajara@utmb.edu

A hallmark of cardiovascular diseases and injuries, including myocardial infarction and reperfusion injury, is the rapid mobilization of circulating neutrophils to the heart. Chemokines play a crucial role in recruiting neutrophils to initiate repair, but a dysregulation in this process leads to failed resolution and collateral tissue damage, and in severe cases, death. Neutrophil-activating chemokines (NACs) exist as monomers and dimers, mediate function by activating G-protein-coupled receptors (GPCRs) expressed on neutrophils and binding glycosaminoglycans (GAGs) on endothelial cells and the extracellular matrix. Studies from our lab have shown that NACs also form heterodimers, that monomers, homodimers, and heterodimers differentially bind GAGs, that monomer-dimer equilibrium regulates *in vivo* recruitment, and that high dimer levels could be responsible for uncontrolled recruitment and tissue damage. We propose molecules that inhibit chemokine dimerization and/or inhibit dimer function could be highly beneficial in a clinical setting. I will discuss how our structural studies on chemokine-CXCR2 and chemokine-GAG interactions could be exploited to design drugs for treating neutrophil-mediated diseases.



SL- 148

Track: Drug Discovery in Preclinical Research

THE USE OF MICROSCALE THERMOPHORESIS, VIRTUAL SCREENING AND THERMAL SHIFT ASSAYS FOR THE RAPID IDENTIFICATION OF FRAGMENTS ACTIVE AGAINST HUMAN KINASE

Dennis Breitsprecher, Kwame Amaning, Stefan Duhr and Alexey Rak

Structure-Design-Informatics/LGCR, Sanofi R&D, France; E-mail: alexey.rak@sanofi.com

Biophysical approaches are routinely used to assess the binding constants of molecular interactions as integral part of the drug discovery process being essential for efficient lead optimization. Furthermore, modern drug discovery operations require characterization of biomolecular interactions to be both time- and cost-effective. Here we report the analysis of an in-house fragment screening campaign for the oncology target - MEK1. The application of virtual screening (VS) as a primary fragment screening approach was followed by biophysical validation using Differential Screening Fluorimetry (DSF) and MicroScale Thermophoresis (MST), with resultant binding mode determination by X-ray crystallography (X-ray). Using the Monolith NT.Automated instrument, we screened a library containing 193 pre-selected fragments for their interaction with MEK1. We identified > 70 binders with dissociation constants (K_ds) ranging from the low μ M to low mM values. 16 fragments displayed K_ds below 100 μ M. Importantly, 7 out of 8 previously determined hits were among the top-fifteen fragments from our MST-ranking and yielded X-ray co-crystal structures with MEK1. Moreover, the MST ranking showed a very strong correlation with a qualitative DSF screening. We demonstrate the effectiveness of the VS-DSF-MST workflow for the early identification of fragments to both 'jump-start' the drug discovery project and to complement biochemical screening data.

Keywords: Small molecules, fragments, screening, biophysical methods.

**SL- 69**

Track: CNS Drug Discovery and Therapy

DEVELOPMENT OF CIRCADIAN RHYTHM BASED FORMULATION OF ATOMOXETINE HYDROCHLORIDE FOR THE TREATMENT OF ATTENTION DEFICIT HYPERACTIVITY DISORDER

Akila Ramanathan, P. Chandran, Rao Gopal and T.K. Ravi

College of Pharmacy, Department of Pharmaceutics, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, Tamilnadu, India; E-mail: ktakila@yahoo.co.in

Attention-deficit/hyperactivity disorder (ADHD) is a common neurological disease affecting 5-8 percent of school going children with symptoms persisting into adulthood in about 60 percent of cases. ADHD severity correlates positively with circadian delay *i.e.* retarded sleep timing and day time sleepiness, implying that treatment interventions focussed at advancing circadian phase may make better day time sleepiness.

So, the present research work was aimed to formulate pulsatile release tablets of atomoxetine hydrochloride in morning hours by increasing adrenergic hormones in the brain. The core tablets were prepared by direct compression and press coated with HPMC K100M and MCC and the release was compared with natural polymers Guar gum (6000 cps) and Xanthan gum (1800 cps). The 32 full factorial design and one-way Anova were employed to evaluate the contribution of natural and synthetic polymers in drug release and lag time. IR spectrophotometer study



showed that all the excipients were compatible with the drug. The stability study was carried out for the desired optimized formulation for a period of 3 months and showed insignificant difference.

Keywords: ADHD, atomoxetine hydrochloride, circadian rhythm, factorial design, pulsatile.

SL- 260

Track: Pharmaceutical Research & Development

FROM PATIENT BIOSPECIMENS TO THERAPEUTIC SOLUTIONS: NEW DEPARTURE IN DRUG DISCOVERY

Araz A. Raoof and Jeroen Aerssens

Discovery Sciences, Janssen Research & Development, USA; E-mail: ARAOOF@its.jnj.com

The pharmaceutical industry is struggling with how to innovate drug discovery research and improve R&D productivity. Whether it is phenotypic or target-centric, the drug discovery process currently used does not predict clinical efficacy and its sustainability is questionable in view of high clinical attrition rates and associated costs. In this presentation we propose an alternative approach that starts with patients and aims at identifying biologic pathways involved in human diseases as a turning point in the discovery of novel therapeutics. This approach utilizes emerging biomedical and technology advances and builds further on the industry's existing knowhow in chemistry and biology. Our new model calls for changes in the rather linear R&D activity chain currently in practice.



Keywords: New Drug Discovery Model, R&D Productivity.

SL- 242

Track: Hot Topics in Medicinal Chemistry

MOLECULAR HYBRIDS: AN INNOVATIVE APPROACH IN DRUG DISCOVERY

Diwan S. Rawat

*Department of Chemistry, University of Delhi, Delhi-110007, India;
E-mail: dsrawat@chemistry.du.ac.in*

The design of new molecules with improved ADME properties along with effective pharmacological potency; lack of toxicity and devoid of resistance for the treatment of infectious diseases has remained a big challenge for the scientific community. In order to address these issues concept of hybrid molecules was put forward which deals with the covalent hybridization of two or more distinct pharmacophores into a single molecule that may lead to a hybrid molecule with improved efficacy. [1-3]. This approach may solve the problem of drug resistance and reduce the undesired side effects [4]. The development of such molecular frameworks with synthetic selectivity and economic viability is still a challenging task for the pharmaceutical industry. Drugs developed through this approach can be used for the cure of infectious diseases where treatment is limited to few drugs and the known drugs have limitations such as toxicity, pharmacokinetics, pharmacodynamic and drug resistance. The benefit of using molecular hybrid is to activate different or same targets by a single molecule, and increase the therapeutic efficacy and to improve the bioavailability. Molecular hybridization approach has already resulted many drug candidates with improved activity profile and some of these compounds are in clinical trials. Towards these goals we have synthesized various molecular hybrids and tested these for antimalarial, anti-TB and anti-cancer activities and efforts will be made to present our recent work [5-17].

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SL-217(a)

Track: Drug Discovery in Preclinical Research

NANOSCALE DRUG CARRYING SYSTEMS TO PREVENT PLACENTAL PASSAGE OF HARMFUL DRUGS TO THE FETUS IN A PREGNANT RODENT MODEL

Jerrie S. Refuerzo

University of Texas Health Science Center at Houston, USA; E-mail: Jerrie.S.Refuerzo@uth.tmc.edu

Preterm labor resulting in preterm birth affects up to 12% of pregnancies in the United States and is the major contributor to neonatal morbidity and mortality. Indomethacin is a drug used clinically to stop preterm labor, but can lead to serious fetal side effects because it crosses the placenta. Nanovectors are nanoscale particles or integrated systems with a variety of physico-chemical and biological properties, such as size, charge and targeting moieties, that can be customized based on the intended use. These properties allow the design of the nanovectors to preferentially deliver a drug to the tissue of interest and prevent its distribution to unwanted locations.

This presentation focuses on the application of nanotechnology to enable targeted administration of indomethacin to the uterus for treatment of preterm labor and reduction of the drug passage to the fetus. First, we will illustrate how modification of the physico-chemical properties of porous silicon nanovectors can prevent its transplacental passage in pregnant rodents. Second, we will present our recent work with indomethacin-carrying liposomes ability to reduce indomethacin concentrations in the fetus 7-fold while maintaining its pharmacological effects. We believe that these innovations will pave the ground for a new paradigm-shifting direction in the treatment of high risk pregnancies.

SL- 151

Track: In-Silico Drug Design and In-Silico Screening

SIMILARITY AND DIFFERENCE IN MECHANISMS OF ACTION OF ANTISTAPHYLOCOCCAL ANTIBIOTICS MUPIROCIN AND BATUMIN REVEALED BY IN-SILICO MOLECULAR DOCKING

J.Y. Kim, V.V. Klochko, E.V. Zhuravel, M.A. Soldatkina and O.N. Reva

Bioinformatics and Computational Biology Unit, Department of Biochemistry, University of Pretoria, Pretoria, South Africa; E-mail: oleg.reva@up.ac.za

Mupirocin and batumin antibiotics synthesized by *Pseudomonas* are encoded by large NRPS-PKS operons. Mupirocin is widely used as a topical treatment of open wounds against *Staphylococcus aureus* infection including MRSA. Cases of mupirocin resistance have been reported. Batumin is a recently discovered antibiotic with an order of magnitude higher activity against *S. aureus*. Despite some chemical similarity between batumin and mupirocin, and sequence similarity of encoding operons, an alternative mechanism of action of batumin was reported in literature as an inhibitor of FAS-II fatty acid biosynthesis pathway, while mupirocin was believed to inhibit isoleucyl-tRNA synthetase. Molecular docking analysis by Accelrys Discovery Studio showed that batumin molecules cannot bind directly to the target protein FabI, but have strong affinity to a broader spectrum of tRNA synthetases. Possible association between inhibition of tRNA synthetases and down-regulation of the fatty acid biosynthesis was considered. Search through the database PharmaDB revealed that mupirocin and batumin may bind to other important drug targets including the N-terminal transcriptional activation domain of p53 tumor suppressor with a binding energy comparable to that of designed p53 inhibitors. This study provided new details on antibacterial activity of mupirocin and batumin, and demonstrated other areas of application of these antibiotics.

SL- 261

Track: Neurodegenerative Disorders

COOPERATIVE RNA MODULATION OF NEURODEGENERATIVE DISEASE TRANSCRIPTS TO IMPROVE IRON HOMEOSTASIS WHILE ALSO PROVIDING ANTI-AMYLOID EFFICACY

Jack T. Rogers, Kevin Hodgetts, Yanyan Liu, Qinjun Li, Catherine M. Cahill and Xudong Huang

Neurochemistry Laboratory, MGH, Harvard, USA; E-mail: jack.rogers@mgh.harvard.edu

We characterized a potent neuroprotective activator of translation of the mRNA for the iron-storage protein ferritin in the network of mRNAs encoding iron-associated proteins. These iron homeostatic proteins include the neurodegenerative amyloid precursor protein (APP) that we recently discovered to have a functional role as an activator of protective ferroportin-dependent iron export from iron burdened neurons. In addition to APP mRNA, the prion precursor protein (PrP) was translated *via* iron modulated RNA protein binding events at an iron-responsive element (IRE)-like RNA stem loop in the 5' untranslated regions (5'UTRs) of its transcript. Our lead translation modulator BL-1 was highly trophic as a consequence of activating neural ferritin levels to safely store excess iron and to prevent toxic buildup of metal catalyzed oxidative radical formation. BL-1 is a peptidylbenzimidazole that was first identified after a high throughput screen for 5'UTR inhibitors of the prion-generating PrP mRNA when conducted at the Broad Institute (Cambridge MA). BL-1 was then found to co-inhibit APP while also activating ferritin translation *via* the related, although uniquely folded, IRE RNA stem loops in the 5'UTRs of their transcripts. As proof-of concept for RNA based therapeutics to cure neurodegenerative diseases, we published concerning potent FDA preapproved APP translation blockers that selectively inhibited the unique IRE stem loop in the 5'UTR of APP mRNA. These FDA drugs included N-acetyl cysteine (antioxidant and iron chelator) and paroxetine (SSRI) which each indeed generated anti-amyloid efficacy *in vivo* in the brains of the APP-5'UTR-positive TgCRND8 APP transgenic mouse model of Alzheimer's disease. We will reveal the efficacy and mechanism-of-action of high-throughput screened and highly novel translation



blockers of both APP and the co-seeding prion protein, with a prospectus for interpreting how these anti-amyloid agents also ameliorate brain iron homeostasis. Our therapeutic goal is to develop BL-1 as our lead small molecule activator of ferritin translation to improve neuronal health and to be ultimately boost cognitive and fear conditioned responses in an established mouse model for AD.

SL- 26

Track: Cancer Targeted Drug Delivery

A HIGHLY EFFICIENT *IN VIVO* THERAPY FOR MELANOMA AND OTHER CANCER TYPES

Karli Rosner^{1,2}, Fredric P. Manfredsson⁴, Evangelia Kirou¹, Rhyomi Sellnow⁴, Judith Abrams⁴, Seongho Kim⁴, Tal Rosner¹ and Darius R. Mehregan^{2,5}

¹Laboratory for Molecular Dermatology, Barbara Ann Karmanos Cancer Institute, USA; E-mail: krosner@med.wayne.edu

²Department of Dermatology, Wayne State University School of Medicine, Detroit, MI, USA

³Translational Science & Molecular Medicine, Michigan State University College of Human Medicine, Grand Rapids, MI, USA

⁴Biostatistics Core, Barbara Ann Karmanos Cancer Institute, Detroit, MI, USA

⁵Pinkus Dermatopathology Laboratory, Monroe, MI, USA

We developed a genetic-based therapeutic modality for multiple cancer types. Using a highly efficient FDA approved delivery system, this modality attained more than 75% regression in human melanoma xenografts after single administration. Recently, it also demonstrated 97-100% killing efficiency through apoptosis in multiple cell types originating from human primary and metastatic melanomas.

Our therapy has been devised to overcome cancer resistance to apoptosis, which accounts for the inability of recently developed targeted therapies and immunotherapies to eradicate metastatic disease and achieve cure. The therapy bypasses all known cancer pro-life signaling and triggers apoptosis without activating the apoptosis cascade. This is achieved by inducing apoptosis at the bottom of the cascade. We introduced genetic codes into cancer cells, which in turn translated the codes into human recombinant deoxyribonuclease1 (hrDNase1) protein that avoids being secreted from the cells, resists inactivation by actin, accesses nuclear DNA and initiates apoptosis through DNA damage.

The hrDNase1 approach provides several substantial advantages over other therapies. hrDNase1 achieves high killing efficiency without requiring an external pro-drug or the non-selective cell killing by the 'bystander-effect' mechanism. This will enable hrDNase1 therapy that incorporates tissue-specific genetic signatures to achieve highly, selective targeting of different cancer types with minimal adverse effects.

SL- 322

Track: Drug Delivery and Targeting

PROLONGED ACTING NANOMEDICINE BASED ANTI-HIV DRUG DELIVERY TARGETING GUT ASSOCIATED LYMPHOID TISSUES

Upal Roy, Hong Ding, Sudheesh P Kanthikeel, Andrea Raymond, Elena M. Kaftanovskaya, Elena Batrakova and Madhavan Nair

Department of Immunology, Institute of NeuroImmune Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, Florida 33199, USA; E-mail: uroy@fiu.edu

Present Antiretroviral Therapy has significantly improved the therapeutic outcome of Human Immunodeficiency Virus (HIV-1) infection. Nonetheless, very few current anti-HIV drugs can penetrate at therapeutic level to the viral reservoirs such as gut associated lymphoid tissue (GALT). Thus, we are proposing a prolong acting nanodrug formulation that is targeted towards GALT. In this regard, we have developed a pluronic nanocarrier containing anti-HIV drug efavirenz (EFV) (EFV-F12-COOH or nanodrug) bio-conjugated with a Microfold cell (M-cell) specific marker antibody (anti-Glycoprotein 2) targeted towards GALT. M-cells are specialized epithelial cells that are predominantly present in the gastrointestinal tract that transport different molecules from lumen to cells of the immune system. The present work has exploited the transcytosis property of M cells for targeted delivery of nanodrug to the GALT. Preliminary characterization showed that the nanodrug is of 110 ± 20 nm size and has shown significantly improved sustained release over unformulated drugs in an *in vitro* M-cell model. This model was further used to characterize nanodrug cytotoxicity, and drug loading capacity. Finally, the therapeutic efficacy of the nanodrug against HIV-1 was significantly high compared to unformulated drug. The present study will help in long term remission of HIV-1 in the GALT and improved treatment outcome.

SL- 143

Track: Cardiovascular Drug Discovery and Therapy

RATIONALLY DESIGNED ALLOSTERIC EFFECTORS OF HEMOGLOBIN EXHIBIT SUPERIOR ANTISICKLING PROPERTIES

Martin K. Safo¹, Tanvi Deshpande¹, Mohini Ghatge¹, Guoyan Xu², Apurvasena Parikh³, Jurgen Venitz³, Yan Zhang² and Osheiza Abdulmalik⁴

¹*Institute for Structural Biology and Drug Discovery and Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA, USA; E-mail: msaf@vcu.edu*

²*Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA, USA*

³*Department of Pharmaceutics, Virginia Commonwealth University, Richmond, VA, USA*

⁴*Division of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA*

Sickle cell disease (SCD) causes significant morbidity, mortality and healthcare disparities in the US and in other places. The fundamental pathophysiology of SCD involves the polymerization of deoxygenated sickle hemoglobin (Hb S) under hypoxic condition, which leads to several secondary adverse events, including sickling of red blood cells (RBCs), hemolysis, inflammation, vaso-occlusion, organ damage, and painful crises. Current investigations focus on candidate drugs which bind to Hb S and increase its oxygen affinity to prevent the hypoxic-related polymerization. Recent promising reports from a Phase I/II study on 5-HMF (aka Aes103 or Bax555) renews optimism for this line of investigation.

We rationally modified Vanillin--a previously reported candidate antisickling drug with a known non-toxic profile--into pyridyl derivatives to enhance its pharmacodynamic properties. X-ray crystallography studies of two representative compounds, INN-270 and TD-7 showed them to react with normal hemoglobin (Hb) as predicted. A time- and dose-dependent modification of Hb (by HPLC analyses) showed the compounds exhibited ~10-fold superior binding affinity

compared to Vanillin. At 2 mM concentration, TD-7 modified Hb S ($92.3 \pm 5.2\%$, $n=4$), increase Hb S oxygen affinity ($\Delta p50 = 45.6 \pm 8.2\%$, $n=3$), and inhibited RBC sickling ($95-100\%$, $n=4$), all in a dose-dependent manner. INN-270 showed similar impressive result, though with a lower potency: Hb S modification of $\sim 75\%$; $\Delta p50 = 40.3\%$; sickling inhibition at $\sim 70\%$. Significantly, while the pharmacologic effect (Hb S modification) of INN-270 returned to baseline after 24 hours, modification by TD-7 persisted at high levels ($\sim 50\%$).

In conclusion, both INN-270 and TD-7 exhibited approximately 40-fold superiority in antisickling potency compared to Vanillin, with TD-7 showing a remarkable long lasting effect. Our findings justify a structure-based approach to designing novel antisickling agents with enhanced pharmacodynamic properties. *In-vitro/ex-vivo* PK/PD studies are currently ongoing to help guide planned animal studies.

SL- 25

Track: Cardiovascular Drug Discovery and Therapy

NATURAL AND SYNTHETIC CAFFEIC ACID-BASED OLIGOMERS: ANTI-OXIDATIVE, ANTI-ELASTASE AND VEGF MODULATORY ACTIVITIES FOR TREATMENT OF EMPHYSEMA

Masahiro Sakagami¹, Sneha Dhapare¹, Norbert F. Voelkel¹ and Umesh R. Desai²

¹Department of Pharmaceutics, and ²Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia, USA; E-mail: msakagam@vcu.edu

Caffeic acid is a key intermediate in the biosynthesis of lignin and a natural anti-oxidant phenolic molecule. Its natural dimeric and tetrameric oligomer derivatives, rosmarinic acid and salvianolic acid B (Sal-B), however, exert distinct pharmacological activities that are not shared by caffeic acid, in addition to the anti-oxidative activities. Such activities include anti-coagulation, anti-inflammation, anti-apoptosis/cytoprotection and angiogenesis. Thus, their therapeutic potentials have been studied primarily for the treatment of cardiovascular and cerebrovascular diseases, but not lung diseases like emphysema and COPD. Meanwhile, we have recently discovered that our ~ 3 kDa synthetic oligomers of caffeic acid, CD and CDSO₃, further possessed anti-elastase and iron (Fe^{2+}) chelating activities that neither caffeic acid nor its small natural oligomers carry, as shown in Table 1. In these activities, the sulfated oligomer CDSO₃ was 7.0- and 2.1-fold more potent than the unsulfated oligomer CD, in addition to a 1.8-fold greater anti-oxidative activity (Table 1).

To date, emphysema and COPD remain incurable, and drugs presently in use cannot repair the destroyed lung. We have proposed therefore a new pathobiologic concept, “vascular endothelial growth factor (VEGF) deficiency”, yet effective means of VEGF normalization and upregulation have yet to be identified. Here, we report our recent findings of unique lung repairing activities displayed by two caffeic acid-based oligomers, Sal-B and CDSO₃, in a rat model of *established* emphysema, and explain their repair mechanisms as based on restored VEGF expression. As shown in Fig. (1), the 2 weeks lung treatment of Sal-B (0.2 mg/kg) and CDSO₃ (0.06 mg/kg) caused remarkable improvements in the rat treadmill exercise endurance time. Reduced VEGF expression in the lungs of these animals was restored, which appeared to involve transcription factors, *signal transducer and activator of transcription 3 (STAT3)* and hypoxia-inducible factor 1- α (HIF1 α), respectively, the latter specifically due to Fe^{2+} chelation. Therefore, STAT3/ HIF1 α -dependent VEGF increase could be a novel strategy for lung repair in emphysema and COPD.

Table1. *In vitro* anti-oxidative, anti-elastase and Fe²⁺ chelating activities of caffeic acid (CA), salvianolic acid B (Sal-B), unsulfated and sulfated CA oligomers, CD and CDSO3

Molecule	CA Unit	In Vitro Half-Maximal Effective Concentration [μM]		
		Anti-Oxidation ¹	Anti-Elastase ²	Fe ²⁺ Chelation ³
CA	1	16.8 ± 1.2	>100 ^a	>100 ^a
Sal-B	4	1-10	>100 ^a	>100 ^a
CD	10-26	6.2 ± 0.3	2.8 ± 0.2	48.4 ± 12.0
CDSO3	10-26	3.5 ± 0.1	0.4 ± 0.04	23.3 ± 2.0

¹Radical scavenging inhibition; ²Chromogenic substrate elastolytic inhibition.

³Ferrozine-Fe²⁺ chelation inhibition

^aNo activity at ≤100 μM.

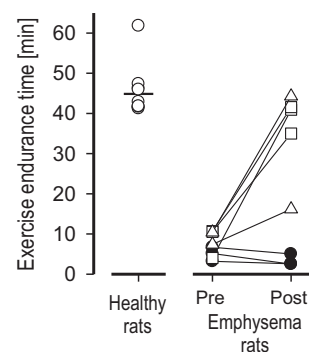


Fig. (1). Improved treadmill exercise endurance times following 2 weeks lung treatment of Sal-B (0.2 mg/kg) and CDSO3 (0.06 mg/kg), compared to placebo (saline) treatment, in an *in vivo* rat model of established emphysema.

SL- 76

Track: CNS Drug Discovery and Therapy

SOLUTE CARRIER 6A1 (SLC6A1; GAT1) TRANSPORTER SUBFAMILY AS A TARGET FOR NOVEL MEMORY-ENHANCING DRUGS

Kinga Salat

Department of Pharmacodynamics, Jagiellonian University, Kazimierz, Poland;

E-mail: salat.kinga@gmail.com

Background: GABAergic neurotransmission is involved in long-term potentiation, a neurophysiological basis for learning and memory. On the other hand, GABA-enhancing drugs may impair memory and learning. In this study the influence of a GAT1 inhibitor on cognition was investigated.

Methods: Albino Swiss (CD-1) and C57BL/6 mice were used in the passive avoidance test (PA), Morris water maze (MWM) and radial-arm water maze (RAWM). Scopolamine (1 mg/kg ip) was applied to induce cognitive deficits.

Results: In the retention trial of PA GAT1 inhibitor showed no effect on the step-through latency. In MWM mice treated with GAT1 inhibitor compared to scopolamine-treated control mice demonstrated improved learning abilities in the acquisition trial. In RAWM on day 1 scopolamine-treated control mice made nearly two-fold more errors than vehicle-treated mice and mice that received combined scopolamine and GAT1 inhibitor. Learning abilities in latter group were similar to these of vehicle-treated mice in the corresponding trial block on day 1. GAT1 inhibitor had no effect on memory retrieval on day 2.

Conclusion: These results show that GAT1 may be a promising drug target in the search for drugs attenuating cognitive impairments.

This work was financially supported by DEC-2012/05/B/NZ7/02705.

Keywords: Cognitive deficits, fear-motivated task, GAT1, mice, spatial memory.



SL- 202

Track: Drug Metabolism

USE OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING TO ASSESS DRUG INTERACTION POTENTIAL OF ANTIBODY-DRUG CONJUGATES (ADCs)

Divya Samineni, Yuan Chen, Sophie Mukadam, Harvey Wong, Ben-Quan Shen, Dan Lu, Cornelis Hop, Sandhya Girish, Jin Yan Jin, Bert Lum and Chunze Li

*Clinical Pharmacology, Genentech Inc, 1 DNA Way, South San Francisco, CA, USA;
E-mail: samineni.divya@gene.com*

Introduction: Antibody–drug conjugates (ADCs) consist of cytotoxic drugs covalently linked to monoclonal antibodies directed to antigens differentially overexpressed in tumor cell. MMAE, a commonly used cytotoxin, which when formed during the ADC catabolism, is a small molecule and may have DDI potential. The objective was to build a physiologically based pharmacokinetic (PBPK) model to assess the potential for drug interactions (DIs) with MMAE ADCs.

Methods: A PBPK model was developed using the *in silico*, *in vitro* ADME and *in vivo* pharmacokinetic (PK) data from the anti-CD22-vc-MMAE ADC. Subsequently, the model was validated and was used to simulate the clinical DI studies between brentuximab vedotin and the prototypical CYP agents.

Results: The PBPK model well described the PK profiles of MMAE for the anti-CD22 ADC and brentuximab vedotin. The model predicted a limited DI potential for brentuximab vedotin with strong CYP3A inhibitors, inhibitors, or substrates and is in close agreement with the observed data.

Conclusion: A PBPK model for MMAE ADCs was developed, validated and applied to predict a low MMAE-DI risk for brentuximab vedotin. This is the first demonstration of the PBPK model application to assess the lack of a need to perform clinical DI studies for MMAE-based ADCs.

SL- 110

Track: CRO/Academic CRO/Industrial Collaborations in Drug Discovery

ULEINE

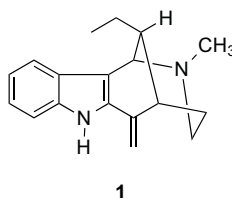
Cid Aimbiré M. Santos

Universidade Federal do Paraná, Curitiba, PR, Brazil; E-mail : cid@ufpr.br

Uleine (**1**) was isolated from *Aspidosperma lei*, Apocynaceae and its chemical structure of uleine was firstly presented by Buchi and Warnoff in 1959 [1]. Two species from Brazil have shown the presence of high content of uleine and its alkaloidal rich-fractions has shown a wide variety of biological activities attributed to uleine. First, a broad spectrum of *in vitro* antimicrobial activities against pathogenic microorganisms has been demonstrated [2]. A gastroprotective effect of this fraction, in which uleine was the main constituent, has been described [3]. In addition, this fraction was able to alter vascular and non-vascular smooth muscle responsiveness [4], and uleine purified from *H. lancifolius* bark was shown to influence the production of nitric oxide [5]. The effects of the alkaloid-rich fraction on normal marrow cells and leukemic cell lines indicated that the cells were not undergoing apoptosis or necrosis, suggesting cytostatic activity for tumor cells [6]. Also the same fraction on human peripheral blood mononuclear cells was able to inhibit the proliferation of phytohemagglutinin-stimulated lymphocytes by blocking their transformation into blast-dividing cells. Furthermore, it inhibited the proliferation of Daudi and Reh cells, two leukemic cell lines of lymphoid origin. The present study widens the biological applications of *uleine* to include its possible use as a modulator of the immune system [7, 8]. More recently, uleine showed an interesting multi-target activity involved in Alzheimer Disease treatment. An initial screening campaign on uleine rich-fractions obtained from *Himatanthus lancifolius*, the primary alkaloid uleine was observed to demonstrate a promising inhibitory activity against AChE from the *electric eel* [9]. The alkaloid could also inhibit both human cholinesterases (hrAChE and hBuChE). More interestingly, uleine also exhibited a strong

inhibitory activity, within the nanomolar range, against BACE-1 and could significantly inhibit β -amyloid ($A\beta_{42}$) self-aggregation. With BACE1 involved in the production of $A\beta$, uleine may interfere with the neurotoxic effects of aggregated amyloid peptides by acting at two different levels, *i.e.* preventing amyloid production and interfering with amyloid oligomerisation into toxic species [10]. The ability of uleine to act on selected targets involved in Alzheimer Disease treatment makes it a promising new chemical scaffold to be further developed for a more effective treatment of this pathology.

On the basis of these premises, the remarkable nanomolar inhibitory potential of uleine on BACE1, with its ability to weakly inhibit $A\beta$ self-aggregation and the two enzymes that regulate ACh, may establish a novel and interesting AD disease modifying profile. Such a profile has not yet been achieved by the current marketed products or by several other more active but selective naturally occurring compounds described so far, and, therefore, deserves further investigation.



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SL- 311

Track: Innovative Drug Discovery and Nanotechnology

THE EFFECT OF ULTRASOUND OF AMPLITUDE AND EXPOSE TIME ON THE PARTICLE SIZE AND SOLUBILITY OF NAPROXENE SODIUM

Gökhan Savaroğlu, Lütfi Genç, Aygün Ceren İldaşer, Evrim Hür and Müjdat Çağlar

Eskisehir Osmangazi University, Department of Physics, Eskisehir, Turkey; E-mail: gsavarog@ogu.edu.tr

Backgrounds: Solubility is the most important parameters for bioavailability of drugs. Low aqueous solubility is the major problem encountered with formulation development of new active molecules. Different techniques have been used for improving of the solubility of poorly soluble drugs which include physical and chemical modifications of drug and other methods like particle size reduction, crystal technology, salt formation, solid dispersion, use of surfactant, complexation, and *etc.* Selection of method depends on drug properties.

Material and Methods: In an attempt to improve solubility of poorly aqueous soluble naproxen sodium, nanoparticle were produced by using solvent/antisolvent technique with ultrasound. In this study, effect of different amplitude levels

and expose times on particle size and solubility of naproxen sodium (Nap.Na) were investigated. A simple, rapid and reliable UV spectroscopy was used for the determination to solubility for Nap.Na with and without imposed ultrasound. Zeta potential (Z.P.) and particle size of Nap.Na with and without imposed ultrasound were measured by using Nano Zeta Sizer. The morphology of drug particles were studied by Scanning Electron Microscopy (SEM).

Results: Such characterizations should prove useful in develop an understanding of the role that ultrasound amplitude and expose times play in solubility and the stabilization/destabilization of nanoparticles in Nap.Na solution with imposed ultrasound. The sonication time and amplitude on particle size of Nap.Na were investigated according to SEM, IR, Z.P., particle size distrubition and UV results.

SL- 237

Track: Hot Topics in Medicinal Chemistry

DEVELOPMENT OF POTENT DUAL BROMODOMAIN-KINASE INHIBITORS FOR CANCER THERAPY

Ernst Schonbrunn

Drug Discovery Department, Scientific Director, Chemical Biology Core, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA

Bromodomain (BRD)-containing proteins are essential for the recognition of acetylated lysine (KAc) residues of histones during transcriptional activation. BRDs of the so-called BET family such as BRD4 have emerged as promising drug targets for a number of diseases, including many cancers that are characterized by changes in the epigenetic cell signature. We and others recently discovered that diverse protein kinase inhibitors also inhibit the KAc recognition site of BRD4. This finding led to the hypothesis that certain kinase inhibitor scaffolds can be designed as equipotent inhibitors of BRD4 and a kinase (or a set of kinases) to specifically target cancer cells that depend on aberrant kinase activity and functionality of BRD4. Here we report the development of highly potent next-generation dual BRD4-kinase inhibitors. Lead compounds showed equipotent activity against BRD4 and kinases such as JAK2, FLT3 and RET ($IC_{50} \leq 10$ nM), potently inhibited cancer cell survival of several solid and liquid tumors including patient-derived samples of myeloproliferative neoplasm, and significantly increased survival of a mouse model of aggressive multiple myeloma. Combined, the data demonstrate the potential of these compounds as cancer therapeutics by inhibiting kinase signal transduction and histone recognition events simultaneously.

SL- 288

Track: Chemistry

IMMUNOMODULATORY POTENTIAL OF ISOLATED CONSTITUENTS FROM *SALIX CAPREA* AND SIMULTANEOUS QUANTIFICATION, METHOD VALIDATION OF FIVE POLAR SECONDARY METABOLITES BY LC/MS

Syed Wajahat Amin Shah

*Department of Chemistry, Kashmir University, Jammu and Kashmir, India;
E-mail: doctorwajaht@gmail.com*

Seven constituents were isolated and identified from medicinally important plant *Salix caprea*, Rutin(R), Kaemferol(K), Luteolin rutinose(CG), Quercetin(Q), β -sitosterol, Stigmasterol, Mannitol, Ethylhenicosanoate. These were screened for *in vitro* immunomodulatory potential employing Con A (40 μ g/well) to stimulate T-cells or LPS (40 μ g/well) to stimulate B- cells proliferation of mice Splenocytes by MTT assay. A rapid, precise, sensitive and validated LC/MS method for simultaneous quantification of five polar natural products (M, R, CG,



Q, K) was developed using solvent system, n-toluene: ethyl acetate: acetic acid (9.8:3.5:0.43, v/v/v). The method was validated for accuracy, precision, LOD, LOQ and all calibration curves showed a good linear relationship ($r > 0.9956$) within test range, accuracy validation recovery 94.53-99.10% with RSDs.

Keywords: LC/MS, immunomodulatory, *Salix caprea*, secondary metabolites.

SL- 329

Track: Drug Delivery and Targeting

MAGNETOFECTION ENHANCING SUICIDAL GENE THERAPY OF UTERINE FIBROID TUMOR CELLS

Shahinaz M. Shalaby, David Curiel and Ayman A. Al-Hendy

Obstetrics and Gynecology, Georgia Regents University, Augusta, Georgia, USA; E-mail: sshalaby@gru.edu

Adenoviruses are among the robust gene delivery systems which have been developed by our group as a potential localized non-surgical alternative for uterine fibroids. Eventhough the safety profile of adenoviruses is outstanding, there is still a need for minimizing any potential spread delivery beyond the lesion avoiding any possible drawbacks. combining viral -based gene delivery with nanotechnology offers the possibility to develop more efficient as well as targeted gene therapy. Magnetic nanoparticles (MNPs) complexed to Adenoviral vectors, in the presence of an external magnetic field, These nanoparticles accelerate the transduction . This approach has not been evaluated yet against human fibroid tumor cells. Magnetic nanoparticles formulations were complexed to a replication defective Adenovirus 5 with GFP as a reporter gene. These complexes were used to transduce human fibroid cells (DDcells) *in vitro* .We have observed a 27% and 31% increase in transduction efficiency of DD cells at 2 different multiplicity of infection (MOI); MOI 1 and MOI 10 respectively, with magnetic nanoparticles compared to adenovirus-alone standard transduction strategy ($P < 0.01$ at MOI 1 and $P < 0.006$ at MOI 10). Applied with suicide gene therapy utilizing herpes simplex thymidine kinase ganciclovir, magnetofection significantly enhanced suppression of proliferation as well as induction of apoptosis.

Keywords: Suicidal Gene Therapy, Uterine Fibroid, Magnetic nanoparticles.

SL- 96

Track: Hot Topics in Natural Products

EVALUATION OF ANTIOXIDANT POTENTIALS AND RADIOPROTECTIVE PROPERTIES OF TROPICAL GINGER IN ALBINO RATS

G.J. Sharma

Centre of Advanced Study in Life Sciences, Manipur University, Imphal-795003, India; E-mail: gjs1951@rediffmail.com

The tropical ginger, *Zingiber montanum* (J. König) A. Dietr, has significant potential in scavenging free radicals and affording protection from radiation-induced chromosomal aberrations. The present investigation aims at determining antioxidant activities and radioprotective properties of the rhizome extract of tropical ginger. Sulphur (thiyl) free radical, DPPH and superoxide scavenging assays were carried out for assessing the antioxidant activities. Radiation-induced (500 cGy) DNA damage in pBR322 *in vitro* test system could be significantly reduced upto 71% ($P < 0.05$) by treatment with 60% ethanol extract (20 μ g). Acute toxicity of the 60% ethanol extract was determined and a suitable injectable dose was selected for intra-peritoneal administration in albino rats (*Rattus norvegicus*, 2n=42). The LD50 of the extract calculated for 72 h was found to be 2.9 g/ kg, and the maximum tolerated dose (MTD) of the rhizome extract was 1.3 g/ kg. Rhizome extract (0.5 g/ kg) in 60% ethanol was intra-peritoneally injected to albino rats and exposed to 100, 300 and 500 cGy, respectively. Radioprotective effect of the extract was determined by alkaline single cell comet assay.

Significant reduction ($P < 0.05$) of comet tail DNA (68%) and length (61%) in rat bone marrow cells was observed at a radiation dose of 500 cGy. The results do demonstrate that tropical ginger has free radical scavenging properties and can protect bone marrow cells from radiation-induced DNA damage in albino rats. The results on radiation-induced DNA damage using plasmid pBR322 DNA obviously justify that the extract at a very low dose can protect DNA from undergoing strand breakage due to gamma radiation exposure. Versatility of *Zingiber montanum* in different chemical assays in terms of its radical scavenging potential shows that this 'non-conventional food plant' has a lot of potential in maintaining human health through dietary supplementation as nutraceutical. This candidate plant also can possibly be a promising candidate in clinical radiotherapy perhaps as a substitute for the well-known radioprotector amifostine.

SL- 218

Track: Biologics

IN SILICO IDENTIFICATION OF PROMISCUOUS CTL EPITOPES OF MYMA OPERON PROTEINS OF *MYCOBACTERIUM TUBERCULOSIS* FOR A EPITOPE BASED VACCINE DESIGN

Sadhna Sharma, Monika Sharma, Iti Saraav, Kirty Pandey, Prasun Dutta and Anshu Bhardwaj

DS KC and Department of Zoology, Miranda House, University of Delhi, Delhi - 110007, India;

E-mail: sadhna.sharma@mirandahouse.ac.in

There is an urgent need for a more effective vaccine against *Mycobacterium tuberculosis* (Mtb). Although CD4⁺ T cells play a central role in host immunity to Mtb, but recent evidences suggest critical role of CD8⁺ T cells in producing protective immunity. Infection of macrophages with Mtb results in upregulation of mymA operon making these proteins important immune targets. In the present study, mymA operon proteins of Mtb were assessed *in silico* for the presence of HLA class I binding CTL peptides. After exclusion of self-peptides they were analyzed for their ability to bind to 33 and 78 Class I HLA alleles in BIMAS and NetMHC 3.4 software respectively. Out of 56 promiscuous epitopes observed in both the software, 41 were predicted to be antigenic using VaxiJen server. The top VaxiJen scoring antigenic peptides were docked to globally relevant HLA allele using CABSdock and Hex program. The docked peptides were found to have significant interactions with the amino acid residues of the HLA class I molecule indicating them to be good CTL epitopes. This information is useful for epitope based vaccine design and can be validated using peptide microarray or MHC tetramer approach.

SL- 50

Track: Medical Imaging

UNDERSTANDING TRACER DIFFUSION IN TUMOR TISSUE FOR HYPOXIA IMAGING USING REACTION-DIFFUSION MODELING: MATCHING COMPUTATIONAL SIMULATION TO DYNAMIC [¹⁸F]FMISO PET MEASUREMENTS IN TUMORS

Kuangyu Shi, Christine Bayer, Constantin-Alin Maftai, Florian C. Gaertner, Sabrina T. Astner, Jan J. Wilkens, Fridtjof Nüsslin, Peter Vaupel and Sibylle I. Ziegler

Department of Nuclear Medicine, Klinikum rechts der Isar, Technical University Munich, Ismaningerstr. 22, 81675 Munich, Germany; E-mail: k.shi@tum.de

Tumor hypoxia occurs mainly due to diffusion limitation of oxygen supply. Positron-emission tomography (PET) with hypoxia-specific tracers provides a noninvasive method to assess the oxygenation status inside tumor. However, a hypoxia-specific tracer needs to reach hypoxic regions usually far away from functional vessels and the diffusion potential of the tracer may influence imaging potential of the radiolabeled molecules. Thus, understanding the diffusion

behavior of hypoxia tracer is important for the development of hypoxia-specific tracers. The diffusion distance of a molecule depends on both the transport and the reaction inside tissue. Reaction-diffusion models have advantages in exploring the diffusion potential of molecules in tissues and revealing the quantitative relation between *in vivo* imaging and the tumor microenvironment. However, it is usually difficult to compare the modeling results with the real PET measurement data quantitatively. This hampers further applications of computational models in tracer development. This study aims to compare the simulation results with a preclinical [^{18}F]FMISO PET study and to optimize the reaction-diffusion model accordingly.

Methods: Nude mice with xenografted human squamous cell carcinomas (CAL33) were investigated with a two hour dynamic [^{18}F]FMISO PET followed by immunofluorescence staining using the hypoxia marker pimonidazole and the endothelium marker CD 31. A large data pool of tumor time-activity curves (TAC) was simulated for each mouse by feeding the arterial input function (AIF) extracted from experiments into the model with different configurations of the tumor microenvironment. A measured TAC was considered to match a simulated TAC when the difference metric was below a certain, noise-dependent threshold. As an extension to the well-established Kelly model, a flow-limited oxygen-dependent (FLOD) model was developed to improve the matching between measurements and simulations. Immunofluorescence imaging of tumor cryosections were employed to monitor the configurations of the simulations.

Results: The matching rate between the simulated TACs of the Kelly model and the mouse PET data ranged from 0 to 28.1% (on average 9.8%). By modifying the Kelly model to a flow-limited oxygen-dependent (FLOD) model, the matching rate between the simulation and the PET measurements could be improved to 41.2 - 84.8% (on average 64.4%). The distribution of modeled intervessel distances was similar to the results of immunofluorescence images.

Conclusion: Using a simulation data pool and a matching strategy, we were able to compare the simulated temporal course of dynamic PET with *in vivo* measurements. By modifying the Kelly model to a FLOD model, the reaction-diffusion simulation was able to approach the dynamic [^{18}F]FMISO measurements in the investigated tumors. The refinement of reaction-diffusion modeling provides the potential to assist the development of hypoxia tracers and the corresponding analysis of hypoxia imaging.

SL- 103

Track: Hot Topics in Natural Products

ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF ENDOPHYTIC FUNGAL EXTRACT OF *RAUVOLFIA SERPENTINA*

Santosh Kumar Singh, Madhu Verma, Amit Ranjan and Rajesh Kumar Singh

Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005 India; E-mail: singhsk71@yahoo.com

Background: Fungal endophytes are symbionts that grow within plant tissues without causing any negative symptoms to their host plant. They produce many bioactive secondary metabolites which are similar compounds of the host which make them an exciting and relatively untapped source of novel compounds. Hence, this method fascinates large scale production of bioactive compounds in easier and economical way than the medicinal plant source. However, one major challenge in drug discovery lies in developing strategies to efficiently recover highly bioactive strains.

Materials and Methods: The plant, *Rauvolfia serpentina*, a well known Indian medicinal plant in Ayurveda, has been used as the source for endophytes in this study. The plant materials have been collected during rainy season (July-August). The source plant materials as leaves, stem and roots were surface sterilized and transferred to PDA media for endophytes isolation. The isolated endophytes were re-cultured for pure strain and the pure separated strains were inoculated in flask containing PDB for mass cultivation. After 15-20 days, the extraction was done using Ethyl acetate. The dried extracts were further analysed for antibacterial activity by disc diffusion method against *E. coli* (ATCC 25922) a gram negative and *Staphylococcus aureus* (ATCC 25323) a gram positive bacteria. Light microscopy & SEM was done for morphologically identification of active strain. The preliminary phytochemical screening was done for host specific chemicals using standard protocols.

Results: There are total 20 endophytic strains were isolated from different parts of *R. serpentina* in which 7 strains were isolated from leaves, 9 from stem and 4 from roots. Out of these strain, RS-R5 was most effective against gram positive

and gram negative bacteria, however, RS-S4 was moderately effective and RS-R1 & RS-S7 were less effective. Alkaloids, flavanoids, polyphenols and steroids were positive while tannin was absent.

Conclusion: Our results suggest that isolated endophytes may be a source of broad spectrum antibacterial molecules.

Keywords: Fungal endophytes, Antibacterial Activity, phytochemical screening and bioactive strains.

SL- 215

Track: Academic CRO/Industrial Collaborations in Drug Discovery

ICCB-LONGWOOD: EXAMPLES OF HOW AN ACADEMIC SCREENING CORE CAN ENABLE INDUSTRY COLLABORATIONS

Jennifer Smith

Assistant Director ICCB-Longwood Screening Facility Harvard Medical School 250 Longwood Ave., SGM 604 Boston, MA 02115, USA; E-mail: jennifer_smith@hms.harvard.edu



The ICCB-Longwood Screening Facility at Harvard Medical School provides resources and the infrastructure for researchers to conduct high-throughput small molecule, siRNA, and microRNA screens, and is staffed with knowledgeable personnel who assist investigators with assay development, lab automation, and data analysis. This biosafety level 2 facility operates on an investigator-initiated, staff-assisted screening model. The investigator is responsible for the screen, retains complete ownership of the project and provides the personnel to help conduct the screen - to prepare reagents for screening, fill assay plates, and read out assays on the plate reader or screening microscope. In addition to maintaining the facility and libraries, staff members provide training in the use of the instruments, assist in developing and optimizing the HTS assay, and perform complex automation tasks. Using this model, hundreds of successful screens have been performed at ICCB-Longwood, leading to many publications in prominent journals. This model has also made ICCB-Longwood amenable to supporting collaborations between academe and industry. This talk will focus on some of the mechanisms by which ICCB-Longwood has been able to foster this, including: industry-led collaborations with ICCB-Longwood to solicit screens focused on specific disease areas; industry-support of academic screens using small molecule and siRNA libraries available at ICCB-Longwood; and facilitating the screening of industry libraries at ICCB-Longwood by particular research groups.

SL- 270

Track: Pharmaceutical Research & Development

INSIGHTS FROM MOLECULAR DYNAMICS STUDIES TO COMBAT ISONIAZID - RESISTANT TUBERCULOSIS

M. Elizabeth Sobhia and Vivek Kumar

Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar - 160 062, Punjab, India; E-mail: mesophia@niper.ac.in



Tuberculosis (TB) is one of the deadliest infectious diseases caused by the air-borne bacillus, *Mycobacterium tuberculosis* (Mtb). In spite of more than four decades of intensive research on TB, it still remains as a life threatening disease and calls for global health concerns. Currently, the MDR-TB eruption is severely daunting the current anti-TB artillery. As a result, a large number of MDR cases are coming into existence and most of them having TB relapse cases. It generally presents a challenge to isoniazid (INH), which is considered as the most coveted ingredient of the chemotherapy given to TB patients. However, rising cases of mutations in *inhA*, the target protein for isoniazid demonstrates new strain of *mycobacterium tuberculosis* that develops resistance towards isoniazid. This presents an urgent need to discover new molecules that would act as an alternative for isoniazid or can potentiate the isoniazid through combination therapy. In this direction, we carried out computational studies using molecular dynamics simulation to find a good combination of isoniazid with other inhibitors. Binding free energy calculations show that these molecules can enhance the binding affinity of isoniazid about two fold in mutants as compared to the wild type. Energy decomposition analysis also shows that binding of the molecules in substrate binding pocket facilitates the strengthening of isoniazid interactions with dinucleotide binding site residues. This study strongly advocates the use of other inhibitors to make a new combination with isoniazid for treating the isoniazid-resistance TB. The study can be conceived for the future direction to stress over the development of this combination to fight against the current drug - resistance complications for TB treatment.

SL- 299

Track: Innovative Drug Discovery and Nanotechnology

GENERIC NANOMEDICINES: PHARMACOKINETIC AND BIOANALYTICAL EVALUATION

Stephan T. Stern

Nanotechnology Characterization Lab (NCL), Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Post Office Box B, Frederick, MD 21702, USA; E-mail: sternstephan@mail.nih.gov

Nanotechnology-based drug delivery platforms offer the potential to rejuvenate traditional chemotherapeutics by improving safety profiles. For example, nanotechnology platforms can target drugs to tumor sites *via* passive mechanisms, resulting from the inherent leakiness of the tumor vasculature, and *via* active ligand-receptor dependent targeting mechanisms. Nanotechnology can also eliminate the need for the toxic vehicles required for administration of

hydrophobic drug entities. Nanotechnology platforms presently undergoing evaluation include nanomicelles, nanoemulsions, nanoliposomes, and metal, polymeric and carbon-based nanoparticles. The success of liposomal doxorubicin, Doxil[®] (Janssen Biotech, Inc.), is highlighted by the recent approval of the first nanomedicine generic, Lipodox[®] (Sun Pharma, FDA approval 2013), developed under the standard generic drug 505(j) regulatory pathway. Another nanomedicine generic, Cynviloq[™] (Sorrento Therapeutics), is currently in development in the US as an alternate, polymeric formulation of albumin-based paclitaxel, Abraxane[®] (Abraxis BioScience), utilizing the 505(b)(2) regulatory pathway. Nevertheless, the regulatory evaluation of generic nanomedicines is far from straight forward. Indeed, the pharmacokinetic complexity of nanomedicines, and associated bioanalytical challenges, adds substantial difficulties to traditional bioequivalence trials. This presentation will specifically address the importance and challenges of monitoring the disposition and *in vivo* integrity of nanotechnology platforms, using case studies to demonstrate the bioanalytical methodologies employed.

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SL- 326

Track: Drug Delivery and Targeting

NANO-FORMULATION MODIFIED DRUG PHARMACOKINETICS AND THERAPY

Stephan Stern

Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research, National Institutes of Health, USA; E-mail: sternstephan@mail.nih.gov

Nanotechnology reformulation can overcome many of the liabilities of current oncology drugs, including insolubility, rapid metabolism, poor bioavailability and off target toxicity. The earliest successful commercialization of nanotechnology was encapsulation of doxorubicin in a nanoscale liposome, Doxil[®] (Janssen Biotech, Inc.), approved by the FDA in 1995. Liposomal doxorubicin decreases systemic free doxorubicin concentrations, reducing cardiac exposure and associated cardiotoxicity. Liposomal doxorubicin commercialization was followed by cremophor-free formulations of the highly insoluble drug paclitaxel, initially as an albumin nanoparticle, Abraxane[®] (Abraxis BioScience), approved in the US 2005, and later a polymeric nanomicelle, Genexol-PM[®] (Samyang Genex Company), approved in Korea 2007. By removing cremophor from the legacy paclitaxel formulation, Taxol[®] (Bristol-Myers Squibb), these nanotechnology reformulations demonstrated dramatic improvements in dose tolerability, as cremophor-dependent dose-limiting hypersensitivity reactions were no longer observed. In addition to eliminating unwanted hypersensitivity side effects, these new cremophor-free formulations also changed the pharmacokinetics of the underlying drugs, and the therapeutic profile. The success of these reformulation efforts have solidified the advantages that nanotechnology offers the pharmaceutical industry, driving the implementation of nanotechnology earlier in the discovery phase of drug development. This presentation will specifically address the importance of formulation-mediated modification of drug pharmacokinetics and how this can lead to improved drug therapy.

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SL- 144

Track: Innovative Drug Discovery and Nanotechnology

NANOTECHNOLOGY-BASED TOOLS FOR THE STUDY OF THE AFFINITY AND SELECTIVITY OF ANTIGENS FOR SMALL MOLECULAR TARGETS UNDERLYING DRUG DISCOVERY AND THERAPEUTICS

Michael A. Stroschio, Mitra Dutta, Xenia Meshik, Sidra Farid, Yi (Elaine) Lan, Souvik Mukherjee, Min Choi, Undarmaa Bat-Erdene, Devanshi Parikh, Saadia Ranginwala, Debopam Datta, Ke (Robin) Xu, Preeti Pratap and TsaiChin Wu

College of Engineering, University of Illinois at Chicago, Chicago, IL, USA;
E-mail: stroschio@uic.edu



It is well recognized that the selectivity of drug delivery depends on the availability of antigens that facilitate the binding of drug-bearing complexes to targets in intended drug delivery regions. Herein, the use of molecular-beacon-based and aptamer-based nanotechnologies are described which facilitate the study of antigens that bind to small molecules as targets. In particular, graphene-based field-effect-transistor-like devices are described along with their application in the study of the affinity and selectivity of DNA- and RNA-based antigens that bind to small molecules as targets. In addition, aptamer-based molecular beacons --- nanosensors based on DNA and RNA aptamers, and semiconductor quantum dots --- and conventional molecular beacons are described along with their applications in the study of the affinity and selectivity of DNA- and RNA-based antigens that bind to small molecules as targets as well as to complementary nucleic acids of interest in drug delivery and therapeutics. As is now well-known, these aptamers bind to a wide range of physiologically important proteins and, in many cases, exhibit affinities and selectivities comparable to those of antibodies. Moreover, aptamers may be produced *via* scalable chemical processes that are not prone to bacterial or viral contamination. For these reasons, aptamers portend advantages for small molecular targets as well as in terms of synthetic accessibility, ease of modification by medicinal chemistry, and more efficient entry into relatively small biological compartments. Accordingly, these properties portend the replacement of antibodies with aptamers in many drug discovery and therapeutic activities. Moreover, aptamer-based molecular beacons, based on fluorescent resonant energy transfer, and graphene-based field-effect transistors are shown to be suited to measuring physiological properties such as the concentration of physiological ions such as potassium over a wide range of concentrations of physiological interest. In summary, the use of nanotechnology-based constructs underlying drug discovery will be described for a number of nano-constructs including: graphene-based and aptamer-based field-effect transistors for small molecules and ion sensing; molecular beacons based on aptamer sensing elements and fluorescent resonant energy transfer between quantum dots and quenchers; and conventional molecular beacons.

Integrated amplifiers that can read signals as fast as the DNA transverses the nanopore; and reducing the capacitive noise of the nanopore membranes so that nucleotide discrimination can be maintained at high-bandwidth.

Keywords: Aptamers, quantum dots, graphene, field-effect transistors, molecular beacons.

SL- 257

Track: Hot Topics in Medicinal Chemistry

NOVEL INHIBITORS AND CHEMICAL PROBES TO STUDY THE PROTEIN ARGININE DEIMINASES (PADS)

Venkat Subramanian

Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA

Protein arginine deiminases (PADs) catalyze the post-translational hydrolysis of arginine residues to form citrulline. This unique modification is now known to play a key role in the etiology of multiple autoimmune diseases (*e.g.*,

Rheumatoid Arthritis, Multiple Sclerosis, Lupus and Ulcerative Colitis) and in some forms of cancer. Among the five human PADs (PAD1, 2, 3, 4 and PAD6), it is unclear which isozyme contributes to disease pathogenesis. The identification of potent, selective, and bioavailable PAD inhibitors can be used to elucidate the specific roles of each isozyme. We recently described “tetrazole” and “imidazole” analogs as suitable backbone amide bond bioisosteres for the parent pan PAD inhibitor Cl-amidine. These analogs are highly potent and show modest selectivity for PAD isozymes. These analogs also exhibit enhanced cell killing in a PAD4 expressing, osteosarcoma bone marrow (U2OS) cell line and can also block the formation of neutrophil extracellular traps (NETs). One of the inhibitors, BB-Cl-amidine arrests NET formation in an MRL/lpr model of lupus mice. The enhanced cell permeability of this inhibitor was functionalized to derive activity based chemical probes to study the *in vivo* roles of PAD family of enzymes. This novel inhibitor design and new chemical probe development will be an important step to study the *in cellulo* role played by PADs.

SL- 73

Track: Traditional Chinese Medicine

STUDY ON ANTI-LUNG CANCER EFFICIENCY OF CENTIPEDE EXTRACTION *IN VITRO* AND *IN VIVO*

Xue-Fei Tian¹, Yuan Chen¹, Zhi-Qi Wang¹, Sha Tian¹, Qing Zhou² and Gang Pei¹

¹*Hunan University of Chinese Medicine, Changsha 410208, China; E-mail: windsame@163.com*

²*The First Affiliated Hospital of Hunan University of Chinese Medicine, Changsha 410007, China*

Objective: To investigate efficiency of Centipede Extraction (CE) on apoptosis induction, proliferation inhibition to Human A549 cell line and growth suppression of subcutaneous transplanted sarcoma in nude mice.

Methods: CE was prepared by Enzymolysis and Acetone precipitation methods and used to treat Human lung cancer A549 cell line. Proliferation inhibition was evaluated by MTT assay and calculate influence of half inhibit concentration (IC₅₀). Cell morphological change and apoptosis were detected by flow cytometry and Hoechst stain. The subcutaneous transplanted sarcoma model were prepared with nude mice and suppression of tumor growth was evaluated compared with cisplatin (CDDP).

Results: After 48 h CE treatment, proliferation of A549 cells were inhibited with dose-dependent and IC₅₀ value was 0.603 mg/mL. The G₀/G₁ phase of cells was down regulated and G₂/M, S phase cells were up regulated. The apoptotic character cells have been found by Hoechst stain. *In vivo* experiment, the tumor weight and volume were decreased significantly compared with model control group ($P < 0.01$).

Conclusion: The CE can inhibit proliferation and induce apoptosis through arresting A549 cells at G₂/M phase and suppress growth of subcutaneous transplanted sarcoma of lung cancer in nude mice.

This study was supported by the grants from the National Natural Science Foundation of China (81473617) and Hunan Natural Science Foundation of China (13JJ2032).

Keywords: Apoptosis, centipede extraction, implanted tumor, lung cancer.

SL- 137

Track: Cardiovascular Drug Discovery and Therapy

INHIBITING C-REACTIVE PROTEIN FOR THE TREATMENT OF CARDIOVASCULAR DISEASE – WAY INTO CLINICAL STUDIES

Jan Torzewski, Oliver Zimmermann, Katharina Weber, Matthias Graf and Oliver Zimmermann

Cardiovascular Center Oberallgäu-Kempton, Robert Weixler Str. 50, 87439 Kempton, Germany;
E-mail: jan.torzewski@kv-keoa.de



Specific C-reactive protein (CRP) inhibition may be a novel approach for reducing cardiovascular mortality. Several expensive attempts to develop specific CRP-inhibitors have not been successful. In 2010, cardiac glycosides have been identified to potently inhibit CRP synthesis in the liver. Cardiac glycosides, for the treatment of cardiac insufficiency, have been in clinical use since the late 18th century. Much is known about their toxicity and side effects. Clinical studies with these long known drugs are ethically easy to justify, and reformulation of established substance classes has become one of the leading strategies in drug development.

Here, we outline our ongoing observational single center study/prospective cohort study investigating whether cardiac glycosides significantly lower CRP plasma levels in decompensated heart failure patients. Key inclusion criteria: NYHA III and IV, acute cardiac failure, LVEF < 40%. Key exclusion criteria: significant concomitant disease (acute renal failure, CRP > 5 mg/dl, leukocyte count > 12 000/μL, AV-block I-III). Experimental intervention: Digoxin in addition to heart conventional heart failure therapy. Control intervention: Conventional heart failure therapy. To be assessed for eligibility: 600 patients. To be assigned to the trial: 120 patients. To be analysed: 60 patients (30 vs 30). Primary efficacy endpoint: CRP plasma levels during follow-up (d21-d0). Statistical analysis: Multiple regression analysis.

This is the first study investigating a CRP synthesis inhibitor in humans. It is also the first study investigating whether cardiac glycosides significantly influence CRP plasma levels in humans. Finally, it serves as a pilot study for subsequent phase III trials.

SL- 252

Track: Hot Topics in Medicinal Chemistry

PEPTOID-COMBINATORIAL STRATEGIES TO TARGET NON-PROTEIN BIOMARKERS IN THE TUMOR MICROENVIRONMENT

D. Gomika Udugamasooriya

Department of Pharmacological & Pharmaceutical Sciences, University of Houston, Houston, TX 77204-5000, USA

Chemotherapies are nonspecific and have side effects. Targeted therapies are expensive and fail on larger patient populations due to the heterogeneity of their target 'protein' biomarkers. This suggests that effective cancer treatments need to be either personalized or find universal biomarkers - probably beyond proteins. We have been exploring peptoids as an emerging class of highly bio-compatible synthetic molecules as future drug leads. Peptoids are serum stable, non-immunogenic, cell permeable, easy to synthesize and optimize. Also, the cost of peptoid development is significantly lower than for small organic molecules, peptides and antibodies. We design and synthesize large on-bead peptoid combinatorial libraries and developed a unique on-bead two-color (OBTC) cell screen to directly identify most specific compounds targeting biomarkers on the cell surface. As an approach to overcome the protein biomarker heterogeneity problem, we applied our OBTC assay at an unbiased fashion and identified a lipid- phosphatidylserine (PS) binding peptoid. PS is universally found on the outer layer of tumor endothelium and on many cancer cells as compared to normal cells. Our peptoid is found to be cytotoxic on various tumor types such as lung, breast and prostate and not on normal cells, indicating a wider but tumor specific treatment method.

SL-326(a)

Track: Inflammation and Immunology

A MEDICINAL CHEMISTRY APPROACH TO GAINING PHARMACOLOGICAL CONTROL OF IMMUNITY

Nicholas Mark Valiante

Department of Immunology & Immunotherapy, GlaxoSmithKline, USA; E-mail: nick.m.valiante@gsk.com

Although activation of the immune response and inflammation in the natural setting provides both benefit and harm to an organism, the overwhelming majority of responses are highly beneficial. Thus, the immune system has been exquisitely tuned by natural selection to maximize protection while minimizing damage to its host. This trade-off between efficacy and on-target toxicity must be addressed when attempting to manipulate the immune system for improved prophylaxis (vaccines) or treatment (immuno-therapy). Our approach to this problem was to focus on pharmacological control of small molecule immune potentiators (SMIPs) and use them to probe the minimal immune stimulation requirements for efficacy. In both prophylactic and therapeutic settings we demonstrate that SMIPs with the best predicted therapeutic indices have low bioavailability, short *in vivo* residence time and induce localized immune activation. Thus, the design and optimization of improved immuno-drugs differs substantially from that of traditional pharmaceuticals. Because of this unique drug discovery and development strategy, we have begun to define new principles for uncoupling the efficacy of immune-based interventions from their inherent immuno-toxicities.

SL- 27

Track: Diabetes and Obesity Drug Discovery & Therapy

DEUTERATED POLYUNSATURATED FATTY ACIDS (dPUFAs) OVERCOME LIPID PEROXIDATION DAMAGE IN CNS DISORDERS

Lex Van der Ploeg, Charles Cantor, Robert De Jager, Harry Saal, Bob Molinari and Misha Shchepinov

Merck Research Laboratories, USA; E-mail: lvanderploeg@rhythmtx.com

Polyunsaturated fatty acids (PUFAs) are easily damaged by reactive oxygen species through a radical chain reaction mechanism of lipid peroxidation [1]. Retrotope's dPUFAs prevent free radical damage, block lipid peroxidation cascades, and can rescue mitochondria from damage [2], thus preventing or reversing cell-damage/-death [3]. This dPUFA mechanism has shown promise in orally dosed animal models of disease, and has thus far not been applied therapeutically by any other drug company.



RT001 (9-*cis*, 12-*cis*-11, 11-d₂-linoleic acid ethyl ester) is a wholly synthetic linoleic acid (an essential fatty acid) analogue in which hydrogens at susceptible carbon atoms are replaced with the twice heavier hydrogen isotope, deuterium. This stabilizes PUFA-containing membranes, which participate in mitochondrial energy generation, against lipid peroxidation. Retrotope is developing RT001 for the treatment of diseases involving damaging lipid peroxidation cascades, including Friedreich's Ataxia. The mechanism of action of RT001 has been demonstrated as detoxifying the damaging presence of well understood disease triggers, like free iron, synuclein, MPTP and beta-amyloid, leading to recovery of cellular function by down-regulating autocatalytic lipid peroxidation damage [4].

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SL- 150

Track: Innovative Drug Discovery and Nanotechnology

PEPTIDE NANOTUBES FOR THE NON-INVASIVE DETECTION OF BIOMOLECULE SECRETION FROM CELLS

Noah S. Franklin, Christian Trippe, Lisa Perreault, Douglas Andrews, Raymond B. Lawton, Joshua Stuckey, Maricris Mayes, Vijaya B. Chalivendra and Milana C. Vasudev

Department of Bioengineering, University of Massachusetts Dartmouth, USA

This study focuses on the use of peptide nanotubes as biocompatible substrates for cellular growth and proliferation as well as biosensors. A broad range of peptides that have been shown to form stable nanowires or nanotubes are based on amyloid proteins attributed to diseases such as Alzheimer's and Type II diabetes. We have examined the ability to sublime different peptides and the subsequent deposition of peptide nanotubes and nanowires using Plasma Enhanced Chemical Vapor Deposition (PECVD). We have designed a custom-built vacuum chamber which was for the deposition of aromatic dipeptide monomers, such as dityrosine and phenylalanine-tyrosine. Specific discharge parameters such as input power, chamber pressure, flow rate of argon gas, substrate position, and the lifetime of the plasma species will be varied to help understand the range of physical and chemical properties of the peptide nanostructures such as their morphology. Plasma used in the PECVD process allows control over the composition of the nanotube growth imparting unique surface properties without modifying the bulk material properties of the substrate. The vertical arrays of nanotubes were studied as a substrate for cellular growth in order to understand the effect of morphology on the cells. By understanding the interactions between these tubes and somatic cells, we will understand how to utilize the tubes as the key component in a biological sensor. With this understanding the sensor will be made which will elucidate the properties of cellular communication that still evade us using conventional methods of detection. Secretion of biomolecules from cells have been conventionally analyzed by biochemical assays which, only offer average measurements from a population of cells with low resolution and sensitivity. Preliminary quantum-chemical studies have also been performed to elucidate the structures and properties of dityrosine dipeptides, the building block for the peptide nanotube formation. This study will provide insights for the subsequent self-assembly atomistic studies of peptide nanotubes.

SL- 35

Track: Medical Imaging

APPLICATION OF PARAMETRIC IMAGING USING ¹⁸F-FDG PET/CT DYNAMIC MULTI-BED SCANNING IN DIFFERENTIAL DIAGNOSIS OF PULMONARY LESIONS

Rong Fu Wang, Wang Qiang, Zhang Jian-Hua and Zhou Yun

Department of Nuclear Medicine, Peking University First Hospital, No.8, Xishiku St., Xichengqu, Beijing 100034, China; E-mail: rongfu_wang@163.com

Objective: The purpose of the study is to investigate the feasibility of using parametric image of influx rate constant Ki generated by multi-bed dynamic F-18-FDG PET/computed tomography (CT) to diagnose pulmonary lesions.

Methods: Multi-bed dynamic FDG PET/CT scanning followed by a routine examination was performed on 21 patients with pulmonary lesions who were divided into two groups with malignant or benign lesions based on biopsy and followup. The number of patients and lesions in the malignant and benign groups were 10 with 15 lesions and 11 with 14 lesions, respectively. The left ventricular blood pool was used for an image-derived input function. The parametric images of influx rate constant Ki was generated with the Patlak plot method with linear regression and spatial constraint algorithm. The inter-group differences for Ki, SUV_{max}, and TAC of F-18-FDG were analyzed. The correlation between Ki and SUV_{max} was also assessed.

Results: The results showed that the maximum diameters of the pulmonary lesions were not significantly different between the malignant and benign groups (P>0.05). Ki and SUV_{max} were significantly higher in malignant lesions

compared to benign lesions ($P < 0.05$). K_i was highly correlated with SUV_{max} in pulmonary lesions ($r = 0.815$, $P < 0.01$). The malignant lesions showed gradually increasing TAC, and benign lesions exhibited gradually decreasing curves.

Conclusion: Our results indicate that parametric images of K_i in F-18-FDG PET/CT dynamic multi-bed scanning could be useful in the diagnosis of pulmonary lesions.

Keywords: Pulmonary lesions, diagnosis, dynamic multi-bed scanning, ^{18}F -FDG PET/CT, Parametric imaging.

SL- 43

Track: Medical Imaging

SITUATION AND PROGRESS OF TUMOR ANGIOGENESIS TARGETED IMAGING AND THERAPY VIA RADIONUCLIDE TRACING TECHNIQUES

Rong Fu Wang

Department of Nuclear Medicine, Peking University First Hospital, No.8, Xishiku St., Xichengqu, Beijing 100034, China; E-mail: rongfu_wang@163.com

Tumor angiogenesis imaging is a new tumor research field, and the key scientific problem is how to find a specific and targeted tumor angiogenesis imaging tracer. It is realized that angiogenesis is an essential step for tumor growth and for the initiation of metastasis. Therefore, angiogenesis imaging methods have been developed for early diagnosis, optimization in personal treatments, and prediction of subsequent clinical response. The small-molecule agents gain access to their target sites more easily than monoclonal antibodies. In our previous study we found that radionuclide labeled small peptide Arg-Arg-Leu(RRL) could combined with tumor derived endothelial cells specifically and has high good imaging in tumor tissue by SPECT and ultrasonic imaging. On the other hand, RGD peptide is a group of small molecular polypeptide which contains Arg-Gly-Asp triple amino acids, a highly selective and affinitive receptor of integrin $\alpha\beta3$. Integrin $\alpha\beta3$ plays a critical role in tumor-induced angiogenesis and metastasis, and has become a promising diagnostic indicator and therapeutic target for various solid tumors. Therefore we assess the impossibility of RRL and RGD as tumor angiogenesis imaging tracer and carrier of tumor targeted therapy in high invasive tumor, a new way to diagnosis and therapy tumor.

SL- 77

Track: Regenerative Medicine

INJECTABLE AND BIODEGRADABLE POLYMERIC BIOMATERIALS FOR REGENERATIVE MEDICINE

Shanfeng Wang

Department of Materials Science and Engineering, and Institute of Biomedical Engineering, The University of Tennessee, Knoxville, TN 37996, USA; E-mail: swang16@utk.edu

Here I present our work on injectable and biodegradable polymeric biomaterials for diverse applications in tissue engineering and regenerative medicine. These photo-crosslinkable biomaterials include copolymers composed of poly(propylene fumarate) (PPF) and poly(ϵ -caprolactone) (PCL), PCL acrylates (PCLAs) with a wide range of molecular weights, polyethylene glycol diacrylate (PEGDA), and polymer nanocomposites containing hydroxyapatite (HA) nanoparticles and polyhedral oligomeric silsesquioxane (POSS) nanocages. These polymeric biomaterials have been fabricated *via* photo-crosslinking into different 2D substrates and 3D scaffolds with controllable chemistry, topology, and stiffness for different tissue engineering applications, such as bone and peripheral nerve regeneration. We have also surface tethered hydrophilic neutral or cationic polymer chains into the polymer networks and fabricated polymer network substrates with microgrooves, micro-pillars, micro-pores, nanowires, or nanopores. Growth factors such as recombinant human bone morphogenetic protein-2 (rhBMP-2) have been incorporated into the polymer scaffolds for promoting tissue ingrowth. Besides their practical applications, the networks formed using these polymeric biomaterials also serve as an excellent platform to investigate how material surface characteristics can be used to regulate the

behavior and functions of mammalian cells, such as cell adhesion, spreading, phenotype, migration, proliferation, differentiation, and integrin/gene/protein expression.

SL- 284

Track: Inflammation and Immunology

PHARMACOLOGICAL MANIPULATION OF SHIP-1 MODULATES HUMAN T LYMPHOCYTE ADHESION AND MIGRATION

Jennifer A Ball, William Wood and Stephen G. Ward

Department of Pharmacy & Pharmacology, University of Bath, Claverton Down Road, Bath, North East Somerset BA2 7AY, UK; E-mail: s.g.ward@bath.ac.uk

Pharmacological modulation of SHIP-1 activity is a promising route to modulate PI3K mediated signalling in leukocytes. The role of SHIP-1 in T lymphocyte function has previously been investigated using genetic strategies in mice and humans. Here we have used a novel, selective allosteric SHIP-1 activating compound and a SHIP-1 inhibitor to explore the role of SHIP-1 in primary human T lymphocyte function. TCR/CD3 mediated Akt phosphorylation was inhibited by pharmacological SHIP-1 activation and the ability of T lymphocytes to proliferate and secrete pro-inflammatory cytokines in response to TCR/CD3 stimulation was also severely impaired. Interestingly, both pharmacological activation and inhibition of SHIP-1 inhibited chemokine induced signalling in activated T lymphocytes, which was associated with a profound reduction in the ability of lymphocytes to migrate towards a chemokine gradient. Consistently, both SHIP-1 activation and inhibition abrogated T lymphocyte adhesion and decreased the activation state of LFA-1. Importantly, SHIP-1 activation and inhibition also impaired cell polarisation, cytoskeletal protein regulation and lamellipodia production. Remarkably, pharmacological activation and inhibition had the same functional consequences providing evidence for a crucial role of SHIP-1 in human T lymphocyte function. This study provides evidence that pharmacological modulation of SHIP-1 may prove therapeutically useful for the treatment of T lymphocyte driven pathologies.

Keywords: SHIP, phosphatase, lymphocytes, TCR, chemokines.

SL- 220

Track: Recent Advances in Patient Treatment and Care

ULEINE, A BOOSTER OF THE IMMUNE RESPONSE: ACTIVITY ON AIDS PATIENTS

A.M. Weffort-Santos¹ and J.D. Federlin²

¹*Universidade Federal do Paraná Curitiba, PR, Brazil; E-mail: almeriane@ufpr.br*

²*Arolab Industrie chimique, Mittelhausbergen, France*

Nitric Oxide (NO) is synthesized by cells of the immune system to inactivate pathogens. Some pathogens as the HIV and Mycobacterium tuberculosis grow in cells of the immune system and inactivate their capacity to synthesize NO. Uleine promotes the synthesis of Nitric Oxide. Uleine was extracted from *Aspidosperma subincanum* and the extract was coated on microbeads for production of the food supplement "Para Pau Aspido" containing 20 mg, 40 mg and 80 mg of uleine expressed in total alkaloids, per capsule. This process liberated the totality of the active ingredients within half an hour. By coating half of the microbeads with a natural product that resists acidity, we increased the time during which the active ingredients were present in the body. An advantage of this is that there is no need to take six caps per day but only three.

Administered daily during a year to aids patients, the food supplement showed no toxicity but a remarkable recovering effect on the patients, for HIV and their opportunistic infections, detected as early as the third month following the beginning of the treatment, even among those patients denied an anti-HIV tri-therapy.



Keywords: AIDS, nitric oxide, tuberculosis, uleine.

SL- 316

Track: Neurodegenerative Disorders

STRESS GRANULES AND NEURODEGENERATION: NOVEL TARGETS FOR THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS AND ALZHEIMER'S DISEASE

Benjamin Wolozin

Department of Pharmacology and Neurology Program in Neuroscience Boston University School of Medicine, Boston, USA; E-mail: bwolozin@bu.edu

Increasing evidence links neurological disease processes to dysfunction of neuronal RNA binding proteins (RBPs), RNA granules and stress granules (SGs). RBPs, such as T-cell intracellular antigen 1 (TIA1), contain prion-like, poly-glycine rich domains, which promote a process of protein aggregation that is normally physiological and reversible. Disease-linked mutations in RBPs increase the tendency of these proteins to aggregate, leading to formation of pathological SGs. Importantly, TIA1 and other SG proteins, co-localize with neuropathology in brain tissue of subjects with AD and ALS.

We have applied this paradigm to identify novel pharmacological approaches for preventing and reversing the pathological processes associated with AD and ALS. ALS is associated with the aggregation of TDP-43, which we have shown associate with stress granules. Using a cell based screening approach, we identified a series of compounds that inhibit TDP-43 inclusion formation in a reproducible and dose-dependent manner, while showing little-to-no cytotoxicity. Inhibition of TDP-43 aggregation is observed in cell lines, primary cortical neurons and in induced pluripotent stem cells from controls and ALS subjects. Biochemical studies indicate that our lead compound preferentially reduces TDP-43 aggregates and cleavage products, while only slightly reducing levels of TDP-43 monomer. The compounds appear to act through a mechanism independent of TDP-43 ubiquitination. The compounds also reduce the deleterious effects of TDP-43 expression in *C. elegans*. This work highlights a novel therapeutic approach for treating ALS and FTLD-TDP.

We have also identified a novel role for tau in regulating SG dynamics, and an equally novel ability of TIA-1 to induce misfolding of tau. Tau associates with TIA-1, a core nucleating RBP, promotes SG formation and reduces the movement of RNA granules containing TIA1, while deleting tau inhibits stress granule formation. TIA1 also regulates tau biology. Overexpressing TIA1 stimulates misfolding and phosphorylation of tau, formation of SGs that co-localize with insoluble tau. The link between tau and translational pathways suggests that pathways regulating translation might also modify tau misfolding and toxicity. Consistent with the model, we observe that small molecules that modify RNA translation also modify tau biology. This work suggests that the process of "Regulated Protein Aggregation" which characterizes the biology of RNA binding proteins can be applied to develop novel approaches for drug discovery in neurodegenerative diseases.

SL- 142

Track: Drug Discovery in Preclinical Research

TARGETING CALCITONIN RECEPTOR EXPRESSED BY BRAIN TUMOUR INITIATING CELLS OF GLIOBLASTOMA MULTIFORME

Peter J. Wookey

Department of Medicine, University of Melbourne, Australia; E-mail: pwookey@unimelb.edu.au

Monoclonal antibodies have been generated that detect a primary epitope within the extracellular domain of human calcitonin receptor (hCTR).

hCTR has been detected expressed by malignant glioma cells and brain tumour initiating cells (BTICs, also known as cancer stem cells) of the deadly brain tumour glioblastoma. Expression was found in >90 percent ($p < 0.05$) of patient samples. Primary cultures of BTICs are grown serum-free *in vitro* as high grade glioma (HGG) cell lines and when introduced into a xenograft mouse model recapitulate the pathology of the original tumour, in particular, the invasive potential. HGG cell lines represent four clonal subtypes including proneural, neural, classical and mesenchymal. hCTR is expressed in >40% HGG cell lines tested so far (immunoblot analysis) and expression was represented in each of the subtypes.

An immunotoxin has been developed by conjugation of MAb2C4 with the plant toxin saporin, a ribosomal inhibitory protein. When introduced into the cytoplasm saporin is highly toxic requiring 1-8 molecules per cell to induce death. In the presence of enhancers (such as saponin SO1861) the EC50 for five HGG cell lines that express hCTR was determined *in vitro* and ranged from 1.6pM to 5.4pM, with one resistant.

These data demonstrate that the immunotoxin based on the anti-CTR antibody:saporin conjugate is a highly effective method for killing BTICs *in vitro* and animal trials are now underway to test its efficacy *in vivo*.

Keywords: Antibody therapy, brain tumour, cancer, immunotoxin.

**SL- 245**

Track: Anti Infection

EFFECTIVE AND LESION-FREE CUTANEOUS INFLUENZA VACCINATION

Mei X. Wu, Ji Wang and Bo Li

Wellman Center for Photomedicine, Massachusetts General Hospital, and Department of Dermatology, Harvard Medical School, Boston, MA 02114, USA;
E-mail: MWU5@mgh.harvard.edu

The skin is a potent site for vaccination, yet at a great cost of skin irritation. We resolve this dilemma by delivering a vaccine into many microzones in the skin *via* a biodegradable microneedle array (MNs) in which any of the two microneedles are set apart enough to prevent spreading vaccine-induced inflammation from one to the other. Constraining vaccine-induced inflammation within individual microzones results in fast healing and lesion-free while evoking sufficient innate immunity. When the inoculation site was treated with non-ablative fractional laser (NAFL), prior to insertion of the MNs, the mice displayed vigorous antigen-uptake, giving rise to strong, Th1-biased immunity, and were fully or strongly protected from a challenge of homologous influenza virus at a high dose as well as heterologous H1N1 and H3N2 viruses. In contrast, mice receiving the MNs only were either poorly or little protected from the viral challenges. The cross-protection is extremely important for seasonal influenza vaccines as mismatches between vaccines and circulation viruses occur frequently, leading to reduced efficacy of flu vaccines and increased morbidity. To the best of our knowledge, this represents the first strategy for lesion-free and efficient vaccination.



SL- 19

Track: Cancer Targeted Drug Delivery

ANTI-TUMOR EFFECT OF THE NEWLY SYNTHESIZED HYDROXYCHLOROQUINE CONJUGATE-AHQ

Hong-tao Xiao, Dong-ke Yu, Rong-sheng Tong, Jun-lan Chuan and Li-juan Zhang

Department of Pharmacy, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610072, China; E-mail: xht927@163.com

Hydroxychloroquine, a natural product, has recently being focused as a potential anti-cancer agent as well as a chemosensitizer when used in combination with anti-cancer drugs. AHQ is a newly synthesized hydroxychloroquine conjugate according to the combination of prodrug theory and targeting tumor theory. The synthetic conjugate AHQ was confirmed by proton nuclear magnetic resonance (NMR) spectroscopy. *In vitro* study, we found that AHQ showed potent antiproliferative activity (0.69- 5.76 $\mu\text{g/mL}$) in a panel of human tumor cell lines. The cytotoxicity of AHQ for human normal cells was much smaller than cancer cell lines. Tumor regression was observed following once daily dosing of AHQ in the HCT116 colon cancer xenograft models. In summary, AHQ will be a potential novel drug for human cancer therapy on the basis of *in vitro* and *in vivo* experiment results. The anti-tumor mechanism of AHQ remains to be further studied.

**SL- 120**

Track: Regenerative Medicine

NANOPARTICLES FOR NEURAL REGENERATION

Qiaobing Xu

Department of Biomedical Engineering, Sackler School of Graduate Biomedical Science, School of Medicine, Tufts University, Medford, MA 02155, USA; E-mail: qiaobing.xu@tufts.edu

Various strategies ranging from biomaterial engineering to cell-based therapy have been explored to repair a damaged neural tissue. For example, physical approaches, including nerve guidance conduits and electrical stimulation, have been applied to facilitate nerve regeneration. Biochemical guidance cues such as neurotrophic factors (which promote the growth and survival of neurons) and adhesion molecules have often been utilized to enhance the regenerative outcomes. However, clinical applications of nerve growth factor (NGF) are still limited due to NGF's deleterious side effects. In this presentation, I will present the application of both synthetic and natural-derived lipid nanoparticles for neural tissue engineering.



SL-70

Track: Regenerative Medicine

BIODEGRADABLE PHOTOLUMINESCENT POLYMERS AND MOLECULES FOR TISSUE ENGINEERING, DRUG DELIVERY, AND IMAGING

Jian Yang

Department of Biomedical Engineering, Materials Research Institute, The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA;
E-mail: jxy30@psu.edu



Despite the exciting progress, some fundamental understandings on the key elements of tissue engineering and drug delivery are still lacking. An *in situ* real-time method to accurately monitor and quantify scaffold degradation and tissue regeneration without traumatically explanting samples or sacrificing animals is urgently needed. Conventional theranostic drug delivery nano systems conjugated or encapsulated with organic dyes or quantum dots (QDs) suffer from multiple challenges for clinical translations such as poor photo-bleaching resistance, low dye-to-particle conjugation ratios, high toxicity, increased particle sizes, added complexity, and high risk of adverse biological reactions. Our recent innovations on the development of citrate-based biodegradable photoluminescent polymers (BPLPs) could potentially address the above concerns. We have identified a unique fluorescence mechanism of BPLPs, which enabled us designing a series of biodegradable photoluminescent polymers and small fluorescent molecules. In this presentation, I will go over a citrate fluorescent biomaterial design methodology for the development of a series of novel biodegradable polymers and fluorescent molecules and their applications in tissue engineering, drug delivery, biosensing, and cancer imaging.

SL-75

Track: Biologics

RESIDUES OF ENROFLOXACIN IN TISSUES OF FRESHLY SLAUGHTERED BROILER CHICKENS AVAILABLE IN LOCAL MARKET OF JHANG CITY (PAKISTAN)

M. Younus, T. Abbas, M. Zafar, S. Raza, A. Khan, M. Aslam, S. Jaleel and A. Idress

College of Veterinary and Animal Sciences, Jhang, Pakistan; E-mail: younusrana@uvas.edu.pk



Use of antibiotics by the poultry industry has the potential to produce residues in edible tissues. This study determined the level of enrofloxacin in breast muscle and liver tissues of broiler chickens available in local market of Jhang, Pakistan. A cross-sectional survey was conducted during August 2014. Forty five samples of breast meat and equal number of liver samples from freshly slaughtered broiler chickens were randomly collected from retail shops. The samples were analyzed through a commercial competitive ELISA kit. Enrofloxacin residues were detected in 80% of the samples. Median concentration in muscles and liver tissues was 103 ng/kg (Inter quartile range [IQR] = 2934), and 1409 ng/kg (IQR = 1819), respectively. Among the positive samples, 53% of the muscle and 62% of the liver tissues contained level of residues more than permissible limit. There was no significant difference in concentration of enrofloxacin between liver and muscle samples ($p < 0.05$). This study provide evidence that about half of the chickens sold in Jhang contained residues more than standard limit thus exposing consumers to it deleterious effects. Use of enrofloxacin must be regulated/controlled.

Keywords: Chicken meat, ELISA, enrofloxacin, residues, Pakistan.

SL- 176

Track: Drug Discovery in Preclinical Research

DISCOVERY OF ORALLY ACTIVE FSHR ALLOSTERIC MODULATORS**Henry Yu**

*TocopheRx Inc., Suite 1087, 15 New England Executive Park, Burlington, MA 01803, USA;
E-mail: henry@tocopherx.com*

Follicle-stimulating hormone (FSH), acting on its receptor (FSHR), plays a pivotal role in the stimulation of follicular development and maturation. Multiple injections of FSH are used in clinics for ovulation induction and for *in vitro* fertilization. An orally bioavailable FSH mimetic would increase patient convenience and compliance. Our effort leading to orally active positive allosteric modulators (PAM) targeting FSHR will be described. One of the PAM molecules has been nominated as a preclinical candidate. We will present SAR, selectivity, DMPK, efficacy, safety and toxicology data from selected examples.

**SL- 190**

Track: Medical Imaging

PRELIMINARY ASSESSMENT OF 99mTc-SALMETEROL XINAFOATE AS A POTENTIAL BONE-IMAGING AGENT**Wafaa A. Zaghary, Mohammed A. Motaleb and Manal M. Anwar**

*Department of Pharmaceutical Chemistry, Helwan University, Cairo Governorate, Egypt;
E-mail: wzaghary@yahoo.com*

Pharmacological modulators of β -adrenoceptors play a vital role in bone mineral density and fracture risk. Salmeterol xinafoate is a selective β_2 -adrenoceptor agonist having the desired pharmacological profile of a long-acting bronchodilator. ^{99m}Tc -salmeterol xinafoate was formulated for the development of a novel potential diagnostic bone-imaging agent with excellent biological properties. Factors influencing the labeling yield such as salmeterol xinafoate amount, pH of the reaction medium, reducing agent amount and reaction time were studied in details. ^{99m}Tc -salmeterol xinafoate was obtained with a high radiochemical yield of $94.6 \pm 0.5\%$ and *in vitro* stability of 6 h when 0.5 mg salmeterol xinafoate was mixed with 10 mg NaBH_4 at pH 9 and reaction time 30 min. The biological distribution showed that, ^{99m}Tc -salmeterol xinafoate was highly concentrated in bone ($36.5 \pm 2\%$ ID/g) at 30 min post injection. The bone uptake of ^{99m}Tc -salmeterol xinafoate was remained high (29.3 ± 2 ID/g) for a time up to 2 h post injection. The results revealed that ^{99m}Tc -salmeterol could be solve the ^{99m}Tc -phosphonate drawback. ^{99m}Tc -salmeterol xinafoate could be used as a new bone-imaging agent.

Keywords: Salmeterol, Technetium-99m, bone imaging.

SL- 15*Track: Medical Imaging***COMPARISON OF TWO METHODS FOR SEMIQUANTITATIVE EVALUATION OF DATSCAN****Jianhua Zhang, Esther Mena Gonzalez, Yan Fan, Rongfu Wang, Melin Vranesic, Zoltan Mari and Zsolt Szabo***Peking University First Hospital, Beijing, China*

Aim: Semiquantitative analysis as a powerful supplement of visual assessment plays an important role in evaluation of DaTSCAN images. Our goal was to compare two semiquantitative methods for evaluation of DaTSCAN and assess their clinical value.

Methods: Sixteen DaTSCAN studies in 11 patients with Parkinson's Disease (PD) and 5 control subjects performed between Jan 2011 and Apr 2013 were retrospectively analyzed. Images were processed with two techniques: 1) Automated semiquantification based on the Brain Analysis Software (BRASS, Hermes Medical Solutions) and 2) Manual regions of interest using PMOD software. The occipital cortex served as reference tissue. Specific binding ratios of the entire striatum, the bilateral caudate nuclei, and the bilateral putamina to the occipital cortex were calculated as (striatum-reference)/reference. Correlations between all these striatal ratios and the Unified Parkinson's Disease Rating Scale (UPDRS) were calculated.

Results: With Brass in controls the average ratios of left caudate, left putamen, right caudate, right putamen and entire striatum were 2.102, 1.934, 2.036, 1.810 and 1.971, while in the PD group they were 1.313, 0.961, 1.257, 0.913 and 1.111, respectively. The striatal binding ratios were significantly higher in the control group ($P < 0.05$) except in the right caudate. With PMOD the average ratios of the left caudate, left putamen, right caudate, right putamen and entire striatum of controls were 2.547, 3.171, 2.546, 2.937 and 2.8 in controls, and 1.921, 1.491, 1.803, 1.473 and 1.452 in PD. Again, these differences were significant ($P < 0.05$), except in the right caudate ($P > 0.05$). UPDRS was higher in the PD group than in the control group (31.125 vs 3.8, $P < 0.05$). The striatal ratios from both methods showed negative correlation with UPDRS ($r: -0.562 \sim -0.877$, $P < 0.05$).

Conclusion: Semiquantitative evaluation of DaTSCAN with either BRASS or PMOD methods can differentiate PD from control subjects. The striatal ratios of both methods show negative correlation with the severity of PD.

Keywords: DatSCAN, semiquantitative evaluation, Parkinson's Disease.

SL-102*Track: Cancer Targeted Drug Delivery***SEPIN-1, A NOVEL SEPARASE INHIBITOR, FOR BREAST CANCER THERAPY****Nenggang Zhang, Ha Do, Lacey E. Dobrolecki, Silviya Demerzhana, Jessica T. Li, Michael T. Lewis, Scott R. Gilbertson and Debananda Pati***Pediatrics, Baylor College of Medicine, Houston, USA; E-mail: nzhang@bcm.edu*

Separase, a chromosomal cohesion-resolving enzyme during cell division, is an oncogene. It is overexpressed in multiple human tumors, including breast, bone and brain. In mouse models, Separase overexpression has been shown to induce aneuploidy, genomic instability, mammary and osteogenic tumorigenesis, and intratumoral heterogeneity. Knockdown of Separase inhibits the growth of Separase-overexpressing mammary tumor cells but has no effect on cells with a normal Separase level. Using a high throughput screen, we identified a small molecule Separase inhibitor (Sepin-1) that inhibits Separase activity in a non-competitive way. Sepin-1 inhibits growth of neuroblastoma, leukemia, and breast tumor cells. It is well tolerated with no significant toxicity or side effect in mice. Studies using patient-derived orthotopic xenografts of triple negative breast cancer (TNBC) render significant survival advantages for Sepin-1- treated mice. Sepin-1 inhibits the growth of Separase-overexpressing human



TNBC xenografts in mice in a Separase-dependent manner, and has no appreciable effect on TNBC tumors with low-Separase expression, suggesting the specificity and efficacy of this compound in targeting tumors addicted to Separase overexpression. Targeting Separase by Sepin-1 results in high level of apoptosis. These results suggest that inhibition of Separase represents a new line of therapy to treat breast and other tumors addicted to Separase overexpression.

SL- 184

Track: Medical Imaging

FIRST MULTI-SITE STUDY ON QUANTITATIVE DYNAMIC WHOLE-BODY FDG PET-CT IN ONCOLOGIC IMAGING

Sulei Zhang, Li Huo, Changjing Zuo, Xueqi Chen, Wenchan Li, Bing Cui, Yubing Wang, Fang Li, Yun Zhou

Department of Nuclear Medicine, Second Military Medical University, Changhai Hospital, Shanghai, China

Background: As temporal and spatial resolution of PET is remarkable improved in advanced PET-CT scanner, dynamic whole-body FDG PET-CT is proposed to improve clinical static whole-body FDG PET-CT imaging technique. The objective of this study is to optimize and simplify dynamic whole-body FDG PET-CT imaging technology in data acquisition and quantification, and evaluate its clinical efficacy.

Method: Total 40 oncology patients were recruited via 3 PET centers. Three PET-CT scanners, Siemens Biograph 64 mCT, Biograph128 mCT, and a GE Discovery PET-CT scanner, were used for image data collection in the 3 PET centers, respectively. Three dynamic whole-body PET scanning protocol were used for the 3 PET scanners for 60 min dynamic PET data acquisition: 11-pass dynamic whole-body scan via multi-bed (1 min/bed, 5 beds/pass) for qualitatively, 17-pass dynamic whole-body scan with varied duration/bed for Biograph128 mCT, and 25 frames with fixed 1-bed dynamic scan for GE Discovery PET-CT. Clinical whole-body PET scan (2 min/bed) were started about 60 min post FDG injection for all patients. The time activity curve of aorta was used as input function for kinetic modeling, and population-based input function method was also evaluated. Patlak plot was used to generate parametric images of FDG uptake rate constant K_i . To reduce the noise levels in the K_i images generated by conventional linear regression method, a linear regression with spatial constraint algorithm was proposed to generate K_i images. Standardized uptake value (SUV) images were generated for different data acquisition window. Both parametric K_i images and SUVs were used for diagnosis separately by different physicians.

Result: The noise levels of K_i images from conventional linear regression method were remarkably reduced by applying spatial constraint algorithm. Linear regression with spatial constraint provided K_i images with lowest noise levels if population-based input function was used. Higher contrast of tumor in K_i images were obtained as compared to SUV images. One-bed dynamic PET followed by a static whole-body scan provided full tissue kinetic information for detection and monitoring tumor therapeutic response.

Conclusion: The Patlak plot with population-based input function and linear regression with spatial constraint is robust and suggested for the quantification of dynamic whole-body FDG PET. The kinetic information obtained from dynamic PET is potentially to improve clinical efficacy of FDG PET-CT which will be examined by the ongoing project.

SL-307

Track: Inflammation and Immunology

CHALLENGES AND CONSIDERATIONS IN NANO-DRUG CHARACTERIZATION**Jiwen Zheng**

Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, U.S. Food and Drug Administration, USA

Nanomaterials are increasingly incorporated into medical products regulated by FDA. The novel properties of nanomaterial such as small size, large surface area, material heterogeneity and polydispersity may lead to additional scientific consideration when these products are being reviewed following current FDA guidelines and practices.

A comprehensive physicochemical characterization of drug products containing nanomaterials is required in order to evaluate product safety and effectiveness. It is also essential for quality control, particularly batch-to-batch consistency during manufacturing. Physicochemical characterization includes, but is not limited to measurement of particle size, size distribution, shape, zeta potential, surface characteristics, composition, purity, drug loading and stability. A wide variety of analytical instruments and methods have been applied to measure these attributes, such as light scattering, laser diffraction, transmission electron microscopy, scanning electron microscopy, atomic force microscopy, *etc.* However, the issues with instrument suitability, sample stability and proper validation pose great challenges on how to interpret the measurement results.

This presentation will review results from a number of case studies involving nano-drug formulations and outline the practical limitations and pitfalls of techniques used for nano-drug characterization.

Keywords: Nano-drug, nanomaterial, physicochemical characterization.

SL-206

Track: Drug Discovery in Preclinical Research

DEVELOPING SMALL-MOLECULE BAX ACTIVATORS FOR CANCER THERAPY**Jia Zhou**

Chemical Biology Program, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555, USA; E-mail: jizhou@utmb.edu

Bax, a central cell death regulator, is a requisite gateway to mitochondrial dysfunction and a major pro-apoptotic member of the Bcl-2 family of proteins that control apoptosis in normal and cancer cells. Several lines of evidence suggest that Bax is a promising target for treatment of human cancers by direct activation of this protein. Accumulating studies support that serine 184 (Ser184) is a critical switch to functionally regulate Bax's proapoptotic activity. Therefore, manipulation of the phosphorylation status at Ser184 represents a novel strategy for treatment of human cancers including triple-negative breast cancer (TNBC) and lung cancer by altering the activity of Bax in tumors. Herein, we report the preclinical development of Bax activators based on our identified lead compounds with the aid of molecular docking around Ser184 site. Several novel direct Bax activators have been developed including SMBA1 (*Nature Communications*, 2014 Sep 17;5:4935. doi: 10.1038/ncomms5935.), CYD-2-11 and CYD-4-61 with low nanomolar binding affinity to Bax protein. More importantly, these compounds not only exhibited potent antiproliferative activity against human cancer cells with low micromolar to nanomolar IC₅₀ values, but also displayed remarkable *in vivo* inhibitory effects against cancer xenograft tumor growth with potential to overcome drug resistance.

SL-3

Track: CNS Drug Discovery and Therapy

DEVELOPMENT OF SEROTONIN 2C RECEPTOR POSITIVE ALLOSTERIC MODULATORS FOR TREATMENT OF NEUROPSYCHIATRIC DISEASES

Christopher Wild, Rachel M. Hartley, Chunyong Ding, Carrie McAllister, Noelle C. Anastasio, Robert G. Fox, Sonja Stutz, Suzanne Tomlinson, Kathryn A. Cunningham and Jia Zhou

Center for Addiction Research, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555, USA; E-mail: jizhou@utmb.edu



Allosteric modulation of G protein-coupled receptors presents a promising approach to selectively target highly homologous receptor subtypes in a site- and event-specific manner conferring several benefits over the traditional targeting of orthosteric binding sites including reduced side-effect profile. Precisely, modulation of the serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) as a target to treat psychiatric disorders characterized by impulsivity is a novel approach with the potential to generate clinically relevant compounds for substance-related disorders, attention deficit hyperactivity disorder, and obesity. Utilizing a drug development team comprised of chemists and biologists, a series of new small molecules have been rationally designed, chemically synthesized, and pharmacologically characterized by using developed lead small molecules as templates, homology modeling and molecular docking techniques, as well as a battery of in-house *in vitro* (functional and radioligand binding studies) and *in vivo* (behavioral studies) assays to assess allosteric modulation of the 5-HT_{2C}R. To date, several advanced drug candidates have been identified to enhance 5-HT_{2C}R-mediated Ca_i⁺⁺ release and ERK1/2 activation induced by the endogenous ligand 5-HT or the selective 5-HT_{2C}R agonist WAY 163909. Our drug development efforts open new avenues in probing the 5-HT_{2C}R function and discovering novel pharmacotherapeutics for neuropsychiatric diseases.

SL-201

Track: Translational Medicine

VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION COMPARED TO CLINICAL AND PATHOLOGICAL FEATURES IN OVARIAN CANCER

Inbar Ben Shachar^{1,2}, Hasan Zedan¹, Svetlana Gandler¹ and Jamal Zidan^{2,3}

¹Department of Ob & Gynecology, Ziv Medical Center, Israel

²Faculty of Medicine in the Galilee, Bar-Ilan University, Safed, Israel; E-mail: Zidan.j@ziv.health.gov.il

³Oncology Institute, Ziv Medical Center, Israel



Background: Vascular endothelial growth factor (VEGF) in ovarian carcinoma acts as both a potent stimulator of angiogenesis and enhancing vascular permeability. There is no consensus in the literature if VEGF can be used as prognostic or predictive factor in ovarian cancer. The aim of this study was to evaluate the expression of VEGF in ovarian cancer and to compare it with both clinical and pathological features of the patients.

Methods: Forty three formalin-fixed, paraffin embedded tissue specimens presenting ovarian cancer were examined using immunohistochemistry (IHC) test for evaluation of VEGF. Recorded clinical and pathological data of each patient were summarized from the patients files in Ziv Medical Center.

Results: All 43 patients with ovarian cancer were evaluated. Twenty three women (53.5%) were Jews, 5 Arabs (11.6%) and 15 (34.9) were other nationalities. At diagnosis stage I was diagnosed in (16.3) pts, stage II (11.6%), stage III (44.2%) and stage IV in (27.9) pts. Median age was 58 years (range: 17-82). Adenocarcinoma was diagnosed in (81.4%), endometrioid in 4 and 4 with other malignancies. Histologically, 16.3% were grade I, 16.3 grade II, 44.2% grade III and in 23.3% grade was unknown. VEGF was negative in 23 (53.5%), weakly positive (+1) in 20 (46.5%) of pts. VEGF +2 or +3 was not found in any of the pts. VEGF was negative in 51.4% pts with adenocarcinoma. VEGF was positive (+1) in 68.4% of 19 pts with grade III tumors (p<0.05) versus 28.6% of 14 pts with grade I and II tumors.

Conclusion: VEGF was higher in grade III adenocarcinoma of ovary compared to grade I and II. As a result VEGF may be used as a prognostic and as a predictive factor for treatment with antiangiogenic drugs. Further studies with more patients are warranted to confirm these results.

POSTERS

PO-39

Track: Pharmaceutical Research & Development

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL COPPER COMPLEXES WITH BITHIAZOLE

Anita Abedi¹, S. Nasser Ostad², Vahid Amani³ and Zohreh Mehri Lighvan⁴

¹Chemistry Department, North Tehran Branch, Islamic Azad University, Tehran 19585-936, Iran; E-mail: anitaabedi@yahoo.com

²Department of Toxicology & Pharmacology, University of Tehran Medical Sciences, Tehran, Iran

³Chemistry Department, Shahid Beheshti University, Evin, Tehran 1983963113, Iran

⁴Department of Chemistry, Isfahan University of Technology, Isfahan 84156/83111, Iran

Bithiazole is selected as a biological ligand in our last researches [1], because it is a moiety of bleomycins (BLMs), an anti-tumor drug employed for treating lymphomas, squamous cell carcinomas, cervical cancers and testicular carcinomas, where the bithiazole tail of bleomycin is involved in the interaction of bleomycin with DNA. On the other hand, copper is an essential element in the body, which plays a specific role in a number of biological processes and copper complexes are used as cleaving reagents and DNA binding molecules [2].

As part of our research on metal complexes with bithiazole derivatives, in this research, we report three neutral copper complexes $[\text{Cu}(\text{4bt})\text{Br}_2(\text{DMSO})]$, $[\text{Cu}(\text{dm4bt})\text{Br}_2(\text{DMSO})]$ and $[\{\text{CuBr}(\text{dm4bt})\}_2(\mu\text{-Br})_2]$, where 4bt is 4,4'-bithiazole and dm4bt is 2,2'-dimethyl-4,4'-bithiazole. The complexes were fully characterized by elemental analysis, IR, UV-Vis and single-crystal diffraction.

Furthermore, these complexes were used for *in vitro* cytotoxicity evaluation against normal cell line as control cell and some cancerous cell cultures, including HT-29, Caco-2 and T47D by MTT assay. To study binding mode of copper complexes with DNA, we examined interaction ability of the complexes with native calf thymus DNA (CT-DNA) by UV-Vis absorption spectrophotometry, fluorescence spectroscopy and circular dichroism (CD).

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**PO-100**

Track: Hot Topics in Drug Targets

DISCOVERY OF NEW AZO DYES AS POTENTIAL NON-GENOTOXIC FOOD COLORS

Olajire A. Adegoke

Department of Pharmaceutical Chemistry, University of Ibadan, Orita UI, Ibadan, Nigeria;
E-mail: ao.adegoke@mail.ui.edu.ng

Diazo coupling reaction between 4-Carboxyl-2,6-dinitrobenzene diazonium ion and propranolol, naproxen and nabumetone, and with α - and β -naphthols yielded a new series of azo dyes. Spectroscopic characterizations established their structure. Three of the four dyes exhibit azo-hydrazone tautomerism. Mechanistic-based binding studies were conducted using BSA and CT-DNA. The potentials of producing specific DNA damages were investigated *in vitro* and *in vivo* using Comet



assays and chromosomal aberrations.

Spectroscopic characterizations showed that α -naphthol and its ether gave *para*-substituted while β -naphthol and its ethers provided *ortho*-substituted dyes. Dealkylation of the naphthalene ether linkage was found to occur upon coupling. This led to the serendipitous discovery of a new class of azo dye series, 4-carboxyl-2, 6-dinitrophenylazohydroxy-naphthalenes. Evidence for the formation of new molecular complexes was established by hypsochromic and hypochromic shifts of the spectra of dye-BSA complexes compared to the spectra of unbound dyes. Thermodynamic considerations for the DNA studies enabled the delineation of the binding modes. The four dyes gave varying results with respect to the parameters of DNA damage studied. AZ-03 and AZ-04 (possessing additional C-7 substituents) did not produce significant genotoxic effect at all concentrations relative to the negative control. In the *in vivo* studies, some non-significant dose-dependent DNA damage occurred. Mitotic indices in all cases revealed lack of cytotoxicity of the monoazo dyes.

The monoazo dyes show the potential of being utilized as colorants, pending further required tests.

PO-81

Track: Cardiovascular Drug Discovery & Therapy

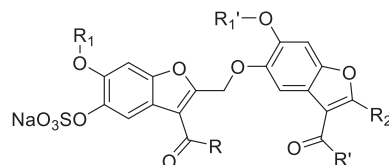
THROMBIN ALLOSTERISM: STRUCTURALLY SIMILAR MONOSULFATED BENZOFURANS DISPLAY VARIABLE REGULATION OF THE PROTEASE

Daniel Afosah^{1,2}, Stephen Verespy^{2,3}, Rajesh Karuturi^{1,2} and Umesh R. Desai^{1,2}

¹Department of Medicinal Chemistry, ²Institute for Structural Biology, Drug Discovery & Development, ³Department of Chemistry, Virginia Commonwealth University, Richmond, VA, USA; E-mail: afosahd@vcu.edu

Sulfated benzofuran dimers (SBDs, see Figure) have been proposed to inhibit thrombin through an allosteric mechanism by binding to a site in the region of Arg173. We employed a computational approach to validate the predicted binding site, understand the SAR, and also develop more potent inhibitors. Using GOLD, a genetic algorithm for docking and scoring, we studied a diverse virtual library of SBD analogs to identify a group of putative allosteric thrombin inhibitors. Chemical synthesis followed by biological assays of targeted inhibitors resulted in interesting insight into thrombin allostery.

Molecular modeling predicted an increase in potency upon substitution of the R₁'-methyl group with benzyl groups due to an added pi-pi ligand-protein interaction. Despite these very favorable molecular modeling results, the putative inhibitors did not show the predicted inhibition potency. None of the 14 putative inhibitors were found to exhibit potencies in the nM range, as predicted. However, interesting discoveries were made when efficacies were considered. Majority of the R₁' analogs displayed efficacy of only 50-60% at saturation, which is dramatically different from all reports available in the literature. A maximal efficacy of 50% is likely to reduce the risk of bleeding as it can only depress prothrombotic state in a regulated manner without the fear of knocking out clotting completely. Substitution of the R₁-methyl group with a cyclohexylmethyl group led to thrombin inhibitors with a minimal efficacy of 80%. This suggests a completely different thrombin regulation property from the agents that offer 50 – 60% inhibition. Thus, although the structures of the two class of thrombin inhibitors are very similar, their allosteric regulatory properties are completely different. We hypothesize that the two groups of molecules utilize significantly different sites and/or modes of binding on thrombin. Further work is necessary to identify the structural basis for the different allosteric regulation mechanisms.



Keywords: Thrombin, inhibitors, allostery, virtual library, computational approach, efficacy, bleeding.

PO-53

Track: Cardiovascular Drug Discovery and Therapy

PATTERNS OF DRUG USE AND ADHERENCE IN THE MANAGEMENT OF HYPERTENSION IN A HEALTH CARE FACILITY IN WARRI, DELTA STATE, NIGERIA

Ogheneovo Clement Aghoja

Clinical Pharmacy, Delta State University, Abraka Delta State, Abraka, Nigeria; E-mail: stmajella@hotmail.com

Introduction: Hypertension is one of the treatable causes of cardiovascular morbidity and mortality. Management evaluation of hypertension helps to streamline treatment protocols in hospitals that conform to global practice standards. Critical to hypertension management is adherence and lack of knowledge to adhering to prescribed medications may constitute treatment failure.

Objective: To determine the patterns and adherence level of antihypertensives and impacting factors to non-adherence.

Method: 400 case files of adult hypertensive patients attending the Central Hospital, Warri were evaluated for a period of six months for drug use pattern. Information on adherence was elicited using the 8-item Morisky questionnaire. Reasons for non-adherence were also sought.

Results: More women (69.8%) than men (30.2%) were affected, with mean systolic blood pressure (SBP); males (155.3mmHg \pm 17.3), females (153.3mmHg \pm 16.7) and mean diastolic blood pressure (DBP); males (92.0mmHg \pm 9.8), females (91.8mmHg \pm 10.7). After commencing treatment, mean SBP and DBP for males and females were (143.6mmHg \pm 22.1; 87.9mmHg \pm 11.6) and (143.8mmHg \pm 22.3; 86.4mmHg \pm 12.5). Calcium channel blockers were most frequently prescribed antihypertensives (29.7%) followed by angiotensin converting enzyme inhibitors (24.8%). Calcium channel blockers, angiotensin converting enzyme inhibitors were the commonest multitherapy (50%). Adherence level was 84%. Major reasons for non-adherence were affordability, and forgetfulness to take their medications.

Conclusion: Calcium channel blockers and angiotensin converting enzyme inhibitors were mostly prescribed and as dual therapy while diuretics were rarely used. Adherence to medication was relatively high but their blood pressure was poorly controlled. Reasons for non-adherence were the inability of respondents to afford medications and forgetfulness.

PO-87

Track: Cardiovascular Drug Discovery and Therapy

PROFILE OF DRUG THERAPY PROBLEMS AND INTERVENTIONS IN HYPERTENSIVE PATIENTS

Rita O. Akonoghre, Azuka C. Oparah and Jeffrey S. Soni

Department of Clinical Pharmacy and Pharmacy Administration, Faculty of Pharmacy, Delta State University, Abraka, Nigeria; E-mail: ritagbro@yahoo.com

Drug therapy problems (DTPs) were profiled among hypertensive outpatients in a Nigerian secondary health care facility and pharmaceutical care interventions provided and the impact on quality of life assessed.

The study employed a prospective design on a sample of 104 patients, 65 of who were followed up for six months. Drug therapy problems were profiled based on classification by Strand *et al*, while SF 12 assessed health related quality of life.

Mean age of 72.3% of the patients was 63.33 (SD +12.42); 78.5% were females and 61.5% had a family history of hypertension; 40.1% had diabetes co-morbidity. Blood pressure in 46.1% of the patients improved after intervention ($P < 0.05$). Inappropriate adherence was the highest occurring DTP. There was a statistically significant association between DTP resolved/prevented, and the pharmaceutical care intervention ($P < 0.001$). The resolution of DTPs showed a



corresponding improvement in quality of life. Therefore, provision of pharmaceutical care services in out-patients by pharmacists should be encouraged.

Keywords: Drug therapy problem, pharmaceutical care, quality of life.

PO-16

Track: Hot Topics in Natural Products

EVALUATION OF THE ANTI-INFLAMMATORY AND SEDATIVE EFFECTS OF LEAF AQUEOUS EXTRACT OF *WITHANIA SOMNIFERA* (L.) DUNAL (*SOLANACEAE*) IN RATS AND MICE

Oluchi N Mbamalu, Munira Ismail, Nabeel Dudhia, Nadeem Noordien, Zukiswa Mabuya, Oatthostse Kakutsi and George Jimboyeka Amabeoku

School of Pharmacy, Discipline of Pharmacology, University of the Western Cape, Cape Town, South Africa; E-mail: gamabeoku@uwc.ac.za

Purpose: The use of medicinal plants in South Africa is cultural. *Withania somnifera* is one of the medicinal plants used to treat various ailments in the country. The plant species has been used by traditional medicine practitioners to treat inflammation and painful conditions like rheumatism. It is also known to be used as a sedative and hypnotic drug. However, there is no information in literature to verify the effectiveness of *Withania somnifera* in the treatment of inflammation and insomnia. The study, therefore, investigated the anti-inflammatory and central nervous system depressant activities of the leaf aqueous extract of the plant species in mice and rats.



Materials and Methods: Fresh leaves of *W. somnifera* were collected from Kirstenbosch Botanical Gardens, South Africa, authenticated by a taxonomist and a voucher specimen (UWC 005) deposited in the University Herbarium. Leaf aqueous extract was prepared using standard extraction methods. The carrageenan-induced rat paw oedema test was used to determine the anti-inflammatory effects while pentobarbitone-induced sleep test and locomotor activity were used to evaluate the sedative effect of the plant species. Phytochemical qualitative analysis, acute toxicity and HPLC studies of the plant species were also carried out using standard methods.

Results: The phytochemical qualitative analysis carried out on the dried powdered leaves of *W. somnifera* showed the presence of saponins, tannins and triterpene steroids. Leaf aqueous extract of *W. somnifera* (100-200 mg/kg i.p.) significantly prolonged pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice in a dose dependant manner. Diazepam (0.5 mg/kg, i.p.) significantly prolonged pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice. The doses of 100 and 200 mg/kg (i.p.) of the plant species and 0.5 mg/kg (i.p.) of diazepam significantly reduced the locomotor activity of mice. Leaf aqueous extract of the plant species (50-200 mg/kg, i.p.) significantly reduced the oedema produced by carrageenan (1%) in rats over 90 min period of testing. Indomethacin (20 mg/kg, i.p.) significantly reduced carrageenan (1%)-induced oedema in rats over 120 min period of testing. The LD50 value obtained for the leaf aqueous extract of the plant species following inter-peritoneal injection was 1600 mg/kg while that following oral administration was probably over 4000 mg/kg. The HPLC finger-print of the aqueous extract showed distinct peaks at the following retention times 2.977, 3.594, 4.154, 4.406, 4.660 and 15.267 min.

Conclusion: The data obtained shows that the leaf aqueous extract of *W. somnifera* has both sedative and anti-inflammatory effects.

Keywords: Acute toxicity, anti-inflammatory activity, HPLC study, leaf aqueous extract, locomotor activity, mice, pentobarbitone-induced sleep test, phytochemical analysis, rats, *Withania somnifera*, *solanaceae*.

PO-65

Track: Hot Topics in Drug Targets

CHOLESTEROL OXIDASE P450 ENZYMES IN *MYCOBACTERIUM TUBERCULOSIS*: STRUCTURE-GUIDED APPROACH FOR DRUG TARGETING AND SELECTIVE INHIBITION

Cecilia N. Amadi¹, Kirsty J. Mclean¹, Colin W. Levy¹, Anthony G. Coyne², Chris Abell², David Leys¹ and Andrew W. Munro¹

¹Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK; E-mail: cnamadi@rocketmail.com

²Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

Mycobacterium tuberculosis (*Mtb*), a deadly pathogen, has scourged mankind for many decades. Tuberculosis, the disease caused by this bacterium, is a major cause of death in developing nations and there is potential for its re-emergence in developed countries. An alarming rise in cases of multidrug-resistant and extremely-drug resistant tuberculosis (MDR-TB and XDR-TB) that do not respond to the customary first-line antibiotics necessitates the urgent need for development of new anti-TB drugs. *Mtb* becomes engulfed in human macrophages post infection of the host, but persists in the harsh environment of the human lungs by utilization of host cholesterol as a carbon source.

The P450s CYP125, CYP142 and CYP124 play key roles in host cholesterol metabolism for energy generation. Understanding the structure/mechanism of the cholesterol 27-oxidases may help facilitate development of novel inhibitors of these P450s, which are crucial for *Mtb* to infect the host and to sustain infection.

Here we present structural and biochemical characterization of CYP142 and CYP124. Optical titrations show that CYP124 binds tightly to cholesterol, cholestenone and methyl branched lipids, consistent with its physiological role in sterol metabolism. CYPs 124/125/142 bind tightly to a range of azole antifungal drugs, and some of these azoles have been shown to clear *Mtb* infection in mice. The crystal structures of econazole-bound and cholestenone-bound CYPs142/124 have been solved. Preliminary fragment screening studies have identified type I (substrate-like) and type II (inhibitor-like) hits for these P450s, and work is in progress to develop these further, with the aim of building novel types of *Mtb* P450-specific inhibitors that can inactivate two or all of these cholesterol oxidases, and in so doing efficiently block host cholesterol utilization and human infection by *Mtb*.

**PO-82**

Track: Drug Delivery and Targeting

EFFECTS OF PHARMACOLOGICALLY TARGETING THE REVERBs ON SLEEP AND WAKEFULNESS

Ariadna Amador¹, Salvador Huitron-Resendiz², Amanda J. Roberts², Laura S. Solt¹ and Thomas P. Burris³

¹Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL, USA; E-mail: aamador@scripps.edu

²Department of Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA, USA

³Department of Pharmacological and Physiological Science, The Saint Louis University School of Medicine, St. Louis, MO, USA

The nuclear receptors REV-ERB α and REV-ERB β are ligand-regulated transcription factors that have been demonstrated to be key components of the circadian clock. Recently, we characterized REV-ERB-specific synthetic ligands, which display significant *in vivo* exposure, SR9009 and SR9011. Both ligands were effective modulators of the circadian rhythm and metabolism *in vivo*. Importantly, SR9009 and SR9011 were demonstrated to regulate sleep

architecture and emotional behavior in mice, highlighting the therapeutic potential of RevErbs for the treatment of diseases associated with dysregulated clock function. To further explore how pharmacological modulation of REV-ERB activity affects sleep, we conducted studies assessing the effects of SR9009 on sleep beyond acute injections at ZT6, the time point of highest REV-ERB mRNA expression. Through use of electroencephalogram (EEG) recordings in mice, we performed time response curves with SR9009 to determine optimal time(s) for dosing, effectiveness of the drug when administered every 3 hours, and investigated whether tolerance to SR9009 can be acquired. Collectively, our studies indicate that pharmacological targeting of the REV-ERBs may have therapeutic potential for the treatment of sleep disorders as well as aid in re-synchronizing aberrant circadian rhythms imposed by shift work and jet lag induced by traveling across time zones.

PO-70

Track: Tissue Engineering

NANOFUNCTIONALIZATION OF 3D CONSTRUCT HYDROGELS FOR TISSUE ENGINEERING APPLICATION

Rana Kadri¹, Ali Tamayol², Laura Sanchez-Gonzalez¹, Stéphane Desobry¹, Michel Linder¹ and Elmira Arab-Tehrany¹

¹Lorraine University, LIBio, Nancy, France; E-mail: elmira.arab-tehrany@univ-lorraine.fr

²Center for Biomedical Engineering, Department of Medicine, Harvard Medical School, Boston, USA

The hydrogels are 'soft' microscopic particles consisting of cross-linked polymeric molecules. They are valued for their functionality and ability to tune physical properties in industrial applications including controlled drug delivery, cosmetics, pharmaceuticals and tissue engineering. The overall goal of the proposed abstract is to functionalize the 3D hydrogel constructs from alginate and GelMA by nano-liposome. The nanofunctionalization will be improved the physicochemical, mechanical and biological properties. The mechanical properties of IPN disc before and after nanofunctionalization were studied at two scales (nanoscale by AFM and mesoscopy scale by rheometer). The results showed after adding the nanoliposomes, the mechanical properties increased at two scales. The morphological property changed by adding the nanoliposome, the presence of the pore with regular size after nanofunctionalization were observed. This new morphology will be improve the transfer of nanoliposome and the surface contact. The results confirmed a high cellular viability after nanofunctionalization. The IPN discs possess with nanoliposome has stronger mechanical characteristics in comparison to the hydrogel without nanoliposome. We showed by adding the nanoliposome from natural sources, the cells proliferation of NIH 3T3 increases. These new properties are interesting to develop the new matrix used in tissue engineering.

PO-57

Track: Recent Advances in Patient Treatment and Care

FLAVONOIDS; CONCRETE NUTRITIONAL COVERS AGAINST IMMUNOPATHOLOGIES

Lotfollah Behroo, Farideh Shishehbor and Mehri Ghafouriyan/Ghafourian

Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; E-mail: behroo_l.nutr@yahoo.com

Specifically over recent years, in the scope of clinical immunology and along with enterprises that lend themselves to promote health care policies, major emphasis is being placed on the employing of Complementary and Alternative Medicines.

On this concern, medicinal plants thanks to have a variety of effectual/safe components/preparations



still serve as leads for development/progression of novel nutraceutical therapeutic agents.

Currently compounds of interest to immunologists are FLAVONOIDS (in general, and Quercetin in particular), which occur in everyday often-eaten foods, in large levels.

Prevalently, flavonoids exist as aglycones, glycosides and methylated derivatives. The flavonoid aglycone is composed of a benzene-ring (A) condensed with a six-membered ring (C) which in the 2-position carries a phenyl-ring (B) as a substituent-group. The aglycone flavonoids can be divided into various classifications on the basis of their molecular building-arrangements.

Quercetin -a nutritional component belonging to "Flavonol" subgroup of the flavonoid family- is the aglycone of quercitrin, rutin and, other glycoside flavonoids. It has extensively disseminated in the plant-Kingdom such as oak-trees, onions and teas, and is found in edible-substances including apples, berries, and brassica vegetables, as well as, in a variety of seeds, flowers, barks, and leaves.

Contrary to just a few earlier studies alluding of Quercetin toxicity/mutagenicity in *in vitro*, several recent credible/endorsed studies suggest/demonstrate that Quercetin has indeed "Antimutagenic" and "Immunoprotective" properties in *in vivo*.

Keywords: Flavonoids, nutritional compounds, immunopathologies.

PO-68

Track: High-Throughput Screening & Laboratory Automation

PEANUT-ALLERGENS EXTRACTION/CHARACTERIZATION: EVIDENCES FOR CONFORMITY WITH FOOD-LABELING

Lotfollah Behroo, Farideh Shishehbor and Lotfollah Behroo

Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; E-mail: behroo_l.nutr@yahoo.com

Contrary to other food allergies (e.g., allergy to Eggs and Cow's Milk), Peanut (PN) allergy is not often outgrown. Currently, strict avoidance and the prescription of Adrenaline –EpiPen–, in the event of an accidental exposure, are the extant/residual recommended cares.

On the other hand, undeclared PN allergens –due to misformulation and/or contamination during food processing– raise a possible hazard for PN-allergy susceptible/suffering human subjects and, it might be avoided, disproportionately/unduly. Hence, meticulous/reliable characterization and quantification procedures for PN-allergens are necessary in order to guarantee the requisite conformance/compliance with food-labeling and, to improve consumer safety.

In the current study, PN proteins -as the allergens of interest- were extracted from fresh/crude PNs, which is described briefly, as follows:

Primarily, PN-bodies were pulverized by a mill and then, the resulted paste was defatted by n-Hexane (1:3 v/v, 3 times). Subsequent to separation process, residues were dried out and deodorized *via* gentle heat-treatment. After that, the obtained flour was mixed with Phosphate Buffered Saline (PBS), (1:10 w/v) and subsequently, subjected to extraction by shaking over night at 4 °C. Then, the resulted suspension was methodically, centrifuged twice for clarification, as mentioned below:

Firstly: Centrifugation at 3500 r/min. and 4 °C for 30 min.

Secondly: Centrifugation at 5000 r/min. and 4 °C for 20 min.

Afterwards, the supernatant was additionally, filter-sterilized through 0.45-µm pore-size sterile syringe filters and lastly, the collected extract (3 mg/ml.PBS; as quantified by Macro-Micro Kjeldahl method), was stored as frozen at -20 °C until need. In conclusion, extraction of the suspected proteins from allergenic food(s) and food crops constitutes an influential process in the allergen-detection methods. Notably, our study's major outcomes covered much ground and were efficiently, of light and leading.



Keywords: Peanut allergy, extraction, labeling.

PO-61

Track: Pharmaceutical Analysis

ANALYSIS OF FAMOTIDINE AND RABEPRAZOLE IN COMBINED DOSAGE FORM BY RP – HPLC

Kuldeep Singh, Ankur Singh, Prashant Musmade and Krishnamurthy Bhat

Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India; E-mail: km.bhat@manipal.edu



Most of the diagnosed gastroesophageal reflux disease (GERD) are treated with once or twice daily standard doses of proton pump inhibitors (PPI) in the initial stages. It is estimated that about 31% of GERD patients are unresponsive to PPI treatment. Concomitant treatment with PPI and H₂ receptor antagonist might be a promising therapeutic strategy to treat these patients. Hence a new formulation containing Famotidine (a H₂ receptor antagonist) and Rabeprazole (a proton pump inhibitor) was developed by our peers. To evaluate this formulation a suitable analytical method is developed and validated as per ICH Q1A (R2) guidelines.

The method developed is RP – HPLC method in isocratic mode. The mobile phase used was ammonium acetate (10mM, pH 5.5) and acetonitrile in 65:35% v/v. the column used was gracesmart C18, 250mm X 4.6mm, 5m and the detection wavelength is 280nm. The retention times observed under these conditions was 3.8 minutes and 8.8 minutes respectively for Famotidine and Rabiprazole. The method was linear over a concentration range of 0.1 – 25.0 mg/mL for both the drugs with a regression coefficient of more than 0.997. The method was found to be specific (peak purity index of 0.99), precise (Relative Standard Deviation of less than 0.94) and accurate (mean percentage recovery of 99.49 to 101.30). The new method can be successfully applied for the analysis, quality control and dissolution test of the combination formulation.

PO-48

Track: Cancer-Targeting Compounds

AK301: A NOVEL MITOTIC ARREST AGENT THAT PROMOTES THE MITOSIS-TO-APOPTOSIS TRANSITION

Michael Joseph Bond, Avijeet Chopra, Amy Anderson and Charles Giardina

*Molecular and Cell Biology, University of Connecticut, Windsor Locks, USA;
E-mail: michael.bond@uconn.edu*



Precise genomic division during mitosis is a well-orchestrated process, mediated by microtubule spindles. Mistakes in the genomic division may result in apoptosis or tumorigenesis. Mitotic inhibitors are widely utilized chemotherapeutic agents, which take advantage of high proliferative nature of cancer cells and defects in their mitotic checkpoints. We have identified a class of novel piperazine-based microtubule inhibitors, of which AK301 (1-(3-chlorophenyl)-4-(2-ethoxybenzoyl) piperazine), is the most potent derivative identified to date. Compared to other mitotic inhibitors, we show that AK301-arrested cells readily enter apoptosis following compound withdrawal or death ligand treatment (IC₅₀ ≈ 150 nM). This effect was more pronounced in p53-normal colon cancer cells. AK301-treated cells also exhibited higher levels of mitosis-associated ATM signaling and caspase-3 activation. Immunofluorescence confocal imaging of AK301-treated cells revealed the formation of characteristic multiple microtubule organizing centers and reduced microtubule growth. Upon AK301 withdrawal, microtubule networks and spindles reformed, cells exited mitosis, and

readily entered apoptosis. In summary, our data show the development of a new class of mitotic targeting agents that may be useful for further drug development. This class of compounds can also serve as chemical probes for studying microtubule dynamics and cell signaling pathways regulating the mitosis-to-apoptosis transition.

Keywords: Colon cancer, mitotic inhibitor, apoptosis.

PO-84

Track: Diabetes and Obesity Drug Discovery & Therapy

BIOASSAY-GUIDED ISOLATION AND STRUCTURAL ELUCIDATION OF ANTIDIABETIC PRINCIPLE OF METHANOL LEAF EXTRACT OF *NEWBOULDIA LAEVIS* (P. BEAUV)

Joel Aondohulugh Bosha, Aruh Ottah Anaga and Isaac Uzoma Asuzu

*Department of Veterinary Physiology and Pharmacology, University of Agriculture, Makurdi, Nigeria;
E-mail: boshajuel@yahoo.com*

Newbouldia laevis (P. Beauv) (NLE) is used in folk medicine to treat diabetes. The aim of the study was to isolate and characterize the active compound responsible for the antidiabetic activity. This was carried out using standard *in vivo* and *in vitro* models in rats. The antidiabetic activities were evaluated using alloxan-induced diabetes in male albino rats after an overnight fast and various doses of NLE, and glibenclamide, the reference drug 2.0 mg/kg. Bioassay-guided isolation/fractionation techniques were used to isolate the active compound. Characterization of the active compound was carried out including molecular and structural elucidation using Nuclear Magnetic Resonance (NMR) and Gas-Chromatography Mass Spectroscopy.

The extract caused 60.2% reduction in fasting blood sugar (FBS) of diabetic rats. Bioassay-guided fractionation of NLE yielded ten (10) fractions with fraction nine (F9) as the active fraction, which caused 66.0% reduction of FBS in alloxan-induced diabetic rats. Further purification using preparative thin layer chromatography (TLC), gave sub-fraction 9.2 as the active compound. Sub-fraction 9.2 reduced the FBS by 61.4%. The characterization of F9:2 using nuclear magnetic resonance (NMR) and mass spectroscopy (MS) confirmed it to be a polyunsaturated fatty acid 9-(4-Nonyl-phenyl)-non-8-enoic acid with the chemical formula C₂₄H₃₈O₂ and molecular weight of 358.56.

Keywords: *Newbouldia laevis*, antidiabetic, active fraction, leaf.

PO-85

Track: Hot Topics in Natural Products

ORAL ADMINISTRATION OF MONTMORILLONITE-ILLITE CLAY MINERAL ALLEVIATES CLINICAL SYMPTOMS OF COLITIS INDUCED BY DEXTRAN SODIUM SALT IN BALB/C MICE

Anne Breitrück¹, Claus Kerkhoff^{1,2}, Kai Sempert¹, Jacqueline Hofrichter¹ and Steffen Mitzner¹

¹*Extracorporeal Immunomodulatory Unit (EXIM), Fraunhofer Institute for Cell Therapy and Immunology IZI, Schillingallee, D-6818057 Rostock, Germany; E-mail: anne.breitruueck@izi.fraunhofer.de*

²*Department of Biomedical Science FB 8 School of Human Sciences, University of Osnabrueck, Albrachtstrasse, D-2849076 Osnabrueck, Germany*

Introduction: Due to the complexity of aetiology and severe side-effects of commonly used drugs treatment options for inflammatory bowel disease are relatively limited. Therefore, we investigated the gastrointestinal influence of refined montmorillonite-illite clay mineral (smectite) in healthy mice and its ability to attenuate the colonic inflammation following DSS exposure.



Methods: Colitis was induced by 4.5% DSS in drinking water for 7 days. Smectite was administered before (prophylactic) and during (therapeutic) colitis induction. Clinical parameters were monitored daily, histopathological score and the expression level of tight junction proteins (TJ) and adherens junction (AJ) in the colon were evaluated.

Results: Preliminarily, smectite was administered for 7 days without any adverse effects. Furthermore, smectite showed significant effects on TJ expression. DSS colitis was characterized by significant body weight loss, increased DAI and histopathology score compared to healthy control. Furthermore expression levels of TJ and AJ were significantly affected. Weight loss, DAI and histological score of mice treated prophylactically with smectite were significantly lower compared to DSS treatment alone.

Summary: Prophylactic application of smectite had a favourable effect on DSS-induced colitis possibly mediated by barrier strengthening mechanism. Further investigations are in progress to evaluate the potential for smectite as an option for remissions strategy.

PO-59

Track: Nutraceutical Drug Discovery and Therapy

EFFECTS OF METHYLSULFONYLMETHANE ON VIABILITY OF K562 CELL LINE

Zeliha Buyukbingol and Arzu Z. Karabay

Ankara University, Faculty of Pharmacy, Department of Biochemistry, Tandogan, Ankara, Turkey;
E-mail: zbuyukbingol@ankara.edu.tr



Methylsulfonylmethane (MSM) is an organic natural sulfur compound which has been shown to exhibit anti-inflammatory and antioxidative effects. Combination or single products of MSM have been marketed widely as dietary supplements for their acclaimed health benefits especially for the maintenance of joint health and anti-inflammatory action under arthritic conditions. Recent studies showed that MSM induced apoptosis in different cancer cell lines and acted as an apoptotic agent besides its anti-inflammatory actions. In this study, effects of MSM on viability parameters of K562 leukemic cell line were investigated. K562 cells were treated with different mM concentrations of MSM and cell viability was determined with MTT test. It was found that MSM decreased cell viability of K562 cells at high mM concentrations. Decrease in cell viability began at 300 mM concentration of MSM. In our previous study we showed that MSM induced intrinsic pathway of apoptosis in macrophages by activation of caspase-3 and p53 pathway [1]. Therefore, cytotoxic effects of MSM on K562 cells could be seen as a result of its apoptotic actions. We also examine caspase and apoptotic proteins to elicit the mechanism of actions of MSM in terms of diminishment in K562 cell viability.

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PO-36

Track: Hot Topics in Drug Targets

DEVELOPMENT OF A STRUCTURAL MODEL FOR THE HUMAN DOPAMINE TRANSPORTER PROTEIN (hDAT)

Matthew C. Castellana, Tarek M. Mahfouz and Andrea Castellar

Student National Pharmaceutical Association (SNPhA), Ohio Northern University, Ada, OH, USA;

E-mail: bergun@anadolu.edu.tr

Currently, depression affects over 26% of the U.S. adult population. The human dopamine transporter (hDAT) can contribute to depression manifestation and is located in the mesolimbic, nigrostriatal, and mesocortical pathways. Dopamine reuptake inhibitors are an important group of antidepressant drugs that work by inhibiting dopamine symporters, thus regulating the amount of dopamine at the synapses. Their use, however, is associated with side effects due to lack of selectivity. Through rational drug design techniques the selectivity can be enhanced; yet these efforts have been hampered due to a lack of reliable structural models for human monoamine transporters. Using the fruit fly (*Drosophila melanogaster*) dopamine transporter and the program CHIMERA, a model for the hDAT was developed and refined. The model was assessed using the web-based server SWISS-MODEL and the results were used to guide the refinement process in an iterative fashion. The refined model was then functionally validated through docking of known inhibitors. The model was able to reproduce experimental binding modes with great accuracy (an RMSD value of 1.8Å for nortriptyline) and was able to rank inhibitors in the correct order of increasing activity. These results indicate that this model is suitable for structure based drug design for development of selective hDAT inhibitors.

PO-23

Track: Hot Topics in Natural Products

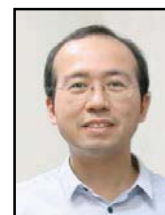
CHEMICAL IDENTIFICATION OF A PROANTHOCYANIDIN POLYMER FROM THE MEDICINAL HERB GRAPTOPETALUM PARAGUAYENSE FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Chia-Chuan Chang, Li-Chen Wu, Chi-Ying F. Huang, Shih-Lan Hsu and Chia-Chuan Chang

School of Pharmacy, National Taiwan University, Taipei, Taiwan; E-mail: chiachang@ntu.edu.tw

Graptopetalum paraguayense (GP) is a traditional Chinese herb used to alleviate hepatic disorders, treat infections and inhibit inflammation. The F3 fraction, a fraction from the 30% DMSO-soluble GP extract (GP-D), induced apoptosis in HCC cells, enhanced PTEN expression, and decreased AKT phosphorylation in our preliminary studies. These results indicate that GP-D and F3 can protect the liver by suppressing tumor growth and could be considered for the treatment of hepatocellular carcinoma. From the bioactive F3 fraction, a novel 3-O-galloyl 4,8-linked-proanthocyanidin polymer (1) with a mean molecular weight (mMW) of 18 kD, a mean degree of polymerization (mDP) of 40, and a procyanidin unit to prodelfinidin unit ratio (PC:PD ratio) of less than 0.05, was isolated by Sephadex LH-20 column chromatography and dialysis. The structural information of 1 including mMW, mean degree of polymerization, PC: PD ratio and stereochemistry, were obtained by chemical (thiolysis), chromatographic (HPSEC) and spectroscopic (colorimetric assay, ¹H and ¹³C NMR) analyses. Its high ratio of the 3-O-galloyl moiety (> 95%) was very similar to that of the compound found in golden root (*Rhodiola rosea*), and 4.7 and 41.3 times than that in seeds (20%) and skin (2%) of grape (*Vitis vinifera*, a common grape species), respectively.

Keywords: *Graptopetalum paraguayense*, proanthocyanidin polymer, structural elucidation.



PO-92

Track: Hot Topics in Drug Targets

CREB BINDING PROTEIN (CBP) INHIBITION AND TARGET ENGAGEMENT

Eugene L. Piatnitski Chekler

Pfizer Inc., 610 Main Street, Cambridge, MA 02139, USA; E-mail: Eugene.Chekler@Pfizer.com



Fully profiled chemical probes are essential to support the unbiased interpretation of biological experiments necessary for rigorous preclinical target validation. We believe that by developing a chemical probe tool kit, chemical biology can have a more central role in identifying targets of potential relevance to disease, avoiding many of the biases that complicate target validation. A development of CREB Binding Protein (CREBBP) selective chemical probe to elucidate biology associated with this bromodomain epigenetic target is presented. Chemical probe optimization is a strategic balance between physiochemical properties and chemistry, to identify high affinity binders that are functionally active and selective, with good permeability properties. The selectivity of the chemical probe against other bromodomain family members was investigated using biochemical and biophysical assays. To address the selectivity issue with BRD4, X-ray crystal structures of the probe candidates bound to CREBBP and BRD4 were used to guide the design. The chemical probes were useful in studies aimed at validating CREBBP as a therapeutic target and for establishing its biological role.

PO-90

Track: Pharmaceutical Research & Development

Wnt/BETA-CATENIN SIGNAL REGULATES VASCULAR CALCIFICATION BY REGULATE ANK EXPRESSION

Beidong Chen¹, Ruomei Qi¹, Cunyu Wang³, Xian Wang⁴ and Gexin Zhao²

¹*Organization: Beijing Institute of Geriatrics, Beijing Hospital, Beijing, China; E-mail: beidong.chen@gmail.com*

²*Organization: UCLA Orthopaedic Hospital Research Center, 615 Charles E Young Dr. South, Los Angeles, CA 90049, USA*

³*Organization: Division of Oral Biology and Medicine, School of Dentistry, University of California Los Angeles, USA*

⁴*Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University Health Science Center, Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing 100191, China*

The purpose of current study is to investigate the mechanism of how the Wnt/Beta-catenin signal regulates the calcification of human vascular smooth muscle cells (VSMCs). We established the renal failure rat model by adding adenine in the diet. There was obvious vascular calcification occurred. Compared with the control group, Wnt1, a canonical Wnt signaling stimulating factor, and ANK, a vascular calcification inhibitor, expression level were significantly reduced in abdominal aorta. This suggested that Wnt signal and ANK level were negatively related with vascular calcification. Then we overexpressed Wnt1, results showed that ANK level was upregulated and Pi (inorganic phosphate) induced VSMCs calcification was inhibited and ANK dependent. Moreover, through ChIP assay we demonstrated canonical Wnt signal increased the binding of Beta-catenin to the promoter of ANK, and induced ANK expression on transcription level, then inhibited VSMCs calcification.

PO-25

Track: Process Chemistry and Drug Manufacturing

RE-CRYSTALLIZATION AND MICRONIZATION OF ACTIVE PHARMACEUTICAL INGREDIENTS USING THE SUPERCRITICAL FLUID TECHNOLOGY

Yan-Ping Chen¹, Chun-Da Chen¹, Pei-Hua Chen² and Muoi Tang³

¹Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan;
E-mail: ypchen@ntu.edu.tw

²Department of Chemical and Materials Engineering, Chinese Culture University, Taipei, Taiwan

The re-crystallization and micronization of active pharmaceutical ingredient (API) is reported using the supercritical antisolvent (SAS) process. One example is the SAS treatment of nitrofurantoin. This API is an antibiotic and is usually used in treating urinary tract infection. The original API has a large mean size of 202 nm. The target of this study is to enhance its dissolution behavior by reducing its mean particle size and crystallinity. The experimental procedures are similar to those of our previous studies [1, 2]. The API was firstly dissolved in a solvent, and was introduced to a precipitation column, together with high pressure carbon dioxide, through a coaxial nozzle. The solution of had a rapid volume expansion and loss of solvent power. Re-crystallization and micronization of the API were resulted in a short period of time. The micronized nitrofurantoin particles were collected and analyzed by SEM, PXRD and DSC. The optimal operation conditions were investigated. The nitrofurantoin particles were micronized from 202 to 2.93 nm. The intensity of crystallinity was reduced after the SAS treatment as observed by the PXRD patterns. The dissolution rate in a simulated intestinal fluid was enhanced by 3.7 times after the SAS process. It is demonstrated that the SAS process yields feasible results for the re-crystallization and micronization of nitrofurantoin.

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PO-5

Track: Drug Delivery and Targeting

BIODEGRADABLE INTERSTITIAL RELEASE POLYMER LOADING A NOVEL SMALL MOLECULE TARGETING AXL RECEPTOR TYROSINE KINASE AND REDUCING BRAIN TUMOUR MIGRATION AND INVASION

Tzzy-Wen Chiou, Jeanne Hsieh, Ssu-Yin Yen, Syuan-Rong Chen, Shuang-En Chuang, Hong-Meng Chuang, Mao-Hsuan Huang, Shinn-Zong Lin and Horng-Jyh Harn

Department of Life Science and Graduate Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan;
E-mail: twchiou@mail.ndhu.edu.tw

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumour. The neoplasms are difficult to resect entirely, because of their highly infiltration property leading to the tumour edge is unclear. Axl is an essential regulator in cancer metastasis and patient survival. In this study, we developed a controlled-release polyanhydride polymer loading a novel small molecule, n-butylidenephthalide (BP), not only increasing local drug concentration and extending its diffusion distance but also reducing tumour invasion, mediated by reducing Axl expression. Firstly, it was found that BP inhibited the expression of Axl in a dose- and time-dependent manner and reduced the migratory and invasive capabilities of GBM cells. In addition, BP downregulated matrix metalloproteinase activity, which is involved in cancer cell invasion. Furthermore, we demonstrated that BP regulated Axl *via* ERK pathway. The overexpression of Axl in GBM cells was used to prove that Axl is a crucial target in the inhibition of GBM EMT (Epithelial-to-mesenchymal transition), migration, and invasion. Most importantly, in an intracranial tumour model with BP wafer *in situ* treatment, we demonstrated that the BP wafer not only significantly increased the survival rate but also decreasing Axl expression,

and led to inhibited tumour invasion. These results contribute to the development of a BP wafer for a novel therapeutic strategy for treating GBM invasion and increasing survival in clinical.

PO-105

Track: Pharmaceutical Research & Development

COMPUTER AIDED FORMULATION AND CHARACTERIZATION OF PROPRANOLOL HCL BUCCAL TABLET USING POLYMERIC BLEND

Muhammad Zeeshan Danish, Nadeem Irfan Bukhari, Sana Hanif, Nadeem Irfan, Nisaar Hussain, Zeeshan Danish, Muhammad Ali, Bushra Nasir, Javed Iqbal, Bilal Tahir, Hamid Saeed, Rubina Ali and Zikrya Saleem

*Department of Pharmacy, University of the Punjab, College of Pharmacy Allam Iqbal Campus, Lahore, Pakistan;
E-mail: pfarmacist@hotmail.com*

The current study was aimed to formulate a continuous release mucoadhesive buccal tablet containing propranolol HCl. The type and quantities of polymers as well as method of compression were set in a preliminary study (F1-F13). So, direct compression method was employed in the main study (F14-F24) using Carbopol® 934P (CP), Ethylcellulose (EC), sodium alginate (SA), hydroxypropylmethylcellulose (HPMC K4M) and carboxymethylcellulose (CMC) as mucoadhesive polymers and were tested for physicochemical tests *i.e.* swellability, surface pH, mucoadhesive time, mucoadhesive strength, *in vitro* release *etc.* Results obtained from the study were used to see the impact of polymers on physicochemical properties using NeuralPower® 3.1, an artificial neural network approach. The software optimized the ingredients, against desirability of physicochemical parameters, as HPMC (150mg), CMC (25mg), CP (20mg) and EC (20mg). Outcome revealed that HPMC primarily contributed to the physicochemical properties of mucoadhesive formulation. To compare prediction, optimized ingredients were formulated (F25) and tested. As predicted, similar release pattern was of F25 was obtained as 26% (0.5hr), 34% (1hr), 40% (2hr), 45% (3hr), 50% (4hr), 62% (5hr), 76% (6hr), 85% (7hr) and 97% (8hr) respectively. For release kinetics, DD solver® was applied which predicted Korsmeyer-Peppas model, indicating non-Fickian release of the drug.

Keywords: Mucoadhesive buccal tablet, artificial neural network, propranolol, HPMC, cellulose.

PO-95

Track: Hot Topics in Drug Targets

NOVEL TREATMENT DESIGNED TO STOP MULTIPLE SCLEROSIS PROGRESSION, BASED ON SYNCITIN INHIBITION

Enrique de Font-Réaulx

*ABC Medical Center, Methodist International, Polanco, Mexico;
E-mail: defontreaulx@hotmail.com*

We developed the first oral treatment designed to stop Multiple Sclerosis (MS) progression that acts directly over a therapeutic target that had never been considered before: Syncitin. We included ferulic acid for this purpose and we added other potent inhibitors of Mitogen Activated Protein-Kinases, apoptosis, caspases and free radicals that acts together to block the most potent known mechanism of disease progression. We called this treatment Cervô.

The results we obtained in our study from 2006 to 2014 show that it is a safe treatment, without any



significant side effect. In MS it is much more effective than any other of the available treatments in the market (interferon or monoclonal antibodies), being effective in 90% of all the cases, if we include all the subtypes of MS. In our experience, in the most prevalent form of MS (Relapsing-Remitting) it particularly effective to stop MS progression.

PO-64

Track: Hot Topics in Drug Targets

A NEW TREATMENT FOR CLINICAL STABILIZATION IN PARKINSON'S DISEASE

Enrique de Font-Réaulx

ABC Medical Center, Methodist International, Polanco, Mexico;

E-mail: defontreaulx@hotmail.com

Previous clinical trials designed to stop Parkinson's Disease (PD) progression have failed to demonstrate significant clinical stabilization. We administrated to PD patients a new oral treatment to stop the disease progression called Cervô. It contains four substances that have a synergic effect in controlling the most important known mechanism of disease progression as: aberrant apoptosis, oxidative damage, mitochondrial degeneration, caspases activation, Mitogen-Activated Protein-Kinases (MAPK) activation. We previously demonstrated that it is safe to use Cervô in humans and that has no collateral effects.

Results: We included 42 patients with Parkinson's disease. Age: 42 to 89 years old (mean 66.12 years), 23 female (54.8%), 19 male (45.2%). Initial United Parkinson's Disease Rating Scale subscale 3 (UPDRS) score: 1-15 (mean 5). Maximum follow-up period: 72 months, mean 29.6 (+/- 22.28 SD). We included only patients with more than 6 months of follow-up for the clinical stabilization analysis (n=37). No disease progression (no increase in the UPDRS score) in 34 (91.8%) patients, and 31 (83.78%) patients improved their basal UPDRS score. There were 2 clinical remissions (5.4%) and the disease progressed in 1 (2.7 %).

Conclusion: Cervô is a new and promising compound that may stop PD's progression.



PO-41

Track: Green Techniques for Medicinal Chemistry

SPILANTHOL EXTRACTION FROM *ACMELLA OLERACEA* USING SUPERCRITICAL CARBON DIOXIDE

Verônica Santana de Freitas, I.A. M. Zelioli, R.A.F. Rodrigues, J.E. Carvalho and F.A. Cabral

Department of Food Engineering, Food Engineering Faculty, State University of Campinas, São Paulo, Brazil; E-mail: ve_freitas@hotmail.com

Jambu (*Acmella oleracea* (L.) RK Jansen) is a plant of South America, widely used in the cuisine of northern Brazil because of its bitter taste. Jambu has several pharmacological properties such as antioxidant, antimalarial, and larvicide, anesthetic and anti-inflammatory. Such properties are related to the presence of spilanthol, an alkylamide found in abundance in this species. In order to explore the potential of this bioactive and due to the high cost of an analytical standard in the market, the aim of this study was to extract spilanthol from the aerial parts of jambu in a fixed bed extractor using supercritical carbon dioxide (scCO₂) coupled to fractionators with a decreasing pressure gradient. The extractions were carried out at 60°C



(140 °F) and 300 bar (4267 psi) pressure. Through this new method it was possible to obtain espilantol with high purity (90%), in less time and with comparable yield to conventional extractions processes. The supercritical technology has the additional advantage of not requiring organic solvents, and therefore, a green methodology.

Keywords: *Acemella oleracea*, green chemistry, spilanthol, supercritical extraction.

PO-53

Track: Drug Delivery & Targeting

ORAL DELIVERY OF BIOPHARMACEUTICS: CHARACTERIZATION AND EVALUATION OF MUCUS PERMEATING NANOPARTICLES

Irene Pereira de Sousa¹, Beatrice Cattoz², Peter C. Griffiths² and Andreas Bernkop-Schnürch¹

¹*Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria;
E-mail: Irene.Pereira-De-Sousa@uibk.ac.at*

²*Department of Pharmaceutical, Chemical and Environmental Sciences, Faculty of Engineering and Science, University of Greenwich, Medway Campus, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK*



Introduction: The intestinal absorption represents a major barrier for oral delivery of biopharmaceuticals which mainly account for the mucus layer covering the GI tract surfaces. The aim of this study was to formulate nanoparticles (NPs) capable to permeate the mucus by cleaving its glycoprotein substructures.

Methods: Papain and bromelain were conjugated to poly (acrylic) acid and NPs were formulated *via* ionic gelation. The carriers were characterized by size, charge, enzyme content and enzymatic activity. The NPs permeation ability was investigated by pulse-gradient-spin-echo NMR (PGSE-NMR) in mucin, by small angle neutron scattering (SANS), spin-echo SANS (SESANS) and rotating tube technique (RTT) in mucus.

Results: The functionalized NPs induced a significant change in the mucin gel network as indicated by PGSE-NMR measurements (2 fold increase in mucin mobility). SANS and SESANS measurements confirmed the NPs ability in changing the mucus structure. The promising permeation properties of these NPs were assessed by RTT, revealing a 4.8 fold higher concentration of bromelain NPs in the inner segments of mucus.

Conclusion: Mucus permeating NPs could be formulated and their potential was assessed *via* several techniques. Bromelain NPs appeared to be the most promising system and, therefore, suitable for the formulation of an oral delivery system for biopharmaceuticals.

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PO-33

Track: Drug Delivery and Targeting

CHARACTERIZATION AND PREPARATION OF BENZOTRIAZOLE - PALLADIUM COMPLEX LOADED SOLID LIPID NANOPARTICLES

Gökhan Dikmen¹, Deniz Hür^{2,3} and Lütfi Genç^{3,4}

¹Eskisehir Osmangazi University, Central Research Laboratory (MERLAB), Odunpazarı, 26480, Eskisehir, Turkey; E-mail: gokhandikmen1@gmail.com

²Anadolu University, Faculty of Science, Department of Chemistry, Tepebaşı, 26470, Eskisehir, Turkey

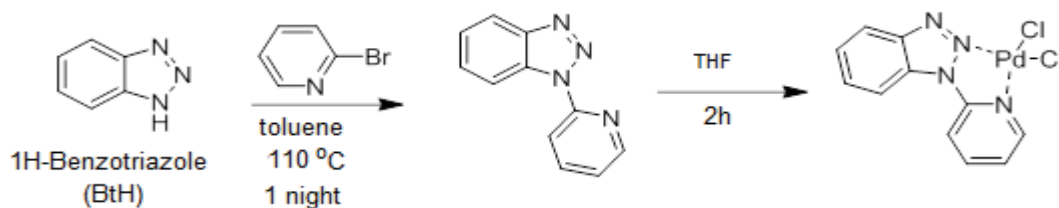
³Anadolu University, Plant, Drug and Scientific Researches Center (AUBİBAM), Tepebaşı, 26470, Eskisehir, Turkey

⁴Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Tepebaşı, 26470, Eskisehir, Turkey



Nano-sized carriers including liposomes, polymeric micelles, dendrimers, solid lipid nanoparticles (SLNs) and nano lipid particles (NLPs) provide controlled release of the drug in the desired time and the region. Recently, palladium complexes have been used as anticancer drug. But, these complexes exhibit a lot of side effects. These side effects can be eliminated using drug delivery systems such as SLNs.

Material and Methods: Benzotriazole - Palladium Complex were synthesized, then benzotriazole - palladium complex loaded SLNs were produced and characterized by Zeta Sizer, FT-IR, NMR, SEM, LC-MS, HPLC and DSC.



Results: Benzotriazole - Palladium Complex loaded SLNs was proven good stability (-27.6 mV) and small size (nearly 214 nm). SLN formulations were compared with the freshly prepared formulations of complex and Tween 80 by FT-IR spectroscopy. Any chemical shift or deformation in the bands and any stability problems were not observed. In addition, nuclear magnetic resonance (NMR) spectra of SLN formulations have been compared to those of the freshly prepared samples of the Tween 80 and complex. According to NMR spectra, any new peaks were not observed for complex loaded SLN and placebo SLN.

Conclusion: This formulation may be suitable as a nano drug carrier system for cancer treatment.

PO-7

Track: Diabetes and Obesity Drug Discovery & Therapy

EVALUATION OF THE METHANOLIC EXTRACT OF MISTLETOE (*TAPINANTHUS BANGWENSIS*) LEAVES GROWN ON ORANGE TREES FOR THE PHYTOCHEMICAL PROPERTIES AND ITS PHYSIOLOGICAL EFFECTS ON STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN LABORATORY ANIMALS

F.O. Ekhaïse, K.M. Agboh and S. Uanseojè

Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria; E-mail: aiyevbosa.ekhaïse@gmail.com

Mistletoe (*Tapinanthus bangwensis*) a semi –parasitic evergreen plant has been used traditionally in Nigerian and other parts of Africa as antihypertensive and antidiabetic agents. The phytochemical analysis revealed the presence of saponins, flavonoids tannins and glycosides. Treatment with aqueous *Tapinanthus bangwensis* (mistletoe) extract at the dose of 500mg/kg body weight showed that the concentration of blood glucose levels in the diabetic test (treated rats) were significantly reduced as compared to the diabetic control (untreated rats). In streptozotocin induced diabetic experimental animals (rats), maximum reduction in blood glucose levels was observed after 14 days of treatment with methanolic crude extract of *Tapinanthus bangwensis* (mistletoe). The result showed the concentrations of blood glucose in the diabetic test (treated) group was significantly reduced to 163.75 ± 46.327 ($p \leq 0.05$) (mg/dl) after 14 days of administration of aqueous *Tapinanthus bangwensis* (mistletoe) extract at 500mg/kg body weight as compared to 377.50 ± 0.50 (mg/dl) of the diabetic control (untreated) group. The result indicated that the methanolic crude extract of *Tapinanthus bangwensis* leaves possesses significant anti-diabetic activity.

PO-35

Track: Others - Case report

A CASE OF INSULIN NEUROPATHY: RARE ADVERSE EFFECT OF INSULIN THERAPY

Bülent Erğün

*Pharmaceutical Toxicology, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey;
E-mail: bergun@anadolu.edu.tr*

Objective: Insulin neuropathy is a rare adverse effect of insulin therapy in patients with long-term uncontrolled hyperglycemia. Neuropathic pain appears after the correction of blood glucose level.

Case: A 34 years old woman with severe neuropathic pain appeared immediately after the beginning of insulin therapy and could not be responded to neuropathic pain relief drugs is reported.

Conclusion: Insulin treatment may cause severe neuropathic pain that cannot be result with neuropathic pain relief drugs.

Keywords: Insulin, adverse effect, neuropathy.

PO-44

Track: Drug Discovery in Preclinical Research

MYCOTOXINS IN DRUG DISCOVERY: EXPOSURE ASSESSMENT BY *IN VITRO* BIOTRANSFORMATION FOR THE PREDICTION OF RELEVANT TEST CONCENTRATIONS

Christiane Kruse Fæste¹, Silvio Uhlig^{1,2} and Lada Ivanova¹

¹Norwegian Veterinary Institute, Oslo, Norway; E-mail: christiane.faste@vetinst.no

²The National Institute of Occupational Health, Oslo, Norway

Strategies for the prediction of *in vivo* kinetic data from *in vitro* biotransformation experiments are well-established for pharmaceutical compounds. Using hepatic microsomes or hepatocytes from humans and different animal species, test compounds are metabolised under the conditions of first-order kinetics, which allows the prediction of *in vivo* kinetic parameters by extrapolating the *in vitro* assay half-lives and clearances with the help of species-specific conversion factors. The application of this method to other xenobiotics such as mycotoxins is, however, unusual.

In the present study, we have developed *in vitro* metabolism assays for Fusarium mycotoxins with an interesting pharmacological potential such as enniatin B and demonstrate their applicability by comparing the predictive results to existing *in vivo* data. The establishment of *in vitro* toxicokinetic assays allows differentiating the expected effect dose from the toxic dose and can significantly reduce the need for *in vivo* animal studies.

Keywords: Mycotoxins, exposure assessment, *in vitro* metabolism.

PO-75

Track: Natural Toxins in Drug Discovery

THE IONOPHORIC MYCOTOXIN ENNIATIN B INDUCES NON-APOPTOTIC CELL DEATH BY LYSOSOME DESTABILIZATION

Christiane Kruse Faeste, Lada Ivanova and Anita Solhaug

Department of Chemistry and Toxicology, Norwegian Veterinary Institute, Oslo, Norway;

E-mail: christiane.faste@vetinst.no

Enniatins are cyclic hexadepsipeptidic mycotoxins with ionophoric properties that have shown antibiotic and anti-fungal properties. Enniatin B (EnnB), the most important analogue, is produced by many Fusarium species and has a high prevalence in grain grown in Northern climate. Beside its importance as a food contaminant with potential health risk for consumers EnnB is considered as an interesting candidate in drug discovery due to its cytotoxic activity. In the present study we have studied the uptake of EnnB via ABC transporters and the EnnB-initiated intra-cellular event cascade in different human cell lines. Lysosomal functionality was already affected after 3 h of toxin exposure and was followed by cell cycle arrest and production of reactive oxygen species (ROS). The data suggested that lysosomal destabilization was an upstream event in EnnB-initiated cytotoxicity followed by a certain extent of translocation of cathepsins into the cytosol and the release of inflammatory cytokines. Cell death was delayed and did not occur as a massive lysosomal breakdown. Instead it was probably progressing and leading to partial and selective lysosomal membrane permeabilization (LMP), starting a non-apoptotic cell death pathway with morphological features considered as necrotic.

Keywords: Enniatin B, mycotoxin, lysosomal destabilization, non-apoptotic cell death.

PO-31*Track: In-Silico Drug Design and In-Silico Screening***RATIONAL DESIGN OF NEW HIV REVERSE TRANSCRIPTASE INHIBITORS****T. Frączek and P. Paneth***Faculty of Chemistry, Lodz University of Technology, Zeromskiego, Lodz, Poland;**E-mail: tomasz.fraczek@p.lodz.pl*

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are an important drug class used in the first line Highly Active Antiretroviral Therapy (HAART) of human immunodeficiency virus (HIV) infection. NNRTIs are relatively safe and well tolerated and proved to be effective in combination with other antiretroviral drugs, providing long term virologic efficacy. However, the very high mutation rate of HIV causes selection of virus variants that are resistant to first generation NNRTIs such as nevirapine or efavirenz. Second generation inhibitors such as etravirine possess a higher genetic barrier for development of resistant mutants. Unfortunately, many of NNRTIs have a poor pharmacokinetic profile resulting mainly from very low aqueous solubility. The aim of our research is to rationally design novel NNRTIs that are both highly effective and bioavailable. With the combined effort of Computer Aided Drug Design and experimental methods (enzymatic assays, Surface Plasmon Resonance) we were able to find several groups of new HIV reverse transcriptase inhibitors. Important feature of our compounds is their excellent water solubility and the possibility of different prodrug modifications that can be employed to fine-tune their pharmacokinetics.

The reported studies are supported by grant 2011/02/A/ST4/00246 (2012-2017) from the Polish National Science Centre (NCN).

PO-76*Track: Drug Discovery and Preclinical Research***IN VITRO SCREENING OF ANTICANCER ACTIVITY OF 38 SYNTHETIC QUINOID DERIVATIVES ON HT-29 COLORECTAL CANCER CELL LINE****L.A. Franco¹, J.F. Acuña¹, J.P. Castro¹, Y.C. Ocampo¹, D.E. Rivera¹, R. Gaitán², F.A. Aristizabal³ and K. Müller⁴**

¹*Biological Evaluation of Promissory Substances Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia; E-mail: lfrancoo@unicartagena.edu.co*

²*Natural Products Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia*

³*Cancer Pharmacogenetics Research Group, Pharmacy Department, Faculty of Sciences, National University of Colombia, Bogotá, Colombia.*

⁴*Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Germany*

Quinones are compounds with several pharmacological properties, including cytotoxic activity. Finding new quinone-derivatives (QD) constitutes an important strategy for the discovery of alternatives to treat cancer. In this work we obtained 38 QD by chemical synthesis, including: naphthoquinones (eighth), furano-naphthoquinones (ten), dihydrofuran-naphthoquinones (nine), Benzo-indolequinones (seven), thiophene-naphthoquinones (three) and thiazolidine-naphthoquinone (one). The structures of the compounds were elucidated without ambiguity by FT-IR, MNR and MS. Compounds were evaluated in regard to their *in vitro* cytotoxicity against the colorectal cancer cell line HT-29 (ATCC®-HTB-38™) and fibroblast (ATCC®-PCS-201-012™) as normal cell line, using the MTT method and doxorubicin-HCl as positive control. LC₅₀ values were determined for the active compounds and the selectivity index (SI) was calculated (LC₅₀Fibroblast/LC₅₀HT-29). According to the results, 26 compounds of the series showed important effects on the viability of HT-29, with 15 classified as active (<20µg/mL) and 11 as moderately active (20-100µg/mL), the other 12 compounds were cataloged as inactive (>100µg/mL), in agreement to the American National Cancer Institute (NCI). Among the active compounds, 5 exhibited SI values above 1. QD-35, a thiophene-naphthoquinone, was the most active

($LC_{50}=1.73\mu\text{g/mL}$) and selective ($SI=2.63$) compound and represents a candidate molecule for further investigation as a potential anticancer alternative.

Keywords: Quinone derivatives, cancer, HT-29.

PO-77

Track: Hot Topics in Natural Products

COLOMBIAN CARIBBEAN PLANT EXTRACTS WITH CYTOTOXIC EFFECT AGAINST HUMAN CANCER CELLS

L.A. Franco¹, Daneiva Caro Fuentes¹, V. Teherán¹, Y.C. Ocampo¹, R. Salas¹ and F. Diaz²

¹Biological Evaluation of Promissory Substances Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia; E-mail: lfrancoo@unicartagena.edu.co

²Laboratory of Phytochemical and Pharmacological Research, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia



Cancer is a term used to describe a group of diseases characterized by growth and spread of abnormal cells, with an elevated mortality rate. Although there is available drug therapy, its treatment costs and side effects affect the quality of life of patients. In this work, we evaluated the cytotoxic effect of ethanolic extracts from 15 Caribbean Colombian plants against a fibroblast cell line and 3 human cancer cells lines: A549 (Lung), HT-29 (Colorectal) and PC-3 (Prostate), using the MTT method. LC_{50} was calculated to the extracts that showed promising activity, according to the NCI criteria (Active: $LC_{50}<20\mu\text{g/mL}$, moderately active: $20<LC_{50}<100\mu\text{g/mL}$ and inactive: $LC_{50}>100\mu\text{g/mL}$). Among the 15 extracts tested, two showed cytotoxic activity against all three cancer cell lines. The *Mammea americana* extract was active on A549 ($LC_{50}=7.55\mu\text{g/mL}$) and moderately active on HT-29 and PC-3 ($LC_{50}=30.70$ and $28.09\mu\text{g/mL}$, respectively). The *Bursera simaruba* extract was active on the A549 and HT-29 cell lines ($LC_{50}=4.34$ and $18.19\mu\text{g/mL}$, respectively) and moderately active on PC-3 ($LC_{50}=57.11\mu\text{g/mL}$); additionally, this extract had the highest selectivity indexes ($SI=LC_{50}\text{Fibroblast}/LC_{50}\text{CancerCell}$) in all cell lines. This study provides evidence about the use of Colombian Caribbean plants, as valuable sources for the isolation and identification of bioactive compounds for cancer treatment.

Keywords: Cancer, ethnopharmacology, cytotoxic, MTT.

PO-52

Track: Pharmaceutical Research & Development

PREPARATION AND CHARACTERIZATION OF CIS-PLATIN LOADED NANO LIPID PARTICLES AND INVESTIGATION OF THEIR ANTICANCER ACTIVITY

Lütfi Genç^{1,2}, Gökhan Dikmen³ and Şennur Görgülü Kahyaoğlu²

¹Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Tepebaşı, 26470, Eskisehir, Turkey; E-mail: lgenç@anadolu.edu.tr

²Anadolu University, Plant, Drug and Scientific Researches Center (AUBİBAM), Tepebaşı, 26470, Eskisehir, Turkey

³Eskisehir Osmangazi University, Central Research Laboratory (MERLAB), Odunpazarı, 26480, Eskisehir, Turkey



Background: Nano-sized carriers (10-1000 nm) including liposomes, polymeric micelles, dendrimers, solid lipid nanoparticles (SLNs) and nano lipid particles (NLPs) provide controlled release of the drug in the

desired time and the region. NLPs are alternative drug delivery system have unique properties including, a high drug loading capacity and drug release for a long time.

Material and Methods: Cis-platin loaded NLPs were produced and characterized by Zeta Sizer, FT-IR, NMR, SEM, LC-MS, HPLC and DSC. Finally, its cytotoxic effects on MCF-7 cancer cells were evaluated by MTT test.

Results: Cis-platin loaded NLPs was proven good stability (-30.5 mV) and small size (nearly 210 nm). NLP formulations were compared with the freshly prepared formulations of complex and tween 80 by FT-IR spectroscopy. Neither shift/deformation in the bands nor any stability problems were observed. In addition, nuclear magnetic resonance (NMR) spectra of SLN formulations have been compared to those of the freshly prepared samples of the Tween 80 and complex. According to NMR spectra neither difference in chemical shifts nor new peak was observed for complex loaded SLN and placebo SLN. In addition, Cis-platin loaded NLPs reduced MCF-7 cells viability.

Conclusion: This formulation may be used as an alternative dosage form for cancer treatment.

PO-18

Track: Immunology and Inflammation

IMMUNOMODULATION OF SOME SELECTED CYTOKINES IN WISTAR RATS BY A CRUDE EXTRACT OF *HALICLONA (SOESTELLA)* SP, A SRI LANKAN MARINE DEMOSPONGE

Varuni K Gunathilake, Wanigasekara D. Ratnasooriya and Preethi V. Udagama

Department of Zoology, Faculty of Science, University of Colombo, Colombo, Sri Lanka;
E-mail: kvkgunathilaka@yahoo.com

Modulation of cytokine secretion offers novel remedy in healing a variety of diseases of the mammalian immune system. Demospongiae marine sponges contain numerous bioactive compounds including immunomodulators. For the first time in Sri Lanka, we investigated the modulation of cytokines in Wistar rats on post treatment with crude extract of demosponge, *Haliclona (Soestella)* sp.



Samples of *Haliclona (Soestella)* sp collected from Unawatuna, Southern Sri Lanka, were extracted in absolute methanol and dichloromethane. The sponge crude extract (SCE) obtained following rota-evaporation (R 200-USA) at 40°C, was used for *in vivo* cytokine assays. Adult Wistar rats (N=6/group), were orally gavaged with 5, 10, 12.5 and 15 mg/kg of SCE; controls received 5% ethanol. Following 14 consecutive days of treatment, IL-6, TNF α , IFN γ and IL-10 cytokine concentrations in rat plasma was measured by sandwich ELISA using commercial kits.

Compared with controls, a significant reduction of IFN γ was apparent with all treatment doses ($P \leq 0.005$). A biphasic response in TNF α levels were detected relative to controls; reduced levels with 5 ($P < 0.005$) and 10 ($P = 0.06$) mg/kg doses, and seven fold elevated levels with 12.5 and 15mg/kg doses ($P < 0.05$). IL-10 was recorded only with the highest treatment dose. Th₁/Th₂ cytokine ratio for TNF α /IL10 was 117.2 with 15mg/kg dose. Though not significant, IL-6 levels lesser than controls with 5 and 10 mg/kg doses ($P > 0.05$) were recorded, while 12.5 ($P = 0.06$) and 15 ($P < 0.005$) mg/kg doses induced two fold higher levels.

Marked immunosuppression of IFN γ (all doses), TNF α (5 & 10mg/kg), IL-6 (5 & 10 mg/kg), and immunostimulation of TNF α (12.5 & 15mg/kg), IL-6 (12.5 & 15mg/kg) and IL-10 (15mg/kg) was observed. Polarization of Th₁/Th₂ cytokines towards Th₁ was evident, probably triggered *via* IL-6 (Th₁₇ type).

In conclusion, this prototype study reports the immunomodulation of selected cytokines in rats treated with crude extract of demosponge, *Haliclona (Soestella)* sp.

Keywords: Cytokines, immunomodulation, *Haliclona (Soestella)* sp, sponge crude extract.

PO-51

Track: Drug Delivery and Targeting

EFFECT OF DIETARY EPA AND DHA SUPPLEMENTATION ON FATTY ACID METABOLISM IN STATIN-ADMINISTERED SHR.CG-*LEPR*^{CP}/NDMCR RATS, A METABOLIC SYNDROME MODEL

Michio Hashimoto¹, Abdullah Al Mamun¹, Masanori Katakura¹, Yoko Tanabe¹, Setsushi Kato², Kyoko Kato² and Osamu Shido¹

¹Department of Environmental Physiology, Shimane University Faculty of Medicine, Izumo, Shimane 693-8501, Japan; E-mail: karita@gii.co.jp

²Kato Hospital, Jinjukai Healthcare Corporation, Kawamoto, Shimane 696-0001, Japan

Effects of cholesterol-lowering drugs statins, which substantially benefits the future cardiovascular events, on the fatty acid metabolism have remained largely obscured. In this study, we investigated effects of atorvastatin on fatty acid metabolism and effect of TAK-085 containing highly purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl ester on atorvastatin-induced cholesterol lowering action in SHR.Cg-*Lepr*^{CP}/NDmcr (SHR-cp) rats, a metabolic syndrome model.

Supplementation of atorvastatin alone at 10 mg/kg BW/day for 17 weeks significantly reduced the plasma total cholesterol and VLDL cholesterol levels and the hepatic EPA and DHA levels and increased the plasma DHA levels without any alteration of red blood cell EPA and DHA levels of SHR-cp rats. After confirming the reduction of plasma total cholesterol in atorvastatin-administered SHR-cp rats, TAK-085 at 300 mg/BW/day was continuously administered to the rats for 6 weeks. The supplementation of TAK-085 significantly increased the EPA and DHA levels in both the plasma and liver, compared with those of atorvastatin alone-administered SHR-cp rats. Concurrently, supplementation of atorvastatin alone significantly decreased SREBP-1c, $\Delta 5$ - and $\Delta 6$ -desaturases, elongase-5 and stearoyl-CoA desaturase-2 (*scd-2*) mRNA expression and increased HMG-CoA reductase mRNA expression in liver, compared to those of the control rats. TAK-085 supplementation significantly increased *scd-2* mRNA expression.

These results suggest that long-term supplementation of ATS decreases the EPA and DHA levels by inhibiting desaturation and elongation steps of n-3 fatty acid metabolism, while TAK-085 supplementation effectively reverses the decreased n-3PUFAs levels in liver of SHR-cp rats (239).

We investigated effects of atorvastatin on fatty acid metabolism, and effect of TAK-085 containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on atorvastatin-induced cholesterol lowering action in SHR.Cg-*Lepr*^{CP}/NDmcr (SHR-cp) rats, a metabolic syndrome model.

Supplementation of atorvastatin alone at 10 mg/kg BW/day for 17 weeks reduced the plasma total cholesterol and VLDL cholesterol levels and the hepatic EPA and DHA levels and increased the plasma DHA levels of SHR-cp rats. After confirming the reduction of plasma total cholesterol in atorvastatin-administered SHR-cp rats, TAK-085 at 300 mg/BW/day was continuously administered to the rats for 6 weeks. The supplementation of TAK-085 increased the EPA and DHA levels in both the plasma and liver, compared with those of atorvastatin alone-administered SHR-cp rats. Concurrently, supplementation of atorvastatin alone significantly decreased SREBP-1c, $\Delta 5$ - and $\Delta 6$ -desaturases, elongase-5 and stearoyl-CoA desaturase-2 (*scd-2*) mRNA expression and increased HMG-CoA reductase mRNA expression in liver, compared to those of the control rats. TAK-085 supplementation significantly increased hepatic *scd-2* mRNA expression.

These results suggest that long-term supplementation of ATS decreases the EPA and DHA levels by inhibiting desaturation and elongation steps of n-3 fatty acid metabolism, while TAK-085 supplementation effectively reverses the decreased n-3PUFAs levels in liver of SHR-cp rats (199).

PO-63

Track: Pharmaceutical Research & Development

AMINOPHOSPHONATES AS NOVEL ACTIVITY-BASED PROBES FOR MATRIPTASE-2

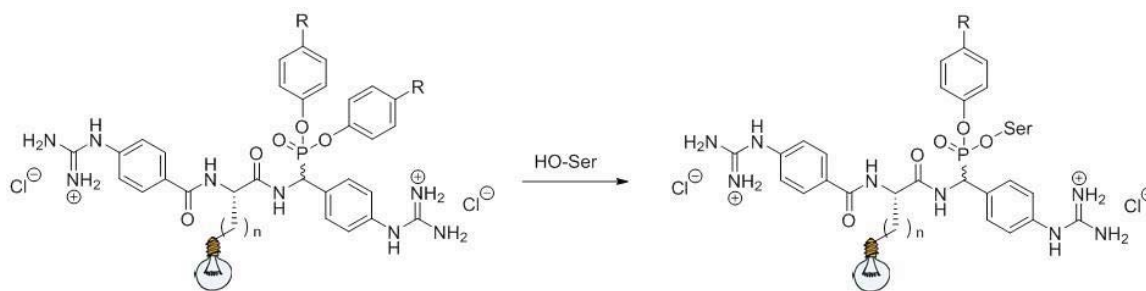
D. Häußler and M. Gütschow

Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg, Bonn, Germany; E-mail: dhaeussl@uni-bonn.de



Activity-based probes form covalent bonds with active enzymes and can be utilized to profile enzyme activities *in vivo*, to identify target enzymes and to characterize their function. They have a reactive head group that covalently binds to the target, a tag that allows detection (e.g. a fluorophore) and a linker to connect both [1].

Matriptase-2 is a transmembrane, multi-domain serine protease with primary substrate specificity for arginine in P1 position, which plays a key role in the human iron homeostasis [2]. Our design of activity-based probes for matriptase-2 is based on linker-connected *bis*-benzguanidines [3]. The two benzguanidine units interact as arginine mimetics with the S1 and the upper part of the S3/S4 pocket, respectively, and direct the inhibitor to the active site of the target enzyme [2]. An amino acid was introduced as a linker, which bears the coumarin fluorophore. Moreover, an incorporated phosphonate allows for a covalent interaction with the active-site serine [4]. The resulting irreversible mode of action was demonstrated, leading to an enzyme inactivation and, simultaneously, to a fluorescence labeling of matriptase-2.



Herein, we present the preparation of coumarin-functionalized amino acids and the subsequent linear synthetic approach to coumarin-labeled *bis*-benzguanidines as activity-based probes for matriptase-2. Spectral properties and kinetic parameters for the reaction with matriptase-2 are reported.

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PO-9

Track: Drug Discovery in Preclinical Research

ANTIOXIDANT ACTIVITY AND FREE RADICAL SCAVENGING PROPERTIES OF CAPTOPRIL

Mohsen Asori and Akbar Hedayatizadeh-Omran

Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran; E-mail: akbar_hedayati@yahoo.com



Several diseases are associated with oxidative stress caused by free radicals and reactive oxygen species. In this study, antioxidant activity of captopril was studied using *in vitro* assays systems. Free radical scavenging and reducing power were determined with diphenyl picryl hydrazyl free radical (DPPH method) and potassium ferricyanide method, respectively. The results of this study showed that captopril possessed a significant free radical scavenging and reducing power properties and there were a clear correlation exists between antioxidant activity and concentration of captopril. Percentage of free radical scavenging of captopril was more than 92% at concentration 0.08 mM.

Keywords: Captopril, oxidative stress, free radical scavenging, reducing power.

PO-93

Track: Drug Discovery in Preclinical Research

THE MAJORITY OF IRANIAN *ASPERGILLUS* ISOLATED FROM CLINICAL SPECIMENS HAS MIC NEAR THE MIC OF STANDARD STRAINS OF THESE FUNGI

Seyed Jamal Hashemi Hezave, Velashjerdifarahani Mohammadreza, Hashemi Farshad and Zakeri Abolfazl

School of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran; E-mail: sjhashemi@tums.ac.ir

Background: Different studies have shown, despite the expanding antifungal agents, opportunistic fungal infections death incidence rate caused by species of *Aspergillus* have increased during recent decades due to the growth of potential factors and immune suppressed individuals. Susceptibility decrease, drug-resistance occurrence, MIC (Minimum Inhibitory Concentration) increase, cross resistance among the isolated *Aspergillus* sp. and lack of the antifungal susceptibility patterns of the most common Iranian isolated *Aspergillus* sp. have become an excuse to design and carry out the present study

Methodology: During 13 months 50 clinically isolated *Aspergillus*, which have been isolated from visceral and cutaneous samples, based on Klich 2002 method and the morphological features were divided into 40 strains of *A. flavus*, 9 strains of *A. niger*, and one strain of *A. fumigatus*. Then their susceptibility test was carried out according to the standard method of NCCLS - M38A Broth Micro dilution.

Results: Through this study we found out that 7.5% of the isolated *A. flavus* with MIC > 2 µg/ml in relation to amphotericin B (AMB), according to CSLI Guideline are probably considered to be as clinically resistant isolated types or treatment failures, and 25% of them in relation to itraconazole (ITR) with MIC = 1 µg/ml and by MIC < 8 µg/ml are considered to be as less sensitive isolated species. On the whole, the domestic isolated *A. flavus* species were less sensitive than those which have been under studies overseas.

The MIC range of 9 strain *A. niger* in relation to AMB, ITR and voriconazole (VRC) respectively came out 0.5 - 1 µg/ml, 0.5 - 2 µg/ml, and 0.25 - 2 µg/ml, that in comparison with similar foreign studies had less sensitivity in spite of being in the standard strain of MIC range and protocol.

The MIC range of 1 strain *A. niger* in relation to AMB, ITR, VRC medicines respectively came out 1, 2, and 0.25 MIC µg/ml, that according to CLSI protocol are considered as high. In comparison with similar foreign studies had less sensitivity.

Conclusion: Through this study we found out that the MIC range Iranian *Aspergillus* isolated from clinical specimens in the majority of the cases go into the reference standard strains of MIC range and the MIC range of some foreign studies. But in some important cases go out of this range that shows lower sensitivity of Iranian isolated *Aspergillus* and their MIC increase.

Keywords: *Aspergillus*, MIC, susceptibility.

PO-66

Track: Hot Topics in Natural Products

MONTMORILLONITE-ILLITE CLAY MINERAL REDUCES UREMIC TOXINS AND VASCULAR PATHOLOGIES IN RATS WITH CHRONIC RENAL FAILURE

Jacqueline Hofrichter, Claus Kerkhoff, Anne Breitrück, Kai Sempert and Steffen Mitzner

*Fraunhofer Institut für Zelltherapie und Immunologie, Perlickstrasse, Leipzig, Germany;
E-mail: jaqueline.hofrichter@izi.fraunhofer.de*

Introduction: Chronic kidney disease (CKD) is highly associated with elevated serum phosphate levels, contributing to an increased cardiovascular risk observed in dialysis patients. Hence, phosphate elimination is essential during ingestion. This study provides the first analyses of Montmorillonite-Illite clay mineral as novel phosphate binding agent since *in vitro* binding studies detected its high adsorbance capacity for phosphate.

Methods: CKD rats induced by 5/6 nephrectomy (5/6 NX) received a high phosphate and calcium diet supplemented either with specific Montmorillonite-Illite clay mineral or Fosrenol. Both, urinary and serum levels of uremic toxins were determined. Pathological arterial changes were reviewed by western blot and histological aortic sections.

Results: Treatment of 5/6 nephrectomised rats with Montmorillonite-Illite clay mineral decreased serum phosphate, creatinine and urinary microalbumin. Severe vascular pathology observed in 5/6NX rats by thickened arterial walls and increased extracellular matrix (ECM), were reduced in 5/6NX rats that received mineral treatment, comparable to sham-operated littermates. αSMA was unaltered and indicates a contractile character of arterial smooth muscle cells.

Conclusion: Montmorillonite-Illite clay mineral is a potent phosphate absorber, comparable to Fosrenol. Moreover, findings of reduced hypertrophy and microalbumin excretion after mineral diet are meaningful since elevated microalbuminuria has been associated with an increased risk for cardiovascular disease.

PO-32

Track: Hot Topics in Natural Products

CHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF *SIDERITIS SYRIACA* SUBSP. *NUSAIRENSIS* AND *SIDERITIS ARGYRAE* ESSENTIAL OIL AND METHANOLIC EXTRACTS

Gokalp Iscan, Yavuz Bulent Kose, Fatih Goger, Betül Demirci

*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey;
E-mail: giscan@anadolu.edu.tr*

The genus *Sideritis* L. (*Lamiaceae*) is represented by more than 150 species mainly found in the Mediterranean basin [1]. In the Flora of Turkey *Sideritis* L. genus represented by 45 species and 15 subspecies, 40 taxa being endemic and this genus known as "*Dagcayi* or *Adacayi*" in Turkey [2].



Traditionally, *Sideritis* species have been widely used as herbal tea in South Anatolia and in folk medicine for their anti-inflammatory, antilulcerative, antimicrobial, antioxidant, antispasmodic, anticonvulsant, analgesic and carminative effects [3, 4].

In the present study hydrodistilled essential oils and the methanol extracts of *Sideritis argyrea* subsp. *Nusairensis* and were subjected to a screening for their antimicrobial and antioxidant activity by using microbroth dilution and DPPH assays respectively. *Sideritis argyrea* oil was demonstrated promising *in vitro* anticandidal activity having a MIC value of 31.0 µg/mL. According to GC-FID and GC/MS results nonacosane (13.2%), hexadecanoic acid (11.5%), hexahydrofarnesyl acetone (11.2%) were determined as main the compounds of *S. syriaca* subsp. *nusairensis* oil, where the β-pinene (35.5%), α-pinene (24.3%) and epi-cubebol (13.0%) in *S. argyrea* oil. 4'-O-methylisoscuteallarein-7-O-(6"-O-acetylallosyl) glucoside and apigenin-coumaroyl glucoside were determined in the methanol extracts of *S. syriaca* while 3'-O-methylhypolaetin 7-O-[6"-O-acetyl]- allosyl glucoside, isoscuteallarein 7-O-[6"-O-acetyl]-allosyl (1-2)-[6"-O acetyl]-glucoside and verbascoside were determined as major compounds in *S. argyrea* extract by using LC/DAD/ESI-MS system.

Keywords: *Sideritis*, Essential oil, Methanolic extract, GC/MS, LC/DAD/ESI-MS, Antimicrobial, Antioxidant.

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PO-26

Track: Inflammation & Immunology

IMMUNE RESPONSE AND MACROPHAGE POLARIZATION ON MINERALIZED CELL SHEETS SEEDED WITH HUMAN MESENCHYMAL AND ENDOTHELIAL PROGENITOR CELLS

Esmaiel Jabbari

Department of Chemical Engineering, University of South Carolina, Columbia, SC, USA;
E-mail: jabbari@mailbox.sc.edu



The inflammatory response after implantation of a tissue engineered cellular (TE) construct plays a critical role in the process of bone formation and healing. The objective of this work was to investigate the effect of mineralized nanofiber sheets, with mineral content close to that of natural bone, covered with devitalized human mesenchymal stem cells (hMSCs) and endothelial colony-forming cells (ECFCs) on the state of polarization of human macrophages. hMSCs, ECFCs, or their combination were seeded on the mineralized sheets and allowed to differentiate to the osteogenic or vasculogenic lineage. After differentiation, mineralized cell sheets were lyophilized to form devitalized, mineralized cell sheets and the effect of secreted growth factors on innate immune response was evaluated by seeding the sheets with human macrophages. Macrophage polarity was characterized by gene and protein expression profiling of cell surface markers (IL1β, CCR7 and TNFα for M1 and CD206, CCL18 and CCL12 for M2). Mineralized cell sheets with devitalized ECFCs differentiated to vasculogenic lineage had highest M2 polarization at late stages whereas hMSCs+ECFCs had lowest M2 initially and highest M1 polarization at late stages. The results demonstrate that cell type and differentiation can be used to control immune response and macrophage polarization in TE constructs.

ACKNOWLEDGEMENTS

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Keywords: Macrophage polarization, inflammatory response, mineralized cell sheet, mesenchymal stem cells, endothelial progenitor cells.

PO-29

Track: Translational

COMPARISON BETWEEN HER2 EXTRACELLULAR DOMAIN IN SERUM AND HER2 OVEREXPRESSION IN BREAST CANCER TISSUE IN THE SAME PATIENTS

Zidan Jamal, Mzalbat Raneen and Sharabi Adi

Oncology Institute, Ziv Medical Center, Safed. Faculty of Medicine in the Galilee, Safed, Israel;

E-mail: Zidan.j@ziv.health.gov.il

Introduction: Human epidermal growth factor receptor 2 (HER-2) is an important prognostic and predictive factor in women with breast cancer (BC). The importance HER-2 extracellular domain (ECD) in serum is not yet determined. The aim of this study was to explore the correlation between serum ECD and tissue HER-2 expression, to compare ECD levels with clinical and pathological features in primary BC patients with review of the literature.

Patients/Methods: In this prospective study patients with stage I–III BC were included. Serum ECD levels were measured by ADVIA Centaur automated assays before surgical resection of the tumor. Serum ECD >15ng/ml was considered to be positive. Immunohistochemistry (ICH) and chromogenic *in situ* hybridization (CISH) tests were used for the detection of HER2 in tumor tissues.

Results: 80 patients with breast tumors were included. Stage I–III BC was diagnosed in 64 patients, Ductal carcinoma *in situ* in 9 and benign tumors in 7 patients. HER-2 overexpression was observed in 8 of 64 patients (16.4%). Mean value of serum ECD was 10.9 ng/ml (range: 6.7 to 21.5). 4 (6.2%) of the 64 patients had high ECD levels and in 60 (93.8%) patients lower levels were found. No significant relationship was found between ECD levels and tissue HER2 overexpression. ECD was higher in women aged > 40 than in women aged < 40. No significant relation was found between ECD and clinical and pathological features.

Conclusion: The sensitivity of HER2 ECD for the diagnosis of HER2 overexpression in primary breast cancer is poor. No significant correlation was found between ECD levels and tissue HER2 expression, clinical and pathological characteristics of primary BC.

PO-4

Track: Cancer Targeted Drug Delivery

MOLECULAR EXPRESSION AND TRANSPORT ACTIVITY OF A PROMISING TARGET FOR TUMOR SPECIFIC DRUG DELIVERY---OLIGOPEPTIDE TRANSPORTER 1 IN HUMAN HEPATOCARCINOMA

Xu Jian, Yanxia Gong, Xiang Wu, Zhi Yao, Qingyu Zhang and Jia Zhao

Central Laboratory, Tianjin Medical University General Hospital, Tianjin, China; E-mail: jianxu516@hotmail.com

Proton-coupled oligopeptide transporter 1 (PEPT1) is expressed predominantly in intestine and recognized as the target of dietary nutrients (di/tripeptide) or peptidomimetic drug for delivery. The information on the existence of PEPT1 in carcinomas were limited. Our study aimed to investigate the expression profile and transport activity of PEPT1 both in human hepatocarcinoma tissues and cell lines. Western blot and immunofluorescent assay revealed the high level of PEPT1 protein expression in hepatocarcinoma Bel-7402, SMMC-7721, HepG2, HEP3B, SK-HEP-1 cell lines. Quantitative real time PCR showed the mRNA expression of PEPT1 in SK-HEP-1, Hep3B and Bel-7402 cells. High level PEPT1 expression in hepatocarcinoma patient samples were observed by Immunohistology and showed a significant correlation between protein level and pathological grade. Functional activities were studied in Bel-7402 cells using D-Ala-Lys-AMCA (a substrate of peptide transporter) and YSL (an antitumor tripeptide). The uptake tests performed by fluorescent microscopy suggested that PEPT1 can transport both D-Ala-Lys-AMCA and YSL into the hepatocarcinoma cells and the uptake can be competitively inhibited by three PEPT1 substrates (Gly-sar, Gly-gln and

Glyglygly). In conclusion, our findings provided the novel information on the expression and function of PEPT1 in human hepatocarcinoma and expanded the potential values for tumor specific drug delivery.

Keywords: Proton-coupled oligopeptide transporter 1, hepatocarcinoma, target therapy, expression, function.

PO-56

Track: Process Chemistry and Drug Manufacturing

COMPARATIVE STUDY BETWEEN DIFFERENT EMULSIFICATION METHODS: MULTI-SCALE CHARACTERIZATION

Messaouda Kaci^{1,2}, Elmira Arab Tehrani¹, Guillaume Gillet², Isabelle Lavis² and Stéphane Desobry¹

¹Laboratoire d'Ingénierie des Biomolécules (LIBio), Université de Lorraine, 2 Avenue de la Forêt de Haye - TSA 40602, 54518 - Vandœuvre-lès-Nancy Cedex, France; E-mail: messaouda.kaci@univ-lorraine.fr

²SAS GENIALIS Route d'Achères, 18250 Henrichemont, France

Emulsifier free emulsion was developed with a new process for foods, pharmaceuticals and cosmetics. This process uses high frequency ultrasounds generated by piezoelectric ceramic transducer vibrating at 1.7 MHz. Obtained emulsions were compared with other emulsions made with classical emulsification process: "low frequency ultrasounds (LFU) and high pressure homogenization (HPH)". This work consists to prepare two emulsions with two emulsification process. The first emulsions contained vegetable oil only. For second emulsion, orange essential oil was added as active ingredient. Oil droplets size was measured by static light diffraction and nanoparticles tracking analysis (NTA) for characterization of nanometric and micrometric droplets fraction. NTA was used for calculate of nanometric fraction. Electrophoretic mobility, surface tension, viscosity were measured and stability was monitored during 30 days at 37°C and oxidation of oily emulsion phases was expected. The results showed that the nanometric fraction was more important for HFU emulsions. It represented more than 90% of emulsion oil for HFU emulsion and represents between 50 and 70% of emulsion made with LFU and HPH. HFU emulsion was stable during 30 days at 37°C unlike other emulsions.

PO-96

Track: Hot Topics in Drug Targets

A PROTEOMIC ANALYSIS OF HUMAN FOLLICULAR FLUID: YOUNGER *VERSUS* OLDER WOMEN

Hee-Gyoo Kang¹, Hee-Joung Lim², AeEun Seok¹, Jiyeong Lee¹, Arum Park¹, JuHwan Lee¹ and Yun-Seok Yang³

¹Bio-medical Laboratory, Department of Biomedical Laboratory Science, College of Health Science, Eulji University, Korea; E-mail: kanghg@eulji.ac.kr

²Institute of Cell & Tissue Engineering, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea

³Department of Obstetrics and Gynecology, Eulji University Hospital, Daejeon, South Korea

Age-related infertility is usually considered as a problem that can be solved by assisted reproduction technology. Therefore, the identification of novel biomarkers that are linked to reproductive aging is the subject of this study. The follicular fluid (FF) was obtained from healthy younger (less than 35 years old) and older (more than 35 years old) women undergoing *in vitro* fertilization (IVF) treatment. Using LC-MS/MS, we identified 672 proteins as consistently

present in hFF of younger group and identified 505 proteins in older group. For the protein functional analysis was found in most of the protein network, which was process network, GO process, and pathway map by used MetaCore software. On the basis of the protein network analysis, the pathway maps of the cell adhesion (glycoconjugates, synaptic contact) in the younger group were higher than older group. And also, components of the cell maturation, reproduction (oocyte maturation) were found more abundant in the younger group compared to the older group. The hFF peptide composition is likely to serve not only the inflammatory follicular state as has been previously suggested; rather, it is a highly diverse and multifunctional environment with several interconnected pathways. These results provide us with important knowledge related to the environment in which the oocyte develops as well as the molecular basis for controlling the process independently of blood supply.

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PO-91

Track: Pharmaceutical Research & Development

NEW DIETARY SUPPLEMENTS AND THEIR EVENTUAL USING FOR WEIGHT

Rabbia Khursheed

*Department of Pharmacy, University of Karachi, Karachi, Pakistan;
E-mail: beeya_alikhan@hotmail.com*

The prevalence and incidence of obesity increase during the last 10 year in whole world. Obesity is a risk factor leading to many serious civilization diseases such as Insulin resistance, Diabetes mellitus II. Type, Dyslipidaemia – small dense oxide LDL...

Studies have shown that more than two-third of adults and almost one-third of children and adolescents are overweight and obese. 45% of overweight and 67% of those who are obese is trying to lose weight.

Health experts agree that making life style changes-including following a healthy eating pattern, reducing caloric intake, and engaging in physical activity-is the basic for achieving long term weight loss. But because making diet and life style changes can be difficult, many people turn to dietary supplements promoted for weight-loss in the hope that these products will help them more easily achieve their weight-loss goals.

Using weight-loss supplements is fairly common. Approximately 15% adults have used a weight-loss dietary supplement at some point in their lives, with more women reporting use (20.6%) than men (9.7%).

The aim of work was the investigation of the change of selected anthropometrical and biochemical parameters (BMI, waist circumference, % of body fat, serum concentrations of total cholesterol, HDL and LDL, TAG) during 3 month controlled, non pharmacology regime of weight reduction using dietary supplement Sternax (50 mg dry extract from Leuzea, 100 mg caffeine, calcium 1000/1200 mg/day for adults) 1 dose per day with simultaneous restrict of dietary energy.

Common ingredients in weight loss dietary supplements are Hydroxycitric acid HCA, Chitosan, Whey protein, Beta glucan, Conjugated linoleic acid CLA, and Mango seed fiber).

The result of the study proved that there is a positive effect of a targeted reduction diet on obese person. Following the three-month therapy, weight-loss, decrease of body fat, lower waist circumference, improved lipid profile and deceased of systolic as well as diastolic blood pressure were demonstrated. On the other hand 50% monitored people noted increase of appetite especially of sweet.



Using dietary supplements is one of the way how to reduce body weight.

PO-12

Track: Drug discovery in Preclinical Research

ATYPICAL ANTIPSYCHOTIC ARIPIRAZOLE FACILITATES FEAR EXTINCTION IN ADOLESCENT RATS

D.E. Ganella, H.B. Madsen, L. Lee-Kardashyan, I.C. Zbukvic, S. Luikinga, R.W. Coulthard, A.J. Lawrence and J.H. Kim

Developmental Psychobiology Laboratory, Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, Kenneth Myer Building 30 Royal Pde Parkville, North Melbourne, Australia; E-mail: jeek@unimelb.edu.au

Currently, the most effective treatment for anxiety disorders is some form of exposure-based therapy, which relies on the process of extinction. We have shown previously that extinction of conditioned fear is impaired during adolescence, which may explain vulnerability this age exhibits for anxiety disorders. During adolescence, dopamine receptor 1 (D1R) activity dominates dopamine receptor 2 D2R activity in the prefrontal cortex (PFC) and this imbalance was hypothesised to be the cause of the adolescent deficit in extinction learning. In the present study we increased D2R activity in adolescent rats using the selective D2 partial-agonist Aripiprazole, which we injected systemically prior to extinction. Aripiprazole is currently FDA-approved to treat adolescent bipolar disorder and schizophrenia. Our results showed that Aripiprazole during extinction leads to a reduction of the conditioned freezing response in next-day fear testing. These behavioural differences were accompanied by changes in brain regions critically involved in extinction, the PFC and the amygdala.



PO-8

Track: Diabetes and Obesity Drug Discovery & Therapy

ELUCIDATION OF INVOLVEMENT OF 11- β HYDROXY STEROID DEHYDROGENASE 1 IN DIABETIC CEREBRAL STROKE

Patel Mittalben Kirtikumar

Department of Pharmaceutics, Shri S K Patel Collage of Pharmaceutical, Education and Research, Mehsana, India; E-mail: mittalpatel14492@gmail.com

Context: Stroke is a second leading cause of death worldwide and diabetic patients have 2-3 folds higher risks to cause compared to non-diabetic.

Aim: The aim of present study was to elucidation of involvement of 11- β hydroxy steroid dehydrogenase in diabetic cerebral stroke.

Method: In *in vivo* study, HFD-STZ diabetic rat underwent middle cerebral artery occlusion surgical procedure for stroke induction on 42nd day. Drug (Metyrapone- 11 β -HSD type 1 inhibitor) was administered for 28 days prior surgery. After 72 hours of surgery, animals were sacrificed and various parameters were assessed *i.e.* blood glucose, serum triglycerides, total cholesterol, HDL, LDL level, serum nitrite, cortisol level, testosterone level, total proteins, soluble proteins, lipid peroxidation, anti-oxidant enzymes, neurological deficits, cerebral edema, spatial memory. Histopathological examination of rat's brain was done along with total to live cell ratio in CA1 hippocampal region.

Results: In the *in vivo* study, blood glucose, total cholesterol, triglycerides, LDL level, serum nitrite, malonaldehyde and cortisol, had significantly decreased whereas HDL level, antioxidant enzymes, total proteins, soluble proteins and



testosterone level had significantly increased in compound treated animals compared to disease control rats. There was also reduction of neurological deficits, cerebral edema enhancement of memory observed significantly in compound treated animals compared to disease control rats. Drug treated rats brain showed significantly higher live cell compared to disease control rats on histopathological examination.

Conclusion: The treatment with 11 β -HSD type 1 inhibitor Metirapone significantly ameliorated the alterations in ischemic pathway and neurodegeneration was delayed.

Keywords: Diabetic stroke, Middle Cerebral Artery Occlusion, Metirapone, 11 β -HSD.

PO-37

Track: Hot Topics in Natural Products

ANTIBACTERIAL, ANTICANDIDAL AND ANTIOXIDANT PROPERTIES OF *TANACETUM ARGENTEUM* (LAM.) WILLD. SUBSP. *FLABELLIFOLIUM* (BOISS. & HELDR.) GRIERSON

Yavuz Bülent Köse, Gökalp İşcan, Fatih Göger, Betül Demirci, Ceren Elmacı

Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey;

E-mail: ybkose@anadolu.edu.tr

Tanacetum L. is the third largest genus of *Asteraceae-Anthemideae* with about 160 species all over the world (1). 46 species are found in Turkey belonging to the genus (2). In the Flora of Turkey, *T. argenteum* (Lam.) Willd. is classified into three subspecies: *Argenteum*, *Flabellifolium* (Boiss. & Heldr.) Grierson and *Canum* (C. Koch) Grierson. *Tanacetum* species are rich in essential oils, bitter substances and sesquiterpene lactones and they are widely used in folk medicine for their antihistaminic, anti-inflammatory and insecticidal effects (3). In the present study hydrodistilled essential oil and total methanol (70%) extracts of *Tanacetum argenteum* subsp. *Flabellifolium* have been evaluated for their antimicrobial and antioxidant effects. The chemical composition of the oil and the crude extract were determined by GC/FID- GC/MS and LC/DAD/ESI-MS systems respectively. B-thujone (47.1%), α -pinene (19.1%) and α -thujone (10.5%) were the main compounds of the essential oil where the 5-caffeoyl quinic acid, 1,5-dicaffeoyl quinic acid, 4,5 (or 3,5) dicaffeoylquinic acid were flavonoid content of the crude extract. The oil and the methanol extract were demonstrated moderate antimicrobial effects (MIC range; 0,062-2, 0 mg/mL) against 21 different pathogenic microorganism. Total phenolic content was determined as 6,25 mg GAE in 100 mg extract and the DPPH radical scavenging effect was determined as 0.16 mg/mL (IC₅₀) and TEAC was determined as 0.21 mMol.

Keywords: *Tanacetum argenteum* subsp. *Flabellifolium*, Essential oil, Methanol extract, Antimicrobial, Antioxidant, GC-FID-GC/MS, LC/MS.



PO-50

Track: Hot Topics in Natural Products

DETERMINATION OF PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF TWO *THYMUS* L. (LAMIACEAE) SPECIES FROM TURKEY**S. Küçük¹ and F. Göger²**¹Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey; E-mail: salan@anadolu.edu.tr²Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

Thymus L. is an important genus due to their essential oils and phenolics. 41 species of *Thymus* are represented by 66 taxa in Turkey and 14 of which are endemic [1, 2]. The Dried herbal parts of *Thymus* species are used as spice and herbal tea in Turkey [3]. Phenol-rich *Thymus* species are used in diabetes, stomach and intestinal diseases, for cough, as herbal tea and also as condiment, whereas, phenol-poor or phenol-less *Thymus* species are used, due to their pleasant aroma, as herbal tea, where they grow [4].

The aim of this study was to determine the antioxidant activity and phenolic profile of the two species of *Thymus* which are *Thymus sipyleus* Boiss and *Thymus leucostomus* Hausskn. et Velen. (endemic).

For this purpose dried herbal plant materials macerated with %70 MeOH. After evaporation and lyophilization steps the extract was analyzed with ABSciex 3200 Q trap LC-MS/MS system. Rosmarinic acid and luteolin were found as major for two species where as naringenin and apigenin glucuronide were also found as the other major compounds.

According to antioxidant activity *Thymus sipyleus* and *Thymus leucostomus* showed weak antioxidant activity with the score of DPPH IC₅₀=0.20mg/ml and DPPH IC₅₀= 0.13 mg/ml respectively. All the extracts showed the similar Trolox Equivalent Antioxidant activity (TEAC) with the score of 1mM TEAC. Total phenolic content of the extracts were also found as same 11mgGAE and 13 mgGAE respectively.

Keywords: *Thymus*, Lamiaceae, Phenolic profile, Antioxidant activity.

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**PO-88**

Track: Pharmaceutical Research & Development

ESSENTIAL OIL COMPOSITION OF ENDEMIC *TRIPLEUROSPERMUM* SPECIES FROM TURKEY**M. Kurkcuoglu¹, H. Inceer² and K.H.C. Baser^{1,3}**¹Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskisehir, Turkey; E-mail: mkurkcuo@anadolu.edu.tr²Karadeniz Technical University, Faculty of Sciences and Arts, Department of Biology, 61080 Trabzon, Turkey³King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia

The genus *Tripleurospermum* Schultz Bip. is represented in Turkey by 25 species, 12 species are endemic in Turkey. Microdistilled essential oils of the dried flowers of three endemic species were analyzed by GC-FID and GC/MS. All the oils were characterized by the occurrence of matricaria esters as main constituents.

The oil of *T. baytopianum* E. Hossain contained (2*E*, 8*E*)-matricaria ester (32.4%), (2*E*, 8*Z*)-matricaria ester (31.4%) and (2*Z*, 8*Z*)-matricaria ester (19.3%) as main constituents. The oil of *T. repens* (Freyn&Sint.) Bornm. was characterized by the occurrence of (2*Z*, 8*Z*)-matricaria ester (84.4%) and (2*E*, 8*Z*)-matricaria ester (4.1%) as major components. Main components in the oil of *T. ziganaense* Inceer&Hayırlıoglu-Ayaz were (2*Z*, 8*Z*)-matricaria ester (73.1%) and (2*E*, 8*E*)-matricaria ester (5.4%).

PO-69

Track: Drug Delivery and Targeting

A NOVEL TARGETING DRUG DELIVERY SYSTEM FOR SPECIFIC DIAGNOSIS AND THERAPY OF LIVER CANCER

Yaoxian Li¹, Yuzhen Wang² and Jingyuan Wang²

¹*College of Chemistry, Jilin University, Changchun 130012, China; E-mail: jingyuan@jlu.edu.cn*

²*Alan G. MacDiarmid Institute of Jilin University, Jilin University, Changchun 130012, China*

In recent years, numerous studies have revealed the potential of stimuli-sensitive polymeric nanocarriers as drug delivery systems in which the drug release can be readily triggered by responding to specific environmental. In particular, the pH-triggered release of drugs has aroused wide concern. What's more, nano-medicines combining anticancer drug delivery and diagnostic functions have emerged as a multifunctional delivery system to enhance therapeutic efficiency. In order to achieve better diagnosis and therapy results, targeting antibodies have been modified to smart polymeric micelle delivery systems. With the targeting agents identifying the tumor cells at the molecular level, the drug or SPIO modified with antibodies can successfully reach the tumor sites, inhibit cancer cells growth, and image the tumor region more efficiently. In this study, we have developed a novel pH-sensitive targeted diagnosis and therapy system anti-VEGF -poly(aspartate)-graft-poly(ethylene glycol)-dodecylamine-hydrazone-(adriamycin-levulinic acid) encapsulating the super-paramagnetic iron oxide nanoparticles (PASP-g-PEG-DDA-Hyd-(ADR-LEV))anti-VEGF-PASP-g-PEG-DDA-Hyd-(ADR-LEV)@IO. A series of studies showed that this novel anti-VEGF-conjugate@IO showed great potential for application in both MRI detection and therapy of liver cancer by virtue of its specific targeting and much stronger EPR accumulation, which allowed the anticancer drug to be orientated directly to target sites.

PO-58

Track: Drug Delivery and Targeting

PREPARATION OF DOX-CONJUGATED DENDRIMER-MODIFIED MAGNETIC IRON OXIDE CONJUGATES FOR DRUG DELIVERY

Yaoxian Li¹, Yuzhen Wang², Jingyuan Wang² and Yapeng Li²

¹*College of Chemistry, Jilin University, Changchun 130012, China; E-mail: jingyuan@jlu.edu.cn*

²*Alan G. MacDiarmid Institute of Jilin University, Jilin University, Changchun 130012, China*

Over the past decade, nanoparticulate drug delivery systems containing anticancer drugs and targeting ligands have received much attention due to their unique accumulation behavior at tumor sites. Dendrimers, due to their unique surface topology, have recently been used successfully in the fields of biomedicine and targeting. Poly-(amidoamine) (PAMAM) is one class of ideal dendrimers because of their unique characteristics such as uniform size distribution,

relatively higher transfection efficiency, and lower cytotoxicity compared to other traditional cationic polymers, when used as drug delivery system. However, the severe harm from systemic administration of bare DOX on healthy tissues and organs has made it less attractive and limited its treatment of cancer. In here, the novel drug release system was constructed from a conjugate of FA-PEG-PAMAM-DOX@IONPs, with FA as the targeting ligand, PAMAM dendrimers as the carrier, and the hydrazine bond (between the PAMAM and DOX) as the key for controlling the release profiles. Studies indicated that the novel nanoparticles containing DOX conjugates showed great potential for application in both MRI detection and cancer therapy by virtue of their targeting function, in addition to EPR accumulation, which allowed the anticancer drug to be orientated directly to the target sites.

PO-30

Track: Hot Topics in Medicinal Chemistry

SYNTHESIS OF PHENYL THIAZOLE ACYL SHIKONIN ESTER DERIVATIVES AS ANTICANCER AGENTS THROUGH MICROTUBULE STABILIZATION

Hong-Yan Lin

Nanjing University, Nanjing, Jiangsu Province, China; E-mail: linhongyan1989@yeah.net

At present, the high incidence of cancer and the side effects of traditional anti-cancer drugs urge us to seek for new and more effective anti-cancer drugs. In this study, we synthesized 17 kinds of phenyl thiazole acyl shikonin ester derivatives and evaluated them as good anti-cancer agents through MTT assay. Among them, C13 showed better antiproliferative activity with an IC₅₀ value as 3.14±0.21 µM against HeLa cells than shikonin (IC₅₀=5.75±0.47 µM) itself.

Then, we did PI staining assay, cell cycle distribution and cell apoptosis analysis for C13 and found it can cause cell arrest at G2/M phase, leading to cell apoptosis. Meanwhile, it can also reduce the adhesive ability of HeLa cells. By docking simulation, we noted that C13 is nicely bound to tubulin at paclitaxel binding site and it can really result in tubulin polymerization, block mitosis through confocal microscopy assay and flow cytometry analysis of the cell-surface polymerized tubulin expression.

PO-45

Track: Innovative Drug Discovery & Nanotechnology

DEVELOPMENT OF POLYMERIC NANOPARTICLES TO IMPROVE THE THERAPEUTIC EFFICACY OF IRON CHELATORS FOR IRON OVERLOAD DISEASES

Tianqing Liu¹, Shanshan Guo², Guangjun Nie² and Greg Anderson¹

¹*Iron Metabolism Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4029, Australia; E-mail: michelle.tianqing.liu@gmail.com*

²*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China*

Significant pathology accompanies body iron accumulation in iron loading disorders such as thalassaemia, and therapy involves the chemical removal of iron using iron chelators. Current chelator treatments remain suboptimal. In the past, subcutaneous desferrioxamine (DFO) has been most widely used in thalassaemia therapy. It has a favourable toxicity profile, but requires lengthy infusion times. Oral chelators (Deferiprone and Deferasirox) are less onerous for the patient, but have more side effects. Herein, we developed amphiphilic copolymer nanoparticles (NPs) as a delivery system for iron chelators. These PEGylated PLGA NPs containing DFO (DFO-NP) were generated by the double emulsion method. Physical characterisation showed a uniform preparation of NPs with an average diameter of 113nm that were stable with varying



pH. DFO-NPs showed much more effective at depleting cellular iron levels than free DFO using macrophage cells. DFO-NPs were at least twice as effective as free DFO in depleting iron from all tissues investigated when compared to free DFO in iron overloaded mouse model. Reductions in hepatic Kupffer cell iron, a key site of iron overload, were particularly noticeable. No apparent toxicity was observed after the treatment of DFO-NPs. This nanoparticle-based chelating system would potentially benefit patients suffering from iron overloading disorders.

PO-17

Track: Drug Discovery in Preclinical Research

TARGETING CANCER STEM CELLS IN ESOPHAGEAL CANCER

Pei-Jung Lu, Cheng-Han Lin, Pei-Fang Kuo and Wan-Chun Hung

Institute of Clinical Medicine, National Cheng Kung University, Tainan, Taiwan;

E-mail: pjlu2190@mail.ncku.edu.tw

Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two major histological types of esophageal cancer. Even after surgery and/or concurrent chemoradiation therapy (CCRT), 5-year survival of esophageal cancer is less than 25%. Moreover, more than 50% of ESCC patients have tumor recurrence within one year after therapy. Cancer stem cells (CSCs) are hypothesized the reason how cancer cells are able to withstand therapeutic assaults, acquire resistant and establish distant metastasis. Therefore, an effective therapy targeting CSCs is urgently needed. Several CSC markers have been shown to associate with poor prognosis of many cancers. However, the CSC marker of ESCC remains unclear. We aimed to identify the CSC-associated markers that may predict the prognosis of ESCC patients who is resistant to CCRT. To achieve these goals, *in vitro* tumor sphere culture system, which enriched CSCs, of ESCC were established. Functional assays demonstrated that the tumor spheres harbored self-renewal, tumor initiation, differentiation, metastasis, and CCRT resistant *in vitro* and *in vivo*. CLDN4, CLDN7, and ABCB1 were identified as the potential CSC markers of ESCC by microarray analysis. High CLDN4 expression was correlated with poor CCRT response in ESCC. Together, CLDN4 was identified as a potential ESCC CSC marker.

Keywords: ESCC, CCRT, cancer stem cells, CLDN4.

PO-21

Track: Hot Topics in Drug Targets

NEW FORMULATION BASED IN ANTI-ATROPHIC PEPTIDES AND DENDRIMERS FOR THE TREATMENT OF SKELETAL MUSCLE ATROPHY. MOLECULAR DYNAMICS STUDIES AND EXPERIMENTAL VALIDATION

Valeria O. Márquez-Miranda^{1,3}, Ingrid D. Araya Durán^{3,1}, Nicolas C. Pacheco¹, Lars Ratjen^{1,3}, Claudio A. Cabello-Verrugio², Gabriela Morales², Juan C. Rivera² and Fernando D. González-Nilo^{1,3}

¹*Universidad Andres Bello, Facultad de Biología, Center for Bioinformatics and Integrative Biology (CBIB), República 239, Santiago, Chile; E-mail: valeria.marquez.m@gmail.com*

²*Laboratorio de Biología y Fisiopatología Molecular, Ciencias Biológicas, Universidad Andrés Bello, Chile*

³*Fraunhofer Chile Research. M. Sánchez Fontecilla 310 piso 14, Las Condes, Chile*

Angiotensin 1-7 (Ang1-7) is a bioactive heptapeptide with beneficial effects on the treatment of circulatory system, skeletal muscle and nervous system diseases. We have determined that Ang1-7 decreases skeletal muscle atrophy. However, due to their instability, peptides cannot be efficiently administered. Therefore, it is required to develop a new method of delivery that increases the half-life of the peptide and improve its bioavailability in target tissues. Dendrimers

have emerged as promissory vehicles to protect and transport a wide range of bioactive molecules. In this work, we explore the use of a neutral, non-cytotoxic PAMAM dendrimer as carrier of Ang1-7 peptide by molecular dynamics simulation (MD). Results showed that, over 60 ns of MD simulation, peptide-binding capacity of the dendrimer was 2:1 molar ratio, in agreement with experimental data. MD analysis also revealed the capacity of neutral PAMAM to protect Ang1-7 and form stable complexes. Peptide coverage ability of the dendrimer was around 50 and 65%. Furthermore, electrophoretic mobility shift assay demonstrated that neutral PAMAM is able to effectively bind peptides, thus it can act as an efficient carrier. Experimental results have shown that Ang (1-7)/neutral-PAMAM complex, but not Ang (1-7) alone, has antiapoptotic activity when is intraperitoneally administered.

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PO-3

Track: Biosafety

PREVALENCE OF PHANTOM VIBRATION SYNDROME AND PHANTOM RINGING SYNDROME (RINGXIETY): RISK OF SLEEP DISORDERS AND INFERTILITY AMONG MEDICAL STUDENTS

**Mehtab Alam, Muhammad Sameer Qureshi, Ayesha Sarwat, Zeba Haque,
Muhammad Salman Masroor, Muhammad Abdullah Makki, Ibad-ur-Rehman Baig,
Muhammad Saad Munir, Moiz Ehtesham and Saad Aslam**

DOW International Medical College, DUHS, Karachi, Pakistan; E-mail: minshawy01@gmail.com

Modern-day market of wireless communications is developed at a very rapid growth. Different features of entertainment like camera, internet, video games, *etc.* are major causes of popularity of mobile phones among young generation. In Karachi, Pakistan almost everyone has a mobile phone. Excessive use of mobile phone is causing serious health issues related to psychology and social behavior. This study was designed to determine the prevalence of newly reported disorders, like Phantom Vibration Syndrome, Phantom Ringing syndrome (Ringxiety), Nomophobia and possible risk for sleep disorders and infertility among medical students in Karachi, Pakistan. The study was based on a questionnaire which was framed after vigorous literature review. Around thirty questions were developed to achieve the objectives. Data was collected from medical students of Dow International Medical College, Karachi, Pakistan. The data was analyzed using software named as Statistical Package for Social Sciences (SPSS). The frequency of Phantom Vibration Syndrome on daily, weekly, rarely and never observed basis was found to be 19%, 18%, 56% and 7% respectively. Overall 93% students felt Phantom Vibration Syndrome but in different frequencies. Majority of the students (70%) kept their mobile phones in their trousers' pockets. Around 10% students kept their mobile phones in upper pockets while 6% students preferred to attach their mobile phones with their belts. Only 14% students answered that they kept their mobile phones in places other than mentioned above. Around 59% students woke up from sleep upon hearing mobile phone ringtone. The percentage of students using mobile phones prior to sleeping was found to be very high, *i.e.*, 93% and 67% students could not live without mobile phones. Mobile phone usage is contributing a major role in increasing psychological stress and related problems among medical students of Karachi, Pakistan.

Keywords: Mobile phones, sleep do.

PO-79

Track: CNS Drug Discovery

IDENTIFICATION OF NATURAL LACTATE EFFLUX INHIBITORS FOR THE DEVELOPMENT OF NOVEL ANTIPROLIFERATIVE AGENTS

Samia S. Messeha, Najla O. Zarmouh, Malak G. Kolta and Karam F. Soliman

College of Pharmacy & Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL, USA;

E-mail: samia.messeha@famu.edu

In cancer cells of the solid tumor and even in the presence of oxygen, most of the pyruvate from glycolysis is removed away from the mitochondria to form lactate (Warburg Effect). To maintain cell hemostasis, lactate is pumped across the cell membrane through specific transporters known as monocarboxylate transporters (MCTs). In the current study, we screened 906 plant extracts for their ability to inhibit lactate efflux using neuroblastoma (N2-A) cell line. Data obtained indicate that the extract of *Fructus chebulae* fruits (FCE), and *Bupleurum chinense* roots (BCE) were the most potent extracts at the lowest tested concentration (50 µg/ml). Furthermore, FCE was more potent than BCE. Lactate efflux inhibition of both plant extracts were higher than the MCT standard inhibitor, phloretin. Furthermore, the obtained data indicate that FCE showed more potency and selectivity in the cytotoxic effects in cancer cells than DI-TNC1 primary rat astrocytes. Moreover, the results show that FCE Inhibited N2-A cell proliferation (IG50 =5.20 µg/ml) and induced apoptotic effect at the concentration level 4.0 µg/ml. We concluded from these results that FCE was very selective to inhibit lactate efflux, decrease N2-A cell proliferation and induce apoptosis and safer than BCE in normal cells.

PO-38

Track: Drug Metabolism

IN VITRO INHIBITORY ACTIVITIES OF PIPLARTINE ON HUMAN CYTOCHROME P450

Fernanda Lima Moreira, Maísa D.Habenschus and Anderson Rodrigo Moraes de Oliveira

Faculdade de Ciências Farmacêuticas, Universidade de São Paulo-USP, São Paulo, Brazil;

E-mail: fer_unifal@yahoo.com.br

Piplartine (PPT) is an alkaloid that has demonstrated promising cytotoxicity activity being considered a drug candidate to be employed for cancer treatment. Therefore, predicting potential drug interactions with piplartine is essential to guarantee PPT safety. In this context, *in vitro* methods are useful and well-established tool to be employed for this purpose. This study approached the *in vitro* reversible and irreversible inhibition of PPT on cytochrome CYP1A2, a relevant CYP isoform on metabolism of several drugs. The inhibition assay was carried out by incubating PPT with human liver microsomal fraction; NADPH and phenacetin (CYP1A2 substrate) in physiological pH at 37°C during 30 min. Dose and time-dependent studies were performed. PPT demonstrated a low IC50 value (8.8 µM). The dose-dependent experiment showed a competitive inhibition with a potent Ki value of 1.5 µM. The time-dependent revealed a KI value of 8 µM and Kinact was 0.014 min⁻¹. The quasi-irreversible inhibition was evidenced through formation of a metabolite intermediate complex (MIC). After assessing the inhibition potential of PPT on CYP1A2 through *in vitro* liver microsomes system, it can be suggested that concomitant intake of PPT with drugs metabolized for CYP1A2 can lead to interactions and should be carefully monitored in humans.

Keywords: Drug-interaction, human liver microsomes, natural product.



PO-40

Track: Regenerative Medicine

CARBOXYTHERAPY AND PLATELET RICH PLASMA: A NEW THERAPY FOR TRIGONITIS, ABACTERIAL AND INTERSTITIAL CYSTITIS

F. Muzi, G. Delicato, D. D'Andria, G. Baffigo, E. Tartaglia, F. Corvese, S. Signore, A. Perla, G. Montagna and G. Tati

Department of Urology, S. Eugenio Hospital, Rome, Italy; E-mail: fabrizio.muzi@yahoo.it

Cystitis often appears even in absence of bacteria colonization. Trigonitis and interstitial inflammation are the most common morphological features of abacterial cystitis in young and post menopausal women.

Arterial obstructive disease and bladder ischemia might play an important role in bladder dysfunction.

Activated inflammatory cells produce radicals of Oxygen (ROS), NF-kB seems involved in ROS synthesis.

Clinical studies have indicated that high CO₂ levels can impact upon periphoreal tissue, reducing ischaemia, responsible of recurrent inflammation and consequently reducing oxydative phenomena.

Platelet-rich plasma (PRP) is a volume of fractionated plasma from the patient's own blood that contains platelet concentrate rich of alpha granules. PRP interacts tissue repair mechanisms by placing supra-physiological concentrations of autologous platelets at the site of tissue damage.

This study proposes a single PRP transvaginal injection followed by 10 weekly applications of carboxytherapy, using subcutaneous injections of sterile CO₂ gas.

We have selected 6 Women (50-75 years), affected by recurrent abacterial cystitis with pain and urge incontinence.

All patients showed a subjective sensible reduction of symptoms.

After 2 months all patients have neither inflammatory symptoms nor endoscopic evidence of trigonitis.

Preliminary qualitative results could encourage the use of carboxytherapy and PRP in treatment of abacterial and interstitial cystitis.

**PO-10**

Track: CNS Drug Discovery and Therapy

THE SYNTHESIS AND EVALUATION OF 4-CHROMANONE DERIVATIVES AS DUAL INHIBITORS OF MONOAMINE OXIDASE AND ACETYLCHOLINESTERASE

Clarina I. N'Da, Anél Petzer and Jacobus P. Petzer

Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom, South Africa; E-mail: 21695814@nwu.ac.za

In the present study we have synthesized a series of 4-chromanone derivatives and evaluated them as inhibitors of the recombinant human monoamine oxidases, MAO-A and MAO-B. The 4-chromanone derivatives are structurally related to a series of α -tetralone derivatives, which have recently been shown to potently inhibit MAO, with selectivities for MAO-B (in preference to the MAO-A isoform). The MAO enzymes are considered drug targets for the treatment of psychiatric and neurological disorders. MAO-A inhibitors have been used in the treatment of depressive illness while MAO-B inhibitors are employed as anti-parkinsonian agents. MAO inhibitors are also under investigation as potential neuroprotective agents, aids to smoking cessation and for the treatment of certain cardiovascular pathologies



and Alzheimer's disease. In an attempt to discover dual-target-directed compounds, which inhibit both MAO and acetylcholinesterase (AChE), the 4-chromanone derivatives were also evaluated as potential inhibitors of AChE. Since AChE inhibitors are used in the therapy of Alzheimer's disease, such compounds may be of enhanced therapeutic benefit.

PO-2

Track: Anti-Infectives

ANTIMICROBIAL PEPTIDES FOR TREATMENT OF BONE INFECTIONS

Ondřej Nešuta^{1,2}, Pavel Melicherčík³ and Václav Čerovský¹

¹*Institute of Organic Chemistry and Biochemistry AS CR, Flemingovám. 2, 166 10 Prague 6, Czech Republic;
E-mail: nesuta@uochb.cas.cz*

²*University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic*

³*Department of Orthopaedics, First Faculty of Medicine, Charles University in Prague and University Hospital Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic*



Antimicrobial peptides (AMPs) are considered a potential therapeutic family of anti-infective agents because of their broad-spectrum of activities and different mechanisms of action compared to conventional antibiotics. It is expected that they should not develop antimicrobial resistance.

In our laboratory we have described a number of novel AMPs belonging to the category of cationic α -helical amphipathic peptides. These peptides were originally isolated from the venom of different wild bees and then characterized. Based on their determined sequences the peptides and their analogues were prepared by solid phase synthesis. They show potent antimicrobial activities against pathogenic bacteria, yeasts, and also microbial biofilms.

We studied the efficacy of our AMPs in the models of induced osteomyelitis utilizing human bone samples. These bones were infected by selected types of bacteria and *Candida* inside the holes bored into the spongy part of the bone. Microbes were grown within the holes and then the holes were filled with the different AMPs mixed with a local carrier used in orthopedics such as calcium phosphate. The focus of the infection in the bone treated with the AMP mixed with the carrier was eradicated much more effectively than the focus treated with antibiotics such as vancomycin or gentamicin mixed with the same carrier.

PO-15

Track: Hot Topics in Natural Products

CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF THE ESSENTIAL OIL OF *FOENICULUM VULGARE* (FENNEL) FROM SOUTH AFRICA (EASTERN CAPE)

M. Nyalambisa, A.O Oyedeji, S.P Songca, O. O Aremu, B.N Nkeh-Chungag, O.O Oyedeji and S.O Oluwafemi

Department of Chemistry, Walter Sisulu University, Mthatha, South Africa; E-mail: nyalambisam@yahoo.com

Foeniculum vulgare is a medicinal and aromatic wild-growing plant found in the Eastern Cape region of South Africa. This study evaluates comparatively the volatile components of different morphological parts of the plant and their anti-inflammatory activity.



Fresh leaf, root and seed as well as dried leaves of the plant were separately processed by hydrodistillation for 4 h and their essential oils (EOs) collected. Extracted EOs were further subjected to GC/GC-MS analysis. The oils were later

evaluated for anti-inflammatory activity on the egg albumin-induced oedema model in rats. Rats pretreating orally with EOs 1 h before subplantar injection with 0.1 ml of 50% (V/V) fresh egg albumin and paw sizes measured with digital caliper hourly over a period of 5 h.

Chemical analysis of EOs showed that the number of compounds detected and identified were 22, 19, 21 and 18 for fresh seed, fresh leaf, fresh root and dried leaf respectively. The oils obtained were light-yellow in colour and aromatic. The percentage yield was in this order: fresh seed>fresh leaf>fresh root>dried leaf. The chemical compositions of obtained EOs also varied among the oils while the major compounds identified included; anethole, fenchone, limonene, α -pinene and methyl chavicol. The results of the anti-inflammatory test showed that all the oils caused significant ($P<0.05$) reduction in oedema size compared to the negative control group mainly at 2-3 h post induction. However, fresh and dried leaf oils showed better activity compared to the standard drug (Ibuprofen, 100 mg/kg).

It can be concluded that various morphological parts of *F. vulgare* contain volatile oils which varied in their yield and chemical compositions. All the oils demonstrated significant anti-inflammatory activity. The sweet aroma and biological activity showed by these oils may be useful in cosmetic and pharmaceutical industries.

Keywords: Anti-inflammatory, chemical composition, essential oil, *Foeniculum vulgare*.

PO-1

Track: Anti-Infectives

IDENTITY OF COMPOUNDS RESPONSIBLE FOR ANTI ADHESION ACTIVITY IN CRANBERRY JUICE

A.C. Neto, A.K. Penndrof, M. Feldman, S. Meron-Sudai, Z. Rones, D. Steinberg, M. Fridman, Y. Kashman, I. Ginsburg, I. Ofek and I.E. Weiss

*Clinical Microbiology and Immunology, Tel Aviv University, Givatayim, Israel;
E-mail: aofek@post.tau.ac.il*

Previous studies have shown that a high MW nondialysable material (NDM) from concentrated cranberry juice exhibit potent anti adhesion activity of a number of microbial species including oral bacteria, uropathogenic *Escherichiae coli*, *Helicobacter pylori* as well as influenza virus. However, the compounds responsible for such activity were not identified. Here we show that a fraction obtained from NDM on LH 20 column and eluted with acetone (NDMac) is responsible for the anti adhesion activity in NDM. NDMac fraction comprises about 1/4 of NDM by weight and also bound at a higher affinity to bacterial surfaces conferring antioxidant activity to whole cells in suspension. MALDI-TOF MS analysis of the NDMac fraction showed the presence of proanthocyanidins, primarily in the 3-6 degrees of polymerization size range. NDMac inhibited co-aggregation of *Streptococcus sanguis* with *Fusobacterium nucleatum* and *Fusobacterium nucleatum* with *Porphyromonas gingivalis* at MIC of 0.47 mg/mL and 0.94 mg/mL, respectively. Further fractionation of NDMac on MCI CHP-20P with water/methanol elution revealed that the anti-adhesion activity in oral bacteria coaggregation assays resides in fractions eluted with at least 70% methanol. Characterization of the active constituents of NDM will be crucial to understanding its full potential as a potent anti-adhesion agent against microbial infections.

Keywords: Anti-adhesion, cranberry, proanthocyanidins.



PO-94

Track: Medicinal Chemistry

CHEMICAL COMPOSITION, FREE RADICAL SCAVENGING AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL OF *MARISCUS ALTERNIFOLIUS* VAHL**Patricia A. Onocha, Ganiyat K. Oloyede and Jamiu A. Akintola**

Department of Chemistry, University of Ibadan, Ibadan, Nigeria; E-mail: triciaonocha@yahoo.com



The study of medicinal plants in different parts of the world has led to major breakthroughs in drug chemistry. Essential oils contain interesting natural plant secondary metabolites which have various medicinal applications in ethno medicine. *Mariscus alternifolius* has been reported to be effective in the treatment of wounds, bacterial and infectious diseases in ethno medicine. The increased interest in discovering drugs from natural source led to the investigation of the chemical composition, antioxidant and antimicrobial activities of the essential oil of *M. alternifolius* Vahl.

The plant essential oil was collected over hexane by Hydro distillation technique and Gas Chromatography-Mass Spectrometry (GC-MS) was employed for analysis. Antioxidant activity of the essential oil was evaluated using the 2, 2-diphenylpicrylhydrazyl (DPPH) method while the antimicrobial screening was determined by agar well diffusion method. GC-MS analysis revealed the presence of a total of 10 constituents representing 71.91% with tricosane (19.45%) as the most abundant constituent in the essential oil of *M. alternifolius* Vahl plant while others with relatively high percentage include z-14-nonacosene (13.37%) and octacosyltrifluoroacetate (10.91%). The free radical scavenging activity of the essential oil gave percentage inhibition of 97.95% at 5 mg/ml which was comparable to that of the standard antioxidant ascorbic acid (97.88%) used for the assay. The essential oil inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* used in this study. The minimum inhibitory concentration (MIC) of 25 mg/ml was observed for *Staphylococcus aureus* and *Escherichia coli*.

This study therefore reports the high antimicrobial and antioxidant activities of the essential oil of *M. alternifolius* Vahl and also provide some scientific basis for its utilization in ethno medicine.

Keywords: Hydrodistillation, tricosane, 2, 2-diphenylpicrylhydrazyl, MIC, *Mariscus alternifolius*.

PO-14

Track: Hot Topics in Natural Products

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF SOME SEMI-SYNTHESIZED OLEANOLIC ACID DERIVATIVES**Sibusiso Rali, Opeoluwa O. Oyedeji, Olukaode O. Aremu, Benedicta N. Nkeh-Chungag and Adebola O. Oyedeji**

Department of Chemistry, University of Fort Hare, Alice, South Africa; E-mail: ooyedeji@ufh.ac.za

Oleanolic acid is a pentacyclic triterpenoid compound widely found in plants. It is a well-known compound with multiple medicinal properties. Oleanolic acid (OA) was isolated from ethyl acetate extract of *Syzygium aromaticum* flower buds and further semi-synthesised to acetyl- and methyl-derivatives. The derived compounds were monitored with thin layer chromatography and confirmed with nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), Fourier infrared spectroscopy (FT-IR) and melting point (Mp). All these compounds were evaluated for analgesic and anti-inflammatory properties. OA-derived compounds were administered at a dose of 40 mg/kg to both rats and mice. Significant (p).

In conclusion, semi-synthesis of oleanolic acid resulted in several derivatives, it also enhanced analgesic, anti-inflammatory and solubility properties of the precursor.

Keywords: Analgesic and anti-inflammatory, derivatives, semi-synthesise, oleanolic acid, *Syzygium aromaticum*.

PO-19

Track: In-Silico Drug Design and In-Silico Screening

IN SILICO STUDIES AND EXPERIMENTAL BINDING ASSAYS OF POTENTIAL ANTI-PRION COMPOUNDS REVEAL AN IMPORTANT BINDING SITE FOR PRION INHIBITION FROM PrPC TO PrPSc

Natraj Sekar Pagadala, Rolando Perez-Pineiro, Trent C. Bjorndahl, Ritesh Kaur Saini, Li Li, Ebrima Gibbs, Neil Cashman and David S. Wishart

*Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada;
E-mail: sekhar@ualberta.ca*

It has been reported that ligands with the same predicted binding site to monomeric prion protein (PrPc) exhibit widely variable stabilizing/destabilizing effects. Furthermore, a correlation was observed between the molecules affinity and its ability to reduce PrPsc titers in *ex vivo* studies. Building off this work, we confirmed experimentally the binding site and further developed a pharmacophore model of the stabilizing molecular interactions. Interestingly, we found that the 2-aminothiazole anti-prion compounds docked within this pocket and satisfied many of the favorable stabilizing interactions. Looking for unique inhibitors of prion misfolding, we screened the MOE database of 60,000 compounds using this scaffold and identified 115 compounds with potential anti-prion effects including novel pyrimidine analogs. Six of the compounds were chosen from the list based on their structural diversity and further assessed for their binding affinities and their effectiveness at reducing PrPsc titres in ScN2a cells. All of which all proved effective. These results and the pharmacophore model are presented. This study demonstrates the usefulness of computational methods to do *in silico* screening for potential anti-prion therapeutics with molecular stabilizing effects.

Keywords: PrPC, PrPSc, NMR, docking, virtual screening.



PO-78

Track: Hot Topics in Drug Targets

FREE RADICAL SCAVENGING AND DIFFERENTIAL RESPONSE OF TROXERUTIN TO NORMAL AND CANCER CELLS

Niranjan A. Panat^{1,2}, Dharmendra K. Maurya², Santosh K. Sandur² and Saroj S. Ghaskadbi¹

¹*Department of Zoology, Savitribai Phule Pune University (SPPU), Pune-411007, India;
E-mail: niranjan.panat@gmail.com*

²*Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India*

Troloxerutin (TRX) is a flavonoid commonly present in tea, coffee, cereal grains, various fruits and vegetables has been traditionally used for treating varicose veins, patches of dark brown pigmentation and hemorrhoids. A systematic study was undertaken to evaluate free radical scavenging ability of TRX against important biologically relevant radicals and also response of TRX to normal and cancer cell types using various cellular models. TRX scavenged superoxide, nitric oxide and also the stable radicals like DPPH[•] and ABTS^{•+}. Its interaction with hydroxyl radicals, carbonate, thiocyanate and ABTS^{•+} was monitored by employing pulse radiolysis and stopped flow techniques. TRX formed a stable transient with [•]OH which did not decay up to 600 μs. It offered protection to critical intracellular macromolecules such as DNA, lipids and proteins against oxidative stress (hydroxyl radicals and peroxy radicals) induced single strand breaks, lipid peroxidation and depletion of protein sulphhydryls respectively. TRX protected intestinal epithelial cells (INT 407 cells), human lung fibroblasts (L132 cells) and splenic



lymphocytes against peroxy radical induced apoptosis and mitotic death. It scavenged basal and inducible ROS, attenuated the decrease in MMP and restored the intracellular GSH levels in INT407 cells. In addition, we explored the ability of TRX to interact with DNA. Studies using UV-VIS spectrophotometry with calf thymus DNA revealed a strong binding of TRX to the DNA (binding constant, $k=5.1905 \times 10^4$) which was eventually confirmed by CD spectropolarimetry. Further the mode of interaction of TRX with DNA was found to be at the minor groove of DNA. The putative mode of interaction was studied by docking the TRX molecule on mammalian DNA isoform which supported the possible TRX-DNA interaction at the minor groove. TRX induced the cytotoxicity in radioresistant and sensitive (DU145 and PC3 respectively) prostate cancer cell types and was found to be localized in the nucleus. The cytotoxicity was exacerbated when exposed in combination with γ -radiation. DNA damage and the ROS levels were found to be elevated in TRX exposed DU145 cells and were further aggravated when cells were exposed to γ - radiation in presence of TRX. Our studies on TRX clearly demonstrate that it can differentially act on normal and tumor cells.

Keywords: Antioxidant, CD spectropolarimetry, DNA binding, flavonoid, free radical scavenging, pulse radiolysis, ROS, trolox, rutin.

PO-58-a

Track: Drug Delivery and Targeting

RELATIONS BETWEEN LEPTIN AND THYROID HORMONES METABOLISM IN WHITE ADIPOSE TISSUE

S. Pavelka^{1,2}

¹*Institute of Physiology, Czech Acad. Sci., Prague; E-mail: pavelka@biomed.cas.cz*

²*Institute of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

Leptin is one of the most important signaling molecules secreted by adipose tissue. And adipose tissue is one of the most important targets for thyroid hormones (TH). However, the metabolism of TH in white adipose tissue (WAT) is so far poorly characterized. In the present studies, therefore, we followed possible changes in activities of the key enzymes of TH metabolism in murine WAT under the conditions that promoted either tissue hypertrophy (*i.e.*, under obesogenic treatment) or involution (*i.e.*, under mild caloric restriction), and in response to the administration of hormone leptin. Especially, enzyme activities of iodothyronine deiodinases (IDs) of types 1 (D1), 2 (D2) and 3 (D3) in WAT, brown adipose tissue (BAT) and in the liver (which served as a control material) were measured, using our newly developed radiometric enzyme assays for IDs. We found that D1 enzyme activity in WAT was stimulated by a high-fat-diet feeding, which also increased plasma levels of leptin. Caloric restriction decreased D1 activity in WAT, but not in the liver, and reduced leptin levels. In return, leptin injections increased D1 activity in WAT. In summary, our results demonstrate, for the first time, changes in D1 activity in WAT under the conditions of changing adiposity, and a stimulatory effect of leptin on D1 activity in WAT. We suggest that D1 has a functional role in WAT, possibly being involved in the control of adipose tissue metabolism and/or accumulation of the tissue.

Keywords: Adipose tissue, iodothyronine deiodinases, leptin.



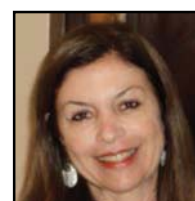
PO-58-b*Track: Drug Delivery and Targeting***THE INFLUENCE OF SUPPLEMENTAL N-3 PUFA IN DIET AND ALTERED THYROID STATUS OF THE RATS ON THEIR LIPID METABOLISM****S. Pavelka^{1,2}**¹*Institute of Physiology, Czech Acad. Sci., Prague; E-mail: pavelka@biomed.cas.cz*²*Institute of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

Marine n-3 polyunsaturated fatty acids (n-3 PUFA) are well known food components recommended for prevention of cardiovascular diseases, atherosclerosis and management of hyperlipidemia. Thyroid hormones (TH) play important roles in lipid metabolism and alteration of thyroid status in animals and humans is associated with changes in serum lipids and lipoproteins concentrations. The results of our previous studies on aged spontaneously hypertensive rats showed benefit from n-3 PUFA supplementation, such as significant decline of blood pressure, suppression of inducible ventricular fibrillation, and improvement of myocardial metabolic state. The aim of the present study was to test how n-3 PUFA supplementation in Lewis male rats with different thyroid status can affect animals' lipid metabolism. Experimentally adjusted hypothyroid, euthyroid, and hyperthyroid status in rats was well defined. In spite of the fact that analyzed parameters such as TH plasma levels, relative heart weight and other biometric data, enzyme activity of liver mitochondrial glycerol-3-phosphate dehydrogenase, and concentrations of plasma lipids were clearly different among particular groups of experimental animals, no significant effect of 6-week-supplementation period of n-3 PUFA (200 mg/kg body weight/day) on the above mentioned parameters was found in this study.

**Keywords:** Lipids, n-3 PUFA, thyroid hormone.**PO-24***Track: Hot Topics in Natural Products***ACTIVITY OF FRACTIONS OF *PIPER UMBELLATUM* HEXANE EXTRACT AGAINST *TRICHOPHYTON RUBRUM*****Rosemeire Cristina Linhari Rodrigues Pietro, Jolindo Alencar Freitas and Rodrigo Sorrechia**

Department of Drugs and Medicines, School of Pharmaceutical Sciences of Araraquara, UNESP – Univ Estadual Paulista, São Paulo State University, Araraquara, Brazil;
E-mail: pietrorc@fcfar.unesp.br

There is an emergent need to develop antifungal drugs with new chemical structures and novel mechanism of action. *Piper umbellatum* (L.) Miq. belonging to Piperaceae family is used in folk medicine for a wide range of ailments such as diarrhea, skin infections, malaria, intestinal parasites and inflammation. In phytochemistry studies was demonstrated the presence of steroids, 4-nerolidylcatechol, sesquiterpenes and essential oils. The objective of this study was to obtain an initial screening of antifungal activity against a clinical isolate of *Trichophyton rubrum* of fractions of *P. umbellatum* hexane extract. *P. umbellatum* powered leaves (200g) were extracted with hexane (2 L). Crude hexane extract (185 mg) chromatographed on silica gel column, was eluted with Hexane/Ethyl Acetate (15 fractions). The minimum inhibitory concentration (MIC) was performed by broth microdilution according to approved standard CLSI M38-A2. The sample range concentration was 1250-9.76 µg/mL. Crude extract MIC was 1250 µg/mL and MIC fractions were 78.1, 312.5, 156.25 and 312.5 µg/mL for F1, F4, F8 and F13, respectively. The results obtained show best activity for Fraction 1 (F1), which presented higher concentration of 4NC in TLC. Therefore, this study is a first step for the isolation and identification of compounds present in the hexane extract.



Support: FAPESP, CNPQ.

PO-13

Track: Hot Topics in HIV Research

EFFECTS OF MELATONIN ON MICE TREATED WITH ANTIRETROVIRAL THERAPY

Áurea Regina Telles Pupulin, Julia B Barbosa and Miguel Spack Jr

Department of Basic Health Sciences, State University of Maringa, Maringa, Brazil; E-mail: artupupulin@uem.br

Introduction: Highly Active Antiretroviral Therapy (HAART) is associated with metabolic complications. Melatonin, the neuro-hormone synthesized during the night, has seen an unexpected extension of its functional implications toward type 2 diabetes development, sleep disturbances and depression. Melatonin has been shown to reduce the toxicity and increase the efficacy of a large number of drugs.



Objective: Current study evaluated the effect of Melatonin on mice treated with antiretroviral therapy.

Material and Methods: Animals were divided into experimental groups consisting of 12 animals each as: (I) animals untreated, (II) animals treated with antiretroviral therapy for 15 days, (III) animals treated with antiretroviral therapy and melatonin 6 mg/kg/day for 15 days. Clinical evaluation (body weight, water intake and ration, excretion products, behavior) was performed before and after treatment and the serum cholesterol, triglycerides, hepatic enzymes (AST, ALT, GGT), creatinine, were assessed by specific methods. Results were analyzed with GraphPad Prism using Student's t test.

Results: Animals treated with antiretroviral therapy and melatonin (III) had higher body weight gain, less hepatomegaly, less anxiety, lower levels of triglycerides, cholesterol and hepatic enzymes when compared to animals treated with antiretroviral therapy.

Conclusion: Considering the low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of drugs its use as a combination therapy with antiretroviral therapy seems important and worthy of pursuit.

Keywords: Metabolic abnormalities, HAART, Melatonin.

PO-106

Track: Drug Delivery & Targeting

FORMULATION AND *IN VITRO* EVALUATION OF CONTROLLED RELEASE MATRIX TABLETS OF DILTIAZEM HYDROCHLORIDE USING DIFFERENT RATE CONTROLLING POLYMERS

Ghulam Razaque, Gul Majid, Zeeshan Danish, Nisar Ahmed, Kamran Ahmad, Junaid Qureshi, Tahmina Rabbani and Mohammad Younis

Department of Pharmacy, University of the Punjab, College of Pharmacy Allam Iqbal Campus, Lahore, Pakistan; E-mail: ppharmacist@hotmail.com

In present study, directly compressed controlled released matrix tablets of Diltiazem Hydrochloride was formulated at drug-to-polymer ratios (D: P) of 10:1, 10:2, 10:3 using polymers Ethocel 45 Premium, K100 LV Methocel, K15M EP Premium Methocel as rate controlling agents. In some formulations of Diltiazem hydrochloride and Ethocel 45 Premium matrices, 30% of filler was replaced by Co-excipient like HPMC, CMC-Na and Starch. The *in vitro* dissolution studies were performed according to USP Method-I (rotating basket method). Phosphate buffer (PH 7.4) was used as dissolution medium and the rotation speed of basket were 100rpm and temperature of the medium was maintained at $37 \pm 0.10^\circ\text{C}$. In order to determine the drug release kinetics various models such as Ist-order, Zero-order, Hixon Crowell, Higuchi and Power Law were applied. Herbesser® tablets were used as reference standard for comparison of dissolution profiles of standard and tests formulation by applying similarity factor (f_2) and difference factor (f_1). The rate controlling agents

extended the drug release rates but Ethocel 45 Premium extended more efficiently than the K100 LV Methocel and K15M EP Premium Methocel containing matrices. These newly prepared formulations released the drug by anomalous non Fickian drug diffusion. The test formulations drug release profiles were different from the release profile of reference standard formulation (Herbesser® tablets). The Co-excipients increased the drug released from the matrices containing Ethocel 45 premiums. Ethocel 45 Premium, K100 LV Methocel and K15M EP Premium Methocel can be effectively used as rate controlling agents in formulation of directly controlled release matrix tablets.

Keywords: Diltiazem Hydrochloride, Ethocel 45 Premium, K100 LV Methocel, K15M EP Premium Methocel, Co-excipients.

PO-99

Track: Hot Topics in Natural Products

PHYSALIS ANGULATA L. CALYCES AS A PROMISING NATURAL SOURCE OF POTENTIAL ANTI-INFLAMMATORY COMPOUNDS

D.E. Rivera¹, Y.C. Ocampo¹, J.P. Castro¹ and L.A. Franco¹

¹Biological Evaluation of Promissory Substances Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia.

Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia; E-mail: david.river8@gmail.com

Physalis angulata is a widely studied plant, with several bioactive components already identified; nevertheless, despite the use of its calyces to treat inflammation in Latin American countries, this organ has been poorly studied regarding this biological activity. In this work, we evaluated the anti-inflammatory potential of the total ethanolic extract from the calyces of *P. angulata* and its fractions in petroleum ether, dichloromethane and methanol; using NO• production on LPS-stimulated RAW264.7 macrophages and the TPA-induced ear edema *in vivo* model. The most active fraction was evaluated regarding its effect over the *in vitro* production pro-inflammatory mediators using ELISA. The total extract significantly inhibited the NO• production on RAW264.7 (IC₅₀=4.063 µg/mL). Consistently, it reduced the inflammation (40%) *in vivo* with improvement of histology score, and MPO activity reduction (29.24%). The dichloromethane fraction was the most active, inhibiting the inflammation by 61.78% and MPO activity by 71.52%. *In vitro* this fraction inhibited the NO• production (IC₅₀=2.48 µg/mL) and reduced the levels of the pro-inflammatory mediators PGE2, IL-1β, IL-6, TNF-α and MCP-1 (IC₅₀<20 µg/mL). These results support the traditional use of the calyces of *P. angulata* to treat inflammatory diseases and place them as a promissory source of new potential pharmacological alternatives.

PO-49

Track: Drug Discovery in Preclinical Research

DISCOVERY OF NOVEL ANTICONVULSANT ACTIVE GABA REUPTAKE INHIBITORS

Kinga Salat¹, Adrian Podkowa¹, Anna Rapacz¹, Paula Zaręba² and Katarzyna Kulig²

¹Department of Pharmacodynamics, Jagiellonian University, Faculty of Pharmacy, Medyczna 9 Krakow, Poland; E-mail: salat.kinga@gmail.com

²Department of Physicochemical Drug Analysis, Jagiellonian University, Faculty of Pharmacy, Medyczna 9 Krakow, Poland

Background: Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system. GABAergic neurotransmission is involved in anticonvulsant effect of antiepileptic drugs and the inhibition of plasma membrane GABA transporters (GAT) involved in its re-uptake enhances GABAergic neurotransmission, which is expected to underlie anticonvulsant activity.



Methods: Five novel derivatives of 4-aminobutanoic acid (GT97, GT98, GT104, GT112, GT123 and GT126) with micromolar affinity for mouse GAT were tested for their protective properties and compared to tiagabine, a selective GAT1 inhibitor in mouse models of seizures induced by pilocarpine, pentylenetetrazole (PTZ) and electroconvulsions (ECT).

Results: In the pilocarpine-induced seizure test the most prominent action was observed for GT104 (100 mg/kg) which prolonged latency time to status epilepticus by 309% ($p < 0.01$) vs the control group. The same compound exerted strong anticonvulsant action in the PTZ, prolonging latency to first clonus, as well as reducing the number of seizures. Similarly, tiagabine (50 mg/kg) and GT123 (100 mg/kg) caused a significant prolongation of latency time to status epilepticus in pilocarpine-induced seizures. In PTZ test strong anticonvulsant properties of tiagabine (dose range: 6.25-100 mg/kg; $p < 0.01$) and GT97 (7.5-100 mg/kg; $p < 0.01$) were shown. In ECT test GT112 (100 mg/kg) elevated the threshold for electroconvulsions by 90% ($p < 0.001$) in comparison to the control group.

Conclusion: The test compounds showed high anticonvulsant activity in rodent models of seizures. The inhibition of mouse GAT1, 2 and 4 subtypes contributes to the anticonvulsant effect observed *in vivo*.

This work was financially supported by DEC-2012/05/B/NZ7/02705.

PO-62

Track: Pharmaceutical Research & Development

ACTIVE SITE MAPPING OF HUMAN CATHEPSIN F WITH DIPEPTIDE NITRILE INHIBITORS

J. Schmitz^{1,2}, N. Furtmann^{1,3}, M. Ponert¹, U. Bartz², J. Bajorath³ and M. Gütschow¹

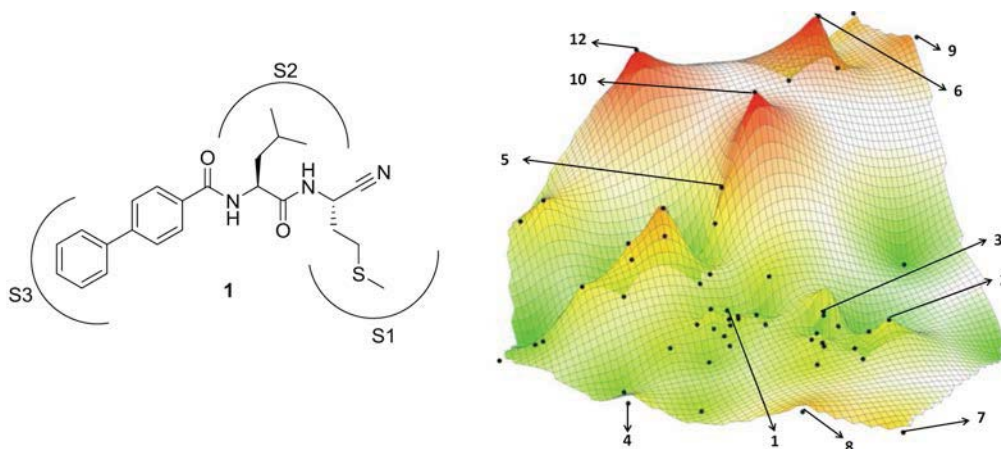
¹University of Bonn, Pharmaceutical Institute, An der Immenburg 4, 53121 Bonn, Germany;
E-mail: janina.schmitz@uni-bonn.de

²Bonn-Rhein-Sieg University of Applied Sciences, von-Liebig-Str. 20, 53359 Rheinbach, Germany

³Department of Life Science Informatics, B-IT, Dahlmannstr. 2, 53113 Bonn, Germany



Cysteine cathepsins are lysosomal cysteine proteases which play roles in many physiological processes. Cathepsin F is predominantly expressed in macrophages. Major histocompatibility complex class II molecules (MHC-II) are expressed by antigen-presenting cell types including macrophages, B cells, and dendritic cells. The cleavage of the invariant chain is the key event in the pathway of MHC-II complexes. Cathepsin S was described as the major processing enzyme of the invariant chain, but it was shown that cathepsin F can adopt its role in cathepsin S deficient mice [1]. Low molecular weight inhibitors for cathepsin F have not been investigated so far. We have chosen the dipeptide nitrile [2] chemotype to develop covalent-reversible inhibitors for this target.



An active site mapping with a library of 52 nitrile-based cathepsin inhibitors was performed at human cathepsin F to draw structure-activity relationships. With the kinetic data in hand, new compounds with optimized residues in P1, P2 and P3 position were synthesized and evaluated. Compound **1** (left) represents such an optimized inhibitor of cathepsin F. With all dipeptide nitriles including the newly synthesized derivatives, a 3D activity landscape was generated to visualize similarity-activity relationships of this series of cathepsin F inhibitors (right).

PO-11

Track: Drug Discovery in Preclinical Research

NOVEL METHOD IN SELECTION OF PRIMARY SETS LEADS TO IDENTIFICATION OF POTENT PIM-1 KINASE INHIBITORS USING LIGAND BASED DRUG DESIGN COMBINED WITH QSAR ANALYSIS TECHNIQUES AND VIRTUAL SCREENING

Rand Omar Shahin

Faculty of Pharmacy, Zarqa Private University, Zarqa, Jordan; E-mail: rshahin@zu.edu.jo

Targeting Human phosphatidylinositol mannoside kinase-1 (PIM-1 kinase) by potent inhibitors is a promising strategy for treating hematopoietic and solid tumor cancers. Headed for this, a total list of 328 previously reported Pim-1 kinase inhibitors has been explored and divided based on the pharmacophoric features of the most active compounds into 10 subsets projected to represent potential binding modes accessible to ligands in the binding pocket of pim-1 kinase. Afterwards CATALYST-HYPOGEN has been employed to identify possible pharmacophoric binding modes assumed by Pim-1 kinase inhibitors. The pharmacophoric models were subsequently allowed to compete within Quantitative Structure-Activity Relationship (QSAR) context. Towards this end, genetic algorithm and multiple linear regression analysis were employed to select an optimal combination of pharmacophoric models and 2D physicochemical descriptors capable of accessing self-consistent QSAR of optimal predictive potential ($r^2_{262} = 0.70$, $F = 119.14$, $r^2_{LOO} = 0.693$, r^2_{PRESS} against 66 external test inhibitors = 0.71). Three orthogonal pharmacophores emerged in the QSAR equation suggesting the existence of at least three binding modes accessible to ligands within Pim-1 kinase binding pocket. Receiver operating characteristic (ROC) curve analyses established the validity of QSAR-selected pharmacophores. Moreover, the successful pharmacophores models were found to be comparable with crystallographically resolved Pim-1 kinase binding pocket. We employed the pharmacophoric models and associated QSAR equation to screen the national cancer institute (NCI) list of compounds several submicromolar Pim-1 kinase inhibitors were identified.

Keywords: Pim-1 kinase, QSAR, Discovery Studio.

PO-43

Track: Hot Topics in Medicinal Chemistry

DIRUTHENIUM-IBUPROFEN MODIFIED METALLODRUGS INVESTIGATED FOR THE ANTICANCER ACTIVITY IN HUMAN GLIOMA CELLS

Hanif-Ur-Rehman¹, Tatiana E. Freitas², Alison Colquhoun² and Denise de Oliveira Silva¹

¹*Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, São Paulo, São Paulo, Brazil; E-mail: deosilva@iq.usp.br*

²*Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, São Paulo, Brazil*

The development of new chemotherapeutic drugs to treat glioma is a clinically recognized urgent need. Glioblastoma multiforme (GBM, WHO grade IV), the most aggressive primary brain tumor,



is responsible for more 50% adult brain cancer cases. Conventional treatments do not allow more than one year patient average survival after surgical resection. Our group has recently reported a novel metallodrug of ruthenium containing the non-steroidal anti-inflammatory drug ibuprofen, which was shown to exert antiproliferative activity in glioma models *in vitro* (in rat and in human glioma cells), and also *in vivo* (rat C6 orthotopic glioma). Here we extend the previous studies by investigating the effects of structurally modified diruthenium-ibuprofen metallodrugs on the proliferation of human glioma cells. Three structurally modified compounds, of the formulas $[\text{Ru}_2(\text{Ibp})_4\text{Cl}]$, $[\text{Ru}_2(\text{Ibp})_4(\text{H}_2\text{O})_2]\text{PF}_6$ and $[\text{Ru}_2(\text{Ibp})_4\text{OTf}]$, where Ibp = ibuprofenate and OTf = trifluoromethanesulfonate, all of them containing the $[\text{Ru}_2(\text{Ibp})_4]^+$ diruthenium-ibuprofen paddlewheel unit, were prepared, characterized, and tested for the antiproliferative activity in the U87 MG human glioma cell line. The effects of the compounds on apoptosis and mitosis processes, as well as the cell uptake of metal, were also investigated. The studies showed distinct behaviors for the three compounds, with interesting findings that point towards the possibility of tuning the anticancer activity in glioma models by modifying the diruthenium-ibuprofen metallodrug structure. (Financial support from FAPESP, CNPq and CAPES is gratefully acknowledged).

Keywords: Ruthenium metallodrugs, anticancer drugs, glioma.

PO-6

Track: Chemistry

SYNTHESIS AND BIOLOGICAL EVALUATION OF DIHYDROARTEMISINYL-CHALCONE ESTERS

Riëtte A. van Biljon, Lyn-Marie Birkholtz, David D. N'Da and Frans Johannes Smit

Pharmaceutical Chemistry, North West University, Potchefstroom, South Africa; E-mail: 20926588@nwu.ac.za

A series of dihydroartemisinin-chalcone esters were synthesized through esterification of chalcones with dihydroartemisinin (DHA). The hybrids were screened against chloroquine (CQ) sensitive (3D7) and CQ resistant (W2) strains of intraerythrocytic *Plasmodium falciparum* parasites, and were all found to be active, with IC₅₀ values ranging between 1.5 - 11 nM against both strains. The esters (7, 10 and 11) featuring oxygenated aryl rings, were found to be equipotent to DHA, but were 2 to 3 times more active than artesunate against the 3D7 and W2 strains of the malaria parasites. They were also screened *in vitro* against a panel of three cancer cell lines consisting of TK-10, UACC-62 and MCF-7. Compound 7, bearing a furan ring, displayed the most potent overall antitumor activity against all three cancer cell lines. During this study, ester 7 was identified as the best candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs.

Keywords: Malaria, *Plasmodium falciparum*, DHA, chalcone, antitumor agents.

PO-80

Track: Drug Delivery

SELECTIVE AND COMPETITIVE MONOAMINE OXIDASE-B INHIBITION BY THE FLAVANONE BAVACHININ

Najla O. Zarmouh, Elizabeth A. Mazzio, Faisel M. Elshami, Samia S. Messeha, Suresh V. K. Eyunni and Karam F.A. Soliman

College of Pharmacy & Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL, USA;
E-mail: karam.soliman@famu.edu

Psoralea corylifolia (PC) is a medicinal plant proposed to have multiple therapeutic activities on the dopaminergic function. PC seeds ethanolic extract (PCSEE) showed potent and selective recombinant human MAO-B (*h*MAO-B)

inhibitory effects that can increase dopamine levels in Parkinson's disease (PD). Furthermore, two of its inherent flavanones, bavachinin (BNN) and its analog bavachin (BVN), were investigated for *h*MAO-A and B inhibition potential. While BVN was not active, BNN inhibited *h*MAO-B selectively with an IC_{50} of 8.82 μ M to be lower than *h*MAO-A IC_{50} by 38.42 folds. The inhibition of BNN was competitive to both *h*MAO-A and B, with a lower *h*MAO-B K_i than *h*MAO-A K_i . BNN and *h*MAO-B K_m/V_{max} ratio results indicated *h*MAO-B efficiency to be reduced more than selegiline. BNN and BVN, docking studies predicted a role of BNN C7-OMe substitution for its higher affinity, and reversibility as an MAO-B selective inhibitor. In PCSEE, BNN was detected with a yield of 0.21% per dry weight indicating BNN significant role in *h*MAO-B selectivity of the extract. These findings indicate that BNN competitive MAO-B inhibition may provide a potential alternative in the therapeutic management of PD. (Supported by NIH grants from NIMHD G12 MD007582 & P20 MD0067).

PO-42

Track: Hot Topics in Natural Products

INDUCED PRODUCTION OF HALOGENATED BIOACTIVE COMPOUND BY THE MARINE-DERIVED BACTERIA AND FUNGI

Byeng W. Son and Keumja Yun

Department of Chemistry, Pukyong National University, Nam-gu, Busan, South Korea; E-mail: sonbw@pknu.ac.kr

Marine-derived microorganisms continue to attract attention as a rich source of structurally novel bioactive metabolites that are potential lead compounds for the development of new drugs [1].

When the marine-derived microorganisms were cultured under saline condition, they rarely produced interesting biological halogenated metabolites (*e.g.*, salinosporamide A3 of a highly potent inhibitor of the 20S proteasome and its halogenated derivatives) [2].

Encouraged by the detection of halogenated marine analogs, we manipulated the fermentation of *Aspergillus* sp. by the addition of metal halides in an effort to gain access to a wider cross-section of halogenated secondary metabolites.

This presentation describes the production, isolation, and identification of halogenated bioactive compounds [3].

We also show the biological activity of new compounds.

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PO-67

Track: Drug Delivery and Targeting

MICROBIAL BROMINATION OF POLYKETIDE BY THE MARINE-DERIVED FUNGUS ASPERGILLUS SP.

Byeng W. Son and Keumja Yun

Department of Chemistry, Pukyong National University, Nam-gu, Busan, South Korea; E-mail: sonbw@pknu.ac.kr

Recently, increased interest in the chemistry of microorganisms isolated from the marine environment has been documented. Marine-derived microorganisms are interesting from an ecological point of view, because they are serious

pathogens in the marine environment. Furthermore, since many can be cultured, they represent an important biomedical resource. During our studies on the chemistry and biology of the marine-derived microorganisms, we have investigated a marine strain of the fungus *Aspergillus* sp.

The addition of metal bromides to the fermentation of the fungus *Aspergillus* sp. resulted in induced production of two new brominated polyketides, methyl dibromodihydroxyphenylacetates.

This presentation describes the production, isolation, and identification of two new metabolites.

We will also show the radical-scavenging activity of these compounds.

PO-55

Track: Anti-Infectives

A NEW CLASS OF POTENT ANTIBACTERIAL AGENTS – 1,4 DISUBSTITUTED THIOSEMICARBAZIDE DERIVATIVES

Aleksandra Strzelczyk¹, Agata Paneth², Magdalena Frysiak¹, Marlena Rasiuk¹ and Paweł Stączek¹

¹*Department of Microbial Genetics, University of Lodz, Lodz, Poland;
E-mail: ola.s@biol.uni.lodz.pl*

²*Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland*

Due to increasing antibiotic resistance of bacterial pathogens, synthesis of new, active chemotherapeutics is one of the main challenges for current medicinal chemistry. Our novel 1,4-disubstituted thiosemicarbazide derivatives are small molecules which are synthesized in the reaction of heterocarboxylic acid hydrazide with a required isothiocyanate.

Antibacterial activity against set of reference Gram-positive and Gram-negative strains was determined by using the microdilution method and the MIC and MBC values were estimated. Considering strong antibacterial activity mainly against *Staphylococcus aureus* (MIC<100 µg/mL), ten of tested compounds were used in research against 12 clinical strains of *S. aureus* and *M. tuberculosis*. Virtually all thiosemicarbazide derivatives had strong antibacterial activity ranging from 16-64 µg/mL. The MIC value in some cases was more than four times lower than control antibiotics; ampicillin and vancomycin. However, all compounds showed bacteriostatic activity rather than bactericidal (MBC>4MIC). The molecular docking and enzymatic studies revealed that the most possible mechanism of action of some derivatives is inhibition of type II topoisomerases in bacterial cells. The microscopic examination of fluorescently tagged replisome protein confirmed that bacteriostatic activity of thiosemicarbazide derivatives is associated with replication process.

In conclusion, evaluation of antibacterial activity of 1,4-disubstituted thiosemicarbazide showed very promising and significant results compared to control antibiotics which makes them good candidates for the development of new drugs mainly against multidrug resistant *S. aureus* and *M. tuberculosis* strains.

This work was supported by National Center of Science under PRELUDIUM 6 grant DEC-2013/11/N/NZ7/00765.

Keywords: Antibiotics, thiosemicarbazides, topoisomerase inhibitors.



PO-54

Track: Drug Delivery and Targeting

DETERMINATION OF MOLECULAR TARGET AND BIOLOGICAL ACTIVITY OF 1,2,4-TRIAZOLE - CIPROFLOXACIN HYBRIDS

Aleksandra Strzelczyk¹, Barbara Kaproń² and Tomasz Plech²

¹Department of Microbial Genetics, University of Lodz, Lodz, Poland;
E-mail: ola.s@biol.uni.lodz.pl

²Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Lublin, Poland

Chemotherapeutics from the fluoroquinolones group are characterized by a broad-spectrum of antibacterial effects however, their usage is currently limited due to progressive bacterial resistance.

The main purpose of our investigations was to obtain derivatives of ciprofloxacin showing considerable activity against both Gram-negative and Gram-positive bacteria, and to find the possible structure-activity relationships connected with inhibitory property of type II topoisomerases. The set of new hybrids containing ciprofloxacin moiety and 1,2,4-triazole derivatives was prepared by using the so-called Mannich reaction. The antimicrobial activity of the compounds was determined, by broth microdilution method, using Gram-negative as well as Gram-positive bacteria. The inhibitory activity against topoisomerases was performed *in vitro* using appropriate gyrase and topoisomerase IV activity assays.

The large majority of the synthesized hybrids possessed significant antibacterial activity that was several times higher as compared to the reference drugs (MIC values ranged between 0.01 and 0.4 μ M). They also displayed a strong inhibitory activity against *E. coli* gyrase (IC₅₀ = 1, 2-3, 5 μ M) and *S. aureus* topoisomerase IV (IC₅₀ = 15, 5-20 μ M).

The presented results revealed that our novel 1,2,4 – triazole – ciprofloxacin conjugates show improved antibacterial activity in comparison to ciprofloxacin which makes them good candidates to become an alternative to commonly used antibiotic.

This research was supported by the Polish Ministry of Science and Higher Education under Iuventus Plus grant no. IP2014 037473

Keywords: 1,2,4-triazole-3-thiones, fluoroquinolones, topoisomerase inhibitors.

**PO-22**

Track: Neurodegenerative Disorders

MILD CONCUSSIVE- AND BLAST-TRAUMATIC BRAIN INJURY (TBI) IN MICE: MODELS FOR DRUG DEVELOPMENT

Ian Tamargo¹, David Tweedie¹, Lital Rachmany², Vardit Rubovitch², Kumar Sambamurti³, Debomoy K. Lahiri⁴, Barry J. Hoffer⁵, Chaim G. Pick² and Nigel H. Greig¹

¹Intramural Research Program, National Institute on Aging, Baltimore, MD 21224, USA; E-mail: ian.tamargo@nih.gov

²Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, 69978 Israel

³Medical University of South Carolina, Charleston, SC 29425, USA

⁴Indiana University School of Medicine, Indianapolis, IN 46202, USA

⁵Case Western Reserve University, Cleveland, Ohio 44106, USA



Pharmacological treatment of TBI represents an unmet medical need, as no effective medication currently exists. Successful drug development requires a fundamental understanding of the pathophysiological mechanisms that underlie sequelae resulting from TBI, particularly the ensuing neuronal cell death and cognitive impairments. To aid in this endeavor two distinct types of mild (m)TBI were evaluated in anesthetized mice: a concussive closed-head weight (30g) drop, representative of a common fall, and a blast shock-wave generated by detonation of an explosive device (500g TNT), emulating blast-mTBI common to warfare. Whereas both are different regarding mechanisms of trauma induction, there are striking similarities in the cognitive and emotional status of survivors. Indices of cognition and the hippocampal gene transcriptome of mice subjected to these mTBIs were evaluated. We identified common behavioral deficits and gene expression regulations, in addition to unique injury-specific forms of gene regulation. Molecular pathways presented a pattern similar to that of gene expression. Interestingly, pathways associated with Alzheimer's disease displayed a markedly different regulation depending on the TBI. Similarities in behavioral outcomes and divergence in hippocampal transcriptome suggest that these two TBIs are different at the molecular level, and provide models to support drug development that will be discussed.

PO-74

Track: Innovative Drug Discovery and Nanotechnology

DEVELOPMENT OF PROTEIN-POLYMER CORE-SHELL NANOPARTICLES (PPCS-NPS) AS EFFICIENT VEHICLES TO DELIVER THERAPEUTIC AGENTS ACROSS BLOOD BRAIN BARRIER (BBB)

Napat Tandikul and Qian Wang

*Department of Chemistry and Biochemistry, University of South Carolina, Columbia, USA;
E-mail: tandikul@email.sc.edu*

Blood Brain Barrier (BBB) plays main role as selective barrier which controls and limits access of chemicals, molecules and therapeutic agents from blood to brain. The BBB endothelial cells are connected by Tight Junctions (TJs) which close intracellular spaces between the endothelial cells and block the free diffusion of substances, therefore many potential drugs for treating human brain diseases cannot reach the brain in sufficient concentration. Recently, many studies have thrown an interest in development of nanoparticles for delivering drugs and imaging agents across BBB. Our research group has developed protein-polymer core-shell nanoparticles (PPCS-NPs) which demonstrate great potential for targeted delivery. In this work, Apolipoprotein E3 (ApoE3), which can be specifically bound to LDLR receptor on BBB endothelial cells, was chosen as targeted motif. Nanoparticles conjugated with ApoE3 and fluorescently labelled ApoE3 (Fl-ApoE3) were successfully synthesized. The synthesis of ApoE3/ Fl-ApoE3 NPs with encapsulation of drugs and dyes is in progress. *In vitro* study of the uptake of ApoE3-NPs, Fl-ApoE3-NPs with and without encapsulation of drugs and dyes will be further investigated by using human umbilical vein endothelial cells (HUVECs) and brain microvascular endothelial cell line (hCMEC/D3) as BBB endothelial cell model.

Keywords: Nanoparticles, Blood brain barrier.



PO-72

Track: Hot Topics in Natural Products

ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF *EMBLICA OFFICINALIS* IN EXPERIMENTAL ANIMALS

Isabella Topno and Jesudoss Prabhakaran

Department of Pharmacology, Pondicherry Institute of Medical Sciences, Pondicherry, India;
E-mail: isabellatopno30@yahoo.co.in



Aim: To evaluate the analgesic and anti-inflammatory effect of *Embllica officinalis* in experimental animals.

Materials and Methods: Peripheral analgesic activity of *Embllica officinalis* (EO) was evaluated using acetic acid induced writhing test. Indomethacin (10 mg/kg, p.o) was used as the standard drug and EO was used at a dose of 300 mg/kg, p.o. Central anti-nociceptive activity of EO was evaluated using eddy's hot plate method. Morphine (5 mg/kg, i.p) was used as the standard drug and EO was used at a dose of 300 mg/kg, p.o. To assess the role of opioid system, the other groups of animals were pretreated with μ opioid receptor antagonist (CTAP-1mg/kg, i.p), δ opioid receptor antagonist (Naltrindole-1 mg/kg,i.p) and κ opioid receptor antagonist (Nor-Binaltorphimine-1mg/kg,i.p) and then treated with vehicle/*Embllica officinalis*. Carrageenan induced hind paw edema in rats was carried out to detect the anti-inflammatory activity of EO extract, using mercury plethysmometer. The rats were divided into nine groups of six in each group. The control group received distilled water at a volume of 5 ml/kg p.o. Indomethacin (10 mg/kg, p.o.), Celecoxib (10 mg/kg, p.o.) and Chlorpheniramine (10 mg/kg i.p.) were used as the standard drugs. EO extract was administered at a dose of 150, 300 and 600 mg/kg p.o. This experiment was carried out with misoprostol to detect the mechanism of action where one group was administered with misoprostol (11.43 μ g/kg p.o.) and was compared with the other group where misoprostol was administered one hr after the administration of EO extract.

Results: Results were analysed using one way ANOVA followed by Tukey's multiple comparison test. p value of <0.05 was considered significant. *Embllica officinalis* increased the latency time significantly (p<0.05) when compared to control group in hot plate model. Further, administration of CTAP prior to EO reversed the anti-nociceptive effect significantly (p<0.001) when compared to EO group. The peripheral analgesic effect of EO extract and standard drug (Indomethacin) in acetic acid induced writhing test. EO extract showed significant analgesic activity at a dose of 300 mg/kg. The number of acetic acid induced writhes decreased significantly (p<0.001), when compared to control group with inhibitory rate of 52.4%. The standard drug, indomethacin (10 mg/kg), also showed significant analgesic activity (p<0.001), when compared to control group with the inhibitory rate of 58.4%.

So the peripheral analgesic activity of EO extract is comparable to that of the standard drug indomethacin.

Conclusion: *Embllica officinalis* possesses analgesic and anti-inflammatory effects in both central and peripheral models of nociception. The central anti-nociceptive activity is probably mediated *via* μ opioid receptor.

PO-46

Track: CNS Drug Discovery & Therapy

HYPOTHALAMIC PHOSPHOLIPIDS (LIPOSOMES FORTE) AND PLATELET-RICH PLASMA IN THE REHABILITATION OF PATIENTS WITH SPINAL CORD INJURY

Saule T. Turuspekova, Anatoliy Ostapenko, Dmitry Mitrokhin, Bakhytzhaz Gaziz, Erlan Kudabaev, Amina Seydanova and Elmira Moldakulova

Department of Internship and Residency in Neurology, Kazakh National Medical University Named After S.D. Asfendiyarov, Almaty, Kazakhstan; E-mail: doctorsaule@mail.ru



Over the past decade, with encouraging results in the treatment method of PRP therapy, the post effects of sports injuries. There is also an evidence that different classes of lipids can significantly improve the

formation and activity of neural networks. The effect of phospholipids liposomes and the method of PRP therapy on the body's regenerative processes in the spinal cord injury (PSMT) is unknown. The present study aims to evaluate the effectiveness of liposome forte and PRP therapy in a rehabilitation program of the patients with PSMT.

There were patients with PSMT, lower paraparesis, conduction disorders of sensitivity, dysfunction of the pelvic organs. We used the scale of the Committee on Medical Research (ISC), the evaluation of muscle strength (for L. McPeak), profile-based questionnaire Recovery locus of control (RLoC).

To the patients of the main group further conducted 5-7 seances of PRP therapy and hypothalamic phospholipid injection for 2 weeks. Indicators show an improvement of motor function by 1.6 points compared to the base period. The results of this study showed a high efficiency of rehabilitation of patients with PSMT in the early recovery period against application of phospholipids of hypothalamus and PRP therapy.

Keywords: Hypothalamic phospholipids (liposomes forte), platelet-rich plasma, the rehabilitation, the spinal cord injury.

PO-60

Track: Pharmaceutical Analysis

HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF SUMATRIPTAN SUCCINATE IN RAT PLASMA

Siddu Bhagawat, Krishnamurthy Baht and S.G. Vasantharaju

Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India; E-mail: sg.vasantharaj@manipal.edu

Sumatriptan succinate was estimated satisfactorily in rat plasma using a validated high-performance liquid chromatographic (HPLC) method with fluorescence detection. Naratriptan was used as an internal standard (IS). The sumatriptan was extracted by liquid-liquid extraction method using methyl tertiarybutyl ether and ethyl acetate mixture (7:3). The analytes were separated using a mobile phase consisting 20 mM phosphate buffer pH 3.0 adjusted with orthophosphoric acid and acetonitrile (88:12 v/v). The flow rate was set at 1.0 mL/min, and the total run time was 18 min. The Hyper C18column (5 µm particle size, 4.6mm×250mm i.d.) was used and maintained at 25 °C. Fluorescence detection was performed at an excitation/emission wavelength of 225/360 nm. The linear calibration curve was plotted in the range from 05 to 200 ng/mL with correlation coefficients of 0.989. The precisions (intra- and inter-day) of the method were not more than 8.0%. The stability study was carried out at -70 °C for seven days and sumatriptan was found to be stable. The proposed method was found to be accurate, precise, robust and specific during the study.



PO-102

Track: Cardiovascular Drug Discovery & Therapy

SULFATED COUMARINS AS POTENT, ALLOSTERIC REGULATORS OF THROMBIN ACTIVITY

Stephen Verespy III, Akul Y. Mehta, Daniel Afosah, Rami A. Al-Horani and Umesh R. Desai

Department of Medicinal Chemistry and Institute for Structural Biology, Virginia Commonwealth University, Richmond, USA; E-mail: verespys@vcu.edu

Utilizing allosterism to regulate hemostasis in thrombotic disorders has the profound advantage of inducing slight conformational changes to the tertiary structure of the target enzyme, thereby altering the orthosteric site to effect a

change in recognition of substrates. This pathway of regulation can ultimately cause enzymes, like thrombin, to display partial activity, which results in depressed catalysis, thus retaining the essential property of hemostasis without the danger of thrombosis. To assess the likelihood of this phenomenon, a small library of coumarin-based sulfated allosteric modulators (C-SAMs) were synthesized and screened against thrombin, FXa, and FXIa to investigate the potential of regulating thrombotic activity by targeting the heparin binding domains (HBDs) of these coagulation enzymes. Several of the compounds were found to possess a variety of potencies and selectivity against the three enzymes. Of the 36 compounds screened, 3g was shown to be at least 200-fold more selective for thrombin with an IC₅₀ of 187 nM and a K_D of 52 nM. Notably with regard to regulation, 3g displayed ~50% efficacy (ΔY) against thrombin in the presence of a small peptide chromogenic substrate. The allosteric nature of the interaction was evaluated and supported by Michaelis-Menten kinetics and exosite competition studies. Plasma clotting assays also identified 3g as the most potent sulfated non-saccharide glycosaminoglycan mimetic discovered. The results presented herein describe 3g as the closest true small molecule allosteric regulator of thrombin to date. Further studies will probe the interactions suggested and lead to 3g derivatives for clinical viability.

Keywords: Allosterism, hemostasis, C-SAMs, HBDs, thrombin.

PO-71

Track: Medical Imaging

CLINICAL STUDY ON TC-99M-MDP WHOLE BODY BONE SCAN COMBINED WITH MRI ON THE DIAGNOSTIC VALUE OF BONE METASTASIS

Rong Fu Wang¹ and An Hui Zhu^{1,2}

¹Department of Nuclear Medicine, Peking University First Hospital, Beijing 100034, China;
E-mail: rongfu_wang@163.com

²Department of Nuclear Medicine, Space Center Hospital, Beijing 100049, China

Objective: The aim of the study is to evaluate diagnostic value of Tc-99m-MDP whole body bone scan combined with MRI on the bone metastasis in patients with malignancies.

Material and Methods: A total of 50 patients with bone metastasis enrolled in this study. All patients underwent MRI and whole body bone scan (BS) with Tc-99m-MDP in two weeks. The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of MRI, BS and MRI combined with BS method to detect bone metastasis were calculated.

Results: (1) On the patient basis: for MRI, BS, and MRI combined with BS, the sensitivity of detecting bone metastasis was 100% (50/50), 96% (48/50) and 100% (50/50), respectively. (2) On the lesion basis: there were 182 and 12 diagnosed with bone metastasis and benign lesion among all 194 foci by MRI and BS, respectively. For MRI, BS and MRI combined with BS, the sensitivity, accuracy and PPV of detecting bone metastasis was 100% (182/182), 100% (194/194), 90.91% (182/182); 67.03% (122/182), 62.89% (122/194), 91.04% (122/134) and 100% (182/182), 100% (194/194), 90.91% (182/182) respectively.

Conclusion: The diagnostic value of MRI combined with BS on bone marrow metastasis and lytic bone metastasis was obviously better than that of BS only.

Keywords: Bone metastatic tumor, bone scan, single emission computed tomography (SPECT), magnetic resonance imaging.

PO-89

Track: Drug Discovery in Preclinical Research

SOLID-STATE NMR CHARACTERIZATION OF S31N M2 TRANSMEMBRANE DOMAIN BOUND TO NOVEL ADAMANTANES WITH PERSISTENT *IN VITRO* EFFICACY

Anna K. Wright^{1,2}, Ivan Hung², Christina Tzitzoglaki³, Harris Ioannidis³, David D. Busath⁴, Antonios Kolocouris³ and Timothy A. Cross^{1,2}

¹Molecular Biophysics, Florida State University, Tallahassee, FL, USA; E-mail: ask09d@my.fsu.edu

²National High Magnetic Field Laboratory, Tallahassee, FL, USA

³Department of Pharmacology and Medicinal Chemistry, National and Kapodistrian University of Athens, Athens, Greece

⁴Physiology and Developmental Biology, Depts. of Microbiology and Molecular Biology and of Physiology and Developmental Biology Brigham Young University, Provo, UT, USA

Antimicrobial resistance is an increasing global threat to public health. Multidrug resistant bacterial and viral infections are on the rise and pose a tremendous challenge for clinical treatment. Since resistance development is an intrinsic trait which happens on a rapid timescale, it must be circumvented with fast and efficient drug development techniques. Solid State Nuclear Magnetic Resonance (ssNMR) is an excellent technique for structural characterization of membrane protein drug targets, in the native-like environment of a lipid bilayer. Coupling ssNMR with computational drug discovery accelerates hit identification and reduces time required for evaluating promising compounds. Here we present structural characterization of the M2 proton channel and elucidating the mechanism of channel inhibition using ssNMR, to aid in computational design of compounds with broad specificity. The M2 proton channel from Influenza A virus is essential for the viral lifecycle and is an important drug target. Structural characterization of M2 binding pocket revealed marked differences in the distances between the drug and the amino acid side chains for different constructs of the protein. Enantiomeric specificity was noted for the inhibitor targeting the wild type channel, and helical tilt in the lipid bilayer was established.

PO-47

Track: Hot Topics in Natural Products

EVALUATION OF TOPICAL FORMULATIONS CONTAINING EXTRACTS OF *ACMELLA OLERACEA* (L.) R.K. JANSEN (JAMBU) AND *ACHYROCLINE SATUREIODES* LAM (MACELA)

Lais Thiemi Yamane and Rodney Alexandre Ferreira Rodrigues

Natural Products Chemistry Division, State University of Campinas, Campinas, Brazil; E-mail: laisthiemi@gmail.com

The species *Acmella oleracea* (L.) R.K. Jansen (Jambu) and *Achyrocline satureioides* Lam (Macela), both native of South America, are very popular in folk medicine. Jambu is used for treating toothaches, mouth and throat problems. Data from the scientific literature showed its efficacy in extract form as an anesthetic and anti-inflammatory. Macela has been used as anti-inflammatory, analgesic, sedative and antioxidant. The objective of this study was to evaluate the topical formulations with different polymers (chitosan, polyvinyl alcohol and hydroxyethyl cellulose) individually or in combination, added with both extracts for the purpose of anesthetic and anti-inflammatory actions. A concentration range of 1 to 5% of polymers was used to obtain a stable and homogeneous formulation. The formulation used was that employed hydroxyethyl cellulose, because it remained stable, homogeneous and with good physical properties. In preliminary tests using vertical Franz cell was found permeability of markers spilanthol and alpha-humulene regarded as responsible for the active pharmacological action. The justification for this study supported in the importance of obtaining a pharmaceutical form in therapy, coupled with the use of plants known to popular wisdom, aiming its use for sustainable management.

Keywords: *Acmella oleracea*, *Achyrocline satureioides*, anesthetic and anti-inflammatory.

PO-86*Track: Protein and Peptide Sciences***STRUCTURAL AND DYNAMIC CHARACTERIZATION ON MUTATED Keap1 FOR VARIED AFFINITY TOWARD Nrf2: A MOLECULAR DYNAMICS SIMULATION STUDY****Pei-Jung Lu and Chia-Ning Yang***Department of Life Sciences, National University of Kaohsiung, Kaohsiung, Taiwan; E-mail: cnyang@nuk.edu.tw*

Keap1 is an adaptor protein that regulates steady-state levels of Nrf2, a bZIP transcription factor, in response to oxidative stress. In basal condition, Nrf2 is negatively regulated through ubiquitination by Keap1. However, upon exposure to oxidative stress, ubiquitination of Nrf2 is inhibited and Nrf2 enters nucleus to turn on cytoprotective genes. Lately, it has been reported that a gene variant G364C and a somatic mutation G430C on Keap1 greatly impair Keap1-Nrf2 interaction and are associated with lung cancer, whereas alanine-scan experiments showed S363A, S508A, S555A, and S602A do not affect Keap1-Nrf2 recognition, regardless that G364 and G430 are not in contact with Nrf2 whereas those four serines interact with Nrf2. Herein, molecular dynamics simulations were applied to describe the structural and dynamic variances among the wild-type and the aforementioned mutants of Keap1. It is concluded that G364C and G430C bring more mobility to D385 toward the binding entrance. Such a movement hampers the Keap1-Nrf2 recognition which relies on Keap1's R380, R415, and R483. In contrast, the mutants with serine replaced by alanine do not alter the hydrogen-bond network formed by the serine backbone and therefore the mutants are almost intact as the wild type dynamically and structurally.

Keywords: Keap1, Nrf2.

PO-104*Track: Pharmaceutical Biotechnology***MICROPLATE-FORMATTED MAMMALIAN PROTEIN EXPRESSION FOR HIGH THROUGHPUT DISSECTING BROAD NEUTRALIZATION ANTIBODIES AND IMMUNOGENS****Yongping Yang, Gabriel Lu and Peter D. Kwong***Vaccine Research Center, NIAID, NIH, Bethesda, MD 20892, USA; E-mail: yongpiny@niaid.nih.gov*

The 454 technology-based genomic scale exploration of broad neutralizing antibody against HIV-1 resulted in thousands and thousands of potent broad neutralizing antibody heavy chains or light chains. Thus, functional pairing of these heavy chains and light chains must be determined before other applications. In addition, structure-based vaccine designs created hundreds and hundreds of immunogen candidates which need to be expressed and characterized. A bottleneck arises with current conventional expression and purification method which remains an inefficient, time-consuming and resource-intensive process.

Presented here is a 96-well microplate-formatted transient transfection, mammalian expression and screen approach that is specifically designed to improve the efficiency of a high-throughput protein expression and screen manipulation and facilitate functional dissection of broad neutralization antibodies and immunogens.

Compared to our conventional protein production, by which the processing time for preparation of 192 antibody chimeras for neutralizing characterization is couple of months and costs about \$1000 for preparation of a single antibody, the 96-well microplate-formatted protein expression requires 500-fold less DNA, expression level achieved is more than 2.4 fold higher, and simultaneous preparation of 192 different antibody chimeras takes less than one week and costs \$1 per antibody, and extremely reduces the labor work burden. Furthermore, the antibodies expressed in a 96-well

microplate are formatted to the 96-well microplate-based neutralizing assay or other 96-well microplate-based function assays.

In conclusion, this platform presented here offers ease of use, time- and cost-efficiency while maximizing protein quality and yield as well as functional analysis outcome. And this method provides a potent tool for rapid mammalian expression and function screen of large amount of proteins. Moreover this technology may be rapidly expanded to systems of greater application, such as dissection of antigens, antibodies and virus-like particles for vaccine development, thus will facilitate high throughput vaccine discovery and development.

PO-83

Track: Protein and Peptide Sciences

ELUCIDATION AND CHARACTERIZATION OF A NOVEL ANTI-TUMOR PROTEIN FROM *ARCA INFLATA*

Rongmin Yu, Jian Xu, Shuangshuang Lv, Zhiyan Chen, Jianhua Zhu, Yue Gao, Jingjing Gao, Jiachen Zi and Liyan Song

Department of Natural Medicinal Chemistry, College of Pharmacy, Jinan University, Guangzhou, China; E-mail: tyrm@jnu.edu.cn

A novel *in vitro* anti-tumor protein (J2-C2) with a molecular weight of 27153.0 Da was isolated from the edible portion of *Arca inflata*. The property and structure of J2-C2 was characterized using physicochemical and instrumental analyses. The results indicated that J2-C2 was identified as a homogeneous compound by native polyacrylamide gel electrophoresis (Native-PAGE), and the isoelectric point of J2-C2 was measured to be 6.3 by isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE). The purity of J2-C2 was over 99% in reversed phase-high performance liquid chromatography (RP-HPLC). Carbohydrate content assay demonstrated that J2-C2 was not a glycoprotein. FT-IR spectrum of J2-C2 was shown that it had characteristic absorption peaks at 1645.71 and 1541.46 cm⁻¹. Partial amino acid sequences of this protein were determined as SSRLMVYLRR, KGEIDMGIVGGASIKR and DCDAVWVLLGHKMP via MALDI-TOF/TOF-MS and *de novo* sequencing. Secondary structural analysis by CD spectroscopy revealed that J2-C2 had 34.0% of α -helix, 27.5% of β -sheet, 13.4% of β -turn and 25.1% of random coil. The anti-tumor effect of J2-C2 against three human tumor cells was measured by MTT assay, and the IC₅₀ values of J2-C2 were 42.38, 45.64 and 48.73 μ M against A549, HepG2 and SPC-A-1 cell lines, respectively.

Keywords: *Arca inflata*, Protein, Characterization; *In vitro* anti-tumor activity.

PO-73

Track: Hot Topics in Natural Products

VASCULAR NORMALIZATION INDUCED BY SINOMENINE HYDROCHLORIDE RESULTS IN SUPPRESSED MAMMARY TUMOR GROWTH AND METASTASIS

Huimin Zhang, Yang Liu, Ligang Niu and Jianjun He

Department of Surgical Oncology, The First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi, China; E-mail: zhmoble@126.com

Solid tumor vasculature is characterized by structural and functional abnormality and results in a hostile tumor microenvironment that mediates several deleterious aspects of tumor behavior. Sinomenine is an alkaloid extracted from the Chinese medicinal plant, *Sinomenium acutum*, which has been utilized to treat rheumatism in China for over 2000 years. Though sinomenine has been demonstrated to mediate a wide range of pharmacological actions, few studies have focused on its



effect on tumor vasculature. We showed here that intraperitoneally administration of 100 mg/kg sinomenine hydrochloride (SH, the hydrochloride chemical form of sinomenine) in two orthotopic mouse breast cancer models for 14 days, delayed mammary tumor growth and decreased metastasis by inducing vascular maturity and enhancing tumor perfusion, while improving chemotherapy and tumor immunity. The effects of SH on tumor vessels were caused in part by its capability to restore the balance between pro-angiogenic factor (bFGF) and anti-angiogenic factor (PF4). However 200 mg/kg SH didn't exhibit the similar inhibitory effect on tumor progression due to the immunosuppressive microenvironment caused by excessive vessel pruning, G-CSF upregulation, and GM-CSF downregulation. Altogether, our findings suggest that SH induced vasculature normalization contributes to its anti-tumor and anti-metastasis effect on breast cancer at certain dosage.

Keywords: Breast cancer, sinomenine hydrochloride, vascular normalization, metastasis.

PO-34

Track: CNS Drug Discovery & Therapy

THE ENHANCEMENT EFFECT OF DA4 ISOLATED FROM BAMBOO (*DENDROCALAMUS ASPER*) SHOOTS ON $\alpha 1\beta 2\gamma 2$ S GABA A RECEPTOR EXPRESSED IN *XENOPUS* OOCYTES

Jingli Zhang, Habsah Mohamad, Muhammad Bilal, Jia Hui Wong, Abdul Hadi Bin Ismail, Amelia Jane Lloyd, Fatin Hilyan Mohamad, Nurnazihah Binti Mohd Noor, Abdul Aziz Mohamed Yusoff, Hasnah Osman, Koktong Wong, Zamzuri Idris and Jafri Malin Abdullah

*Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia;
E-mail: zjlphd@hotmail.com*

$\alpha 1\beta 2\gamma 2$ GABA A receptor represents the most existing GABA A receptors in mammalian cortex and hippocampus. While $\alpha 1\beta 2\gamma 2$ S GABA A receptor expressed in *Xenopus* oocytes is an *in vitro* model for the screen of anti-epileptic drugs as shown in our previous articles. In this article, a chemical of DA (*Dendrocalamus Asper*) 4 (4-hydroxybenoic acid), could increase the GABA A related response, while other extracts from the bamboo shoots DA5 (lauric acid), DA3 (palmitic acid), DA1 & DA2 (they are both major palmitic acid mixed with other minor fatty acid) could not significantly increase the GABA A related response.

Conclusion: 4-hydroxybenoic acid, isolated from bamboo shoots, is hopefully a new drug in treating epilepsy and further investigation should be done to verify its anti-epileptic effect in rodents and human.

Keywords: $\alpha 1\beta 2\gamma 2$ GABA A receptor, $\alpha 1\beta 2\gamma 2$ S, *Xenopus* oocytes, bamboo shoots, 4-hydroxybenoic acid, lauric acid, palmitic acid, anti-epileptic.

PLENARY LECTURES

PL-293**CURRENT STATUS OF OBESITY AND THE NEED FOR A PARADIGM SHIFT****Richard L. Atkinson**

Virginia Commonwealth University, Obetech Obesity Research Center, 800 East Leigh St., Suite 50, Richmond, VA 23219, USA; E-mail: ratkinson2@vcu.edu



There is a worldwide epidemic of obesity that is creating great health problems for individuals and massive economic costs for governments. More than 68% of American adults are overweight or obese. Many government and professional society guidelines for the treatment of obesity state that “diet and exercise are the primary treatment of obesity.” If the search terms “obesity and lifestyle and treatment and human” are used in PubMed, there are 8819 references. None have shown significant long term success of lifestyle treatment of obesity – the 5 year failure rate is >95%. It seems highly unlikely that the 8820th study of lifestyle is suddenly going to prove successful. Only in 2013 did the American Medical Association adopt a statement that obesity is a “disease.” Before the founding of the American Obesity Association, a lay advocacy group, in 1995, the NIH spent about \$30 million on obesity research out of a total budget of about \$12 billion. This was less than 1% of the budget for a disease affecting almost 30% of American adults at that time. Currently, the NIH states on its website that it spends about \$857 million on obesity research, but since the total budget figure is inflated by 5 fold, the true expenditure probably is about \$171 million. A quite significant percentage of this amount is spent on lifestyle research. In contrast to more established diseases, the early research into obesity has not focused on basic science, but on treatment (e.g., \$220 million for the “LOOK AHEAD” trial). We are in our infancy of the understanding of obesity. Since pre-history, humans have thought they knew the etiology of obesity – too much diet and too little exercise. It seems logical, but just because a perturbation affects a variable, does not mean that it is the cause of the variable. It seems quite possible that diet and exercise account for only a very small percentage of obesity. There is no doubt that body weight, or at least body fat, are regulated by the body. Why some people regulate at a high percentage of body fat vs others is not clear. Recent research has identified a number of etiologies of obesity that are NOT the “Big Two” of diet and exercise. This talk will summarize the research on some of these alternate etiologies of obesity and focus on several that may be responsible for large portions of obesity. Genetic factors are very important. At least 60 genes have been shown to contribute to or prevent obesity. Calculating the factorial (60 x 59 x 58, etc.), there are more combinations of genes for obesity than there are people on Earth. Next is virus-induced obesity. Human adenovirus 36 (Adv36) causes obesity in animals and in multiple countries that have been studied, about 30% of obese humans have been infected compared to about 10%-20% of non-obese humans. Scientists have postulated that Adv36 first appeared in the 1970s, just before the prevalence of obesity dramatically increased across the world. Another etiology of obesity that is being recognized as a major contributor to the epidemic are presumably epigenetic factors affecting women of child bearing age. If a woman has the following factors before and during pregnancy, the risk of obesity in her child is 20-40 fold higher than if none of the factors are present: obese at conception, increased weight gain, smoking, eating a high fat or high carbohydrate diet, lack of exercise, older age, taking certain drugs, and perhaps one of the most important, developing gestational diabetes during pregnancy. Almost all of these factors may be avoided or removed, and some evidence suggests that this will prevent a great deal of obesity in her offspring. Other factors that may play a role in causing obesity are certain drugs that are taken much more commonly in the last 30 years, certain industrial pollutants in the environment, and alteration of gut microbiota by changes in the diet favoring processed or refined foods and beverages. The etiology of obesity is so complex that a concerted effort must be made to identify basic biochemical and molecular factors leading to obesity. This information must be used to identify new drugs for treating obesity. Finally, in contrast to most “cookie cutter” treatment of obesity today, individualized treatment must be developed. No two patients are alike and it seems likely that obesity treatment options will be very numerous in the future.

PL-122**APPLICATION OF NITRIC OXIDE RESEARCH TO DRUG DEVELOPMENT****Ferid Murad**

Department of Biochemistry & Molecular Medicine, George Washington University, 2300 Eye Street NW, Suite 530, Washington, DC 20037, USA; E-mail: jsreed83@gwmail.gwu.edu



The role of nitric oxide in cellular signaling in the past three decades has become one of the most rapidly growing areas in biology. Nitric oxide (NO) is a gas and a free radical with an unshared electron that can regulate an ever-growing list of biological processes. Nitric oxide is formed from L-arginine by a family of enzymes called nitric oxide synthases. These enzymes have a complex requirement for a number of co-factors and regulators including NADPH, tetrahydrobiopterin, flavins, calmodulin and heme. The enzymes are present in most cells and tissues. In many instances, nitric oxide mediates its biological effects by activating the soluble isoform of guanylyl cyclase (SGC) and increasing cyclic GMP synthesis from GTP. Cyclic GMP, in turn, can activate cyclic GMP-dependent protein kinase (PKG) and can cause smooth muscles and blood vessels to relax, decrease platelet aggregation, alter neuron function, *etc.* These effects can decrease blood pressure, increase blood flow to tissues, alter memory and behavior, decrease blood clotting, *etc.* The list of effects of nitric oxide that are independent of cyclic GMP formation is also growing at a rapid rate. For example, nitric oxide can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Some of these reactions result in the oxidation of nitric oxide to nitrite and nitrate to terminate the effect and perhaps act as NO reservoir for future NO formations; while other reactions can lead to altered protein structure function and/or catalytic capacity. These effects of (NO) probably regulate bacterial infections, inflammation of tissues, tumor growth, and other disorders. These diverse effects of nitric oxide that are cyclic GMP dependent or independent can alter and regulate numerous important physiological events in cell regulation and function. Nitric oxide can function as an intracellular messenger, an autacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects. Thus, it is a unique molecule with an array of signaling functions. However, with any messenger molecule, there can be too little or too much of the substance, resulting in pathological events. Some of the methods to regulate either nitric oxide formation, metabolism, or function have been in clinical use for more than a century as with the use of organic nitrates and nitroglycerin in angina pectoris that was initiated in the 1870's. Inhalation of low concentrations of nitric oxide can be beneficial in premature infants with pulmonary hypertension and increase survival rates. Ongoing clinical trials with nitric oxide synthase inhibitors and nitric oxide scavengers are examining the effects of these agents in septic shock, hypotension with dialysis, inflammatory disorders, cancer therapy, *etc.* Recognition of additional molecular targets in the areas of nitric oxide and cyclic GMP research will continue to promote drug discovery and development programs in this field. Current and future research will undoubtedly expand the clinician's therapeutic armamentarium to manage a number of important diseases by perturbing nitric oxide formation and metabolism. Such promise and expectations have obviously fueled the interests in nitric oxide research for a growing list of potential therapeutic applications. There have been and will continue to be many opportunities from nitric oxide and cyclic GMP research to develop novel and important therapeutic agents. There are presently more than 150,000 publications in the areas of nitric oxide research. The lecture will discuss our discovery of the first biological effects of nitric oxide and how the field has evolved since our original reports in 1977. The possible utility of this signaling pathway to facilitate novel drug development and the creation of numerous projects in the Pharmaceutical and Biotechnology industries will also be discussed.

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PL-123**EVOLUTIONARY DYNAMICS AND TREATMENT OF CANCER****Martin Nowak**

Program for Evolutionary Dynamics, Harvard University, Cambridge, MA 02138, USA;

E-mail: martin_nowak@harvard.edu

Cancer is an evolutionary process. Cancer initiation and progression are caused by somatic mutation and selection of dividing cells. The mathematical theory of evolution can therefore provide quantitative insights into human cancer. I will discuss the role of chromosomal instability (CIN) and the accumulation of drivers and passengers in growing tumors. I will study success and failure of targeted therapy including combination of different drugs and evolution of resistance. A simple conclusion is that combination treatment can succeed, if the cancer requires at least two point mutations to gain resistance. From the perspective of preventing resistance, simultaneous therapy is highly recommended whereas sequential therapy is a recipe for almost certain treatment failure.

FURTHER READINGS

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PL-224**EMERGING PHARMACO-MPE (MOLECULAR PATHOLOGICAL EPIDEMIOLOGY) PARADIGM FOR GLOBAL PRECISION MEDICINE****Shuji Ogino**

Harvard Medical School, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard T.H. Chan School of Public Health; E-mail: Shuji_Ogino@dfci.harvard.edu

This lecture introduces the evolving paradigm of "Molecular Pathological Epidemiology (MPE)" (= Molecular Pathology + Epidemiology) (S Ogino *et al.* J Natl Cancer Inst 2010; S Ogino *et al.* Nat Rev Clin Oncol 2011; A Field *et al.* JAMA 2013; *etc.*) as simply as possible. Any given human disease represents fundamentally heterogeneous process, as implicated by the "Unique Disease Principle". MPE dissects complex interplay between environmental, dietary, lifestyle factors, molecular pathogenic alterations, and disease occurrence and progression. MPE is a logical next step of genome-wide association studies (GWAS), termed "GWAS-MPE Approach". MPE has proven itself to be a promising approach to identify biomarkers for precision medicine (A Chan *et al.* NEJM 2007; X Liao *et al.* NEJM 2012; R Nishihara *et al.* NEJM 2013, *etc.*). Recently, the pharmaco-MPE paradigm has been utilized to uncover unanticipated effects of medications on health and diseases, using large population-based MPE databases. It is increasingly possible to design MPE database worldwide using routine molecular testing data, as molecular pathology testing is becoming routine clinical practice. It is essential to build large-scale population-based databases including medication use, lifestyle factors, molecular pathology, and clinical outcome. Such databases can generate novel information on potential chemopreventive or therapeutic benefits of drugs, which can be further tested by experimental models and clinical trials. To expand opportunities and address challenges, the "International Molecular Pathological Epidemiology (MPE) Meeting Series" was established in 2013, and the Third International MPE Meeting will be held in Boston on May 12-13, 2016. Because disease heterogeneity is a ubiquitous phenomenon, the MPE and pharmaco-MPE paradigms should become routine to advance biomedical and population health sciences in the 21st century, and move us towards personalized prevention and treatment.

PL-222**RECONSTRUCTION, MODELING AND USE OF GENOME-SCALE NETWORKS IN BIOLOGY****Bernhard Palsson**

*Departments of Bioengineering and Pediatrics University of California, San Diego, San Diego, USA;
E-mail: palsson@eng.ucsd.edu*



Following the availability of full genome sequences in the mid 1990s, an effort was initiated to reconstruct, on a genome-scale, the biochemical reaction networks that underlie cellular functions. After 15 years of intense efforts, we now have highly curated network reconstructions, their experimental validation, and the generation of mathematical and modeling procedures available that allow the computation of cellular functions from genome- and bibliome-wide data sets. This effort has put a mechanistic basis into the most fundamental relationship in the life sciences; the genotype-phenotype relationship. This effort has started with simple organisms and the best characterized cellular functions and it is steadily growing in scope and biological complexity.

PL-223**NOVEL PRO-RESOLVING LIPID MEDIATORS IN INFLAMMATION & INFECTION LEADS FOR RESOLUTION PHYSIOLOGY AND PHARMACOLOGY****Charles N. Serhan**

*Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women's Hospital & Harvard Medical School, Boston, Massachusetts, 02115, USA;
E-mail: cnserrhan@zeus.bwh.harvard.edu*



Endogenous mechanisms controlling inflammation are of paramount importance because persistent and chronic inflammation can impact all organs and tissues throughout the body and are involved in many widely occurring diseases. Recent advances in our appreciation of the molecular mechanisms in resolution of acute inflammation (RoI) and ischemia-reperfusion injury systematically uncovered a novel genus of potent pro-resolving autacoid families, each biosynthesized from essential polyunsaturated fatty acids (PUFA) to activate potent responses not shared by the substrate. These include the *resolvins* (Rv), *protectins* (PD) and *maresins* (MaR), collectively termed *specialized proresolving mediators* (SPM) that act in pico-nanogram range. SPM are temporally and spatially biosynthesized by resolving-inflammatory exudates, which proved to evoke potent anti-inflammatory and pro-resolving actions as well as enhance microbial clearance. The potent SPM actions and complete structures are confirmed, which also permitted use of LC-MS-MS-based metabololipidomics to identify SPM in human and murine tissues (i.e. peripheral blood, breast milk, adipose, lymphoid, placenta), isolated human cells types (e.g. apoptotic human neutrophils, microparticles and macrophage phenotypes M1, M2), fish and diminished SPM in human pathologies e.g. breath condensates, Alzheimer brain, and synovial fluids from rheumatoid patients (CN Serhan Nature June vol 510, 2014 doi:10.1038/nature13479). Specific SPM demonstrate potent and stereoselective actions that involve specific G-protein-coupled receptors and are not immunosuppressive. Lipid mediator-metabololipidomics with self-limited resolving inflammatory exudates and human tissues demonstrated temporal orchestration of the SPM, i.e. RvD1 and RvD2 antecede RvD3, and MaR1 in mice and human tissues (Colas *et al.* AJP 2014). Many of the SPM born in inflammation-resolution are now shown to have potent actions and roles within host defense against bacteria and virus, pain, organ protection, tissue regeneration, exercise and neurobiology/cognitive function. This Plenary Lecture will update advances in SPM mechanisms in RoI, their new sites of formation and novel actions that opened the door for their role(s) in resolution physiology and pharmacology. Together, these new SPM families provide opportunities for resolution-based pharmacology and resolution physiology.

PL-2**MICRORNAs, SMALL INTERFERING RNAs AND MODULAR THERAPEUTICS****Phillip A. Sharp**

Koch Institute for Integrated Cancer Research and Department of Biology, MIT, USA; E-mail: marg@mit.edu

RNA interference was discovered a little over ten years ago and subsequently was shown to be mediated by intracellular double stranded small RNAs approximately 21 nucleotides in length (siRNA). In mammals, these RNAs enter the microRNA pathway, present in all cell types, are loaded into a complex containing the critical Argonaute protein, and target its activity to cleave and cause degradation of specific complimentary mRNA. Thus, in principle with the appropriate design of the siRNA any target gene could be silenced. Over the past years, the challenge of effective delivery of the hydrophilic siRNAs to cells has been advanced to where it is now possible in a therapeutically attractive fashion to silence genes expressed in the liver of humans with a sugar-based conjugate of a chemically modified siRNA. These therapeutic agents are modular in structure, where one constituent provides a gene-specific component whose modulation of expression is beneficial, while the other agent targets and facilitates delivery to the inside of cells. This modular property greatly reduces the time required to develop therapeutics to new disease modifying genes and is an example of future developments in pharmaceuticals. Examples of these types of agents will be discussed. The activities of small RNA based agents will be set in the context of the known biology of small non-coding RNAs.

PL-1**DESIGNING BIOLOGY FOR A HEALTHY WORLD****Pamela A. Silver**

Department of Systems Biology, Harvard Medical School and The Wyss Institute for Biologically Inspired Engineering, Harvard University, USA; E-mail: Pamela_Silver@hms.harvard.edu

The engineering of Biology presents infinite opportunities for therapeutic design, diagnosis, and prevention of disease. Towards these goals, we seek to make the engineering of Biology faster, more predictable and cheaper. This ‘Synthetic Biology’ has deep practical and social consequences for the pharmaceutical as well as the commodity industry. Here, I will present concepts and experiments that begin to address how we approach these problems in a systematic way.

By one strategy, we seek to predictably engineer mammalian cells to produce novel compounds that could potentially act as new therapeutics. For example, we have developed an algorithm for biosynthesis of new steroids that could have increased specificity towards their respective targets. This has implications in treatment of inflammation and clean production of other chemicals of interest.

By a second strategy, we design chimeric proteins to act as specific therapeutics. Specificity in biologics remains one of the outstanding issues in their use. We have again developed an algorithm based on coarse grain modeling for the predictable design on new proteins. Some have been tested in animals and show the predicted effects.

Lastly, we engineer components of the microbiome to act as both diagnostics and therapeutics. In one example, we have engineered natural gut bacteria to record the exposure of animals to antibiotics and to count the number of cell divisions as the bacteria passes through the gut. We can engineer the same bacteria to secrete toxins that could result in localized killing of pathogens and to act in a communal manner. Taken together, these experiments have far-reaching implications for the use of biology to prevent and treat disease in the future.



PL-291**ADVANCED THERAPEUTICS WHILE PRESERVING THE MICROBIOME****Ada Yonath**

*Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel;
E-mail: Ada.Yonath@weizmann.ac.il*



Ribosomes, the universal cellular machines for translation of the genetic code into proteins, possess spectacular architecture accompanied by inherent mobility, allowing for their smooth performance as polymerases that translate the genetic code into proteins. The site for peptide bond formation is located within a universal internal semi-symmetrical region. The high conservation of this region implies its existence irrespective of environmental conditions and indicates that it may represent an ancient RNA machine. Hence, it could be the kernel around which life originated. The mechanistic and genetic applications of this finding will be discussed.

Owing to the key role played by ribosomes in life cycles, almost half of the clinically useful antibiotics paralyze ribosomes by binding to their functional sites. By investigating the three dimensional structures of ribosomes from non-pathogenic bacteria as models for genuine pathogens, common features were identified. Thus, the antibiotics binding modes, inhibitory actions and synergism pathways have been determined for almost all ribosomal antibiotics. These indicated the principles of differentiation between patients and pathogens and suggested common principles of mechanisms leading to bacterial resistance.

The incredible global increase in resistance to antibiotics that we are witnessing recently is a serious medical threat. It seems that the world is approaching a post-antibiotic era, in which common infections and minor injuries that have been treatable for decades could become fatal once again.

As species specific diversity was detected in susceptibility to infectious diseases and in developing specific resistance mechanisms, our structural studies have been extended to ribosomes from genuine pathogens. By determining the high resolution structure of the first and only ribosomal particle from a genuine pathogen with several antibiotics, we identified subtle, albeit highly significant structural elements that can account for the species specificity in resistance, thus could paved ways for improvement of existing antibiotics as well as for the design of advanced therapeutics capable of minimizing antibiotics resistance.

PL-292**SPECIFIC ANTI-TUBERCULIN (IgG) HIGH LEVELS AND LYMPHOCYTE *IN VITRO* PROLIFERATION WITH TUBERCULIN ARE BETTER INDICATORS OF LTBI THAN TST**

Edmond J. Feris^{1,§}, Joaquin Zuñiga^{2,§}, Liliana Encinales³, Joel N.H. Stern^{4,5}, Julio Granados⁶, Marcelo Fernandez-Vina⁷ and Edmond J. Yunis³

¹*Department of Pharmacology and Toxicology, Geisel School of Medicine at Dartmouth, Lebanon, NH 03765, USA*

²*Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico*

³*Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA;
E-mail: edmond_yunis@dfci.harvard.edu*

⁴*Department of Science Education, Hofstra North Shore-LIJ School of Medicine, Hempstead, NY 11549, USA*

⁵*Department of Neurology, North Shore- LIJ, Manhasset, NY 11030, USA*

⁶*Immunology and Rheumatology Department, Instituto Nacional de Ciencias Medicas y Nutricion México City, Mexico*



⁷Department of Pathology, Stanford University School of Medicine, Stanford, CA 94304, USA

In previous report, we reported that anti tuberculin (IgG) antibodies and *in vitro* proliferation assays against tuberculin are better indicators of latent tuberculosis infection (LTBI) than the tuberculin skin test (TST). We concluded that these tests are markers of LTBI regardless of the level of exposure and TST results. We now report that, the blocking of proliferation responses was only produced in the cultures with tuberculin and not *Candida* suggesting a crosstalk between the two antigens influence cellular immunity and not humoral immunity. Most important is the fact that although cellular immunity plays a central role in the pathogenesis of active and latent tuberculosis, humoral immunity is dominant in LTBI. In this regard, recent evidence demonstrated that conserved genes of *Mycobacterium tuberculosis* coding the epitopes that induce T cell dependent are naturally selected; the immune response benefits the mycobacteria. Therefore, we emphasize the need to use humoral immune mechanisms in the diagnosis and control of mycobacterium infection.

[§]These authors contributed equally.

KEYNOTE LECTURES

KNL-82

Track: Pharmaceutical Biotechnology

CREATING BIOACTIVE CHEMICAL SPACE BY BIOTRANSFORMATION**M. Iqbal Choudhary and Atta-ur-Rahman**

International Center for Chemical and Biological Sciences, (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research) University of Karachi, Karachi-75270, Pakistan; E-mail: iqbal.choudhary@iccs.edu



The structural changes in a molecule brought about either by whole cell cultures of fungi or bacteria, isolated enzymes, and animals and plant cells is termed as biotransformation. Creating novel chemical space through biotransformation of existing drugs can be a faster way towards drug discovery. The process can successfully be utilized for the expansion of structural diversity around a pharmacophore. This can be an important tool in medicinal chemistry for the introduction or modification of specific functionalities at the positions difficult to access by conventional chemical methods. The use of biocatalysts offers a remarkable arsenal of highly chemo-, regio- and stereo-selective methods for chemical conversions, which are often difficult to achieve even from state-of-the-art synthetic procedures. In last two decades, this methodology has become an important tool for asymmetric synthesis, not only at the academic level but also at the industrial scale. There is a need to fully exploit the potential of biotransformation in creating new and novel chemical space for the discovery of lead molecules against prevalent diseases.

During our studies of creating chemical space, we structurally modified a large number of existing drugs and other compounds into their novel structural analogues by microbial and plant cell suspension cultures. The resulting metabolites have exhibited interesting biological activities, different from their precursors. The anabolic androgenic drug methenolone enanthate (brand names Primobolan and Primobolan Depot) is used for the treatment of the advanced breast carcinoma in the postmenopausal women as well by athletes to build the muscle strength. The biotransformation of metenolone enanthate by fungi led to the synthesis of six metabolites, these transformed products showed anti-inflammatory potential in the oxidative burst assay and potent to moderate activity on proinflammatory cytokine TNF- α , produced by THP-1 cells. Similarly oxymetholon marketed as anadrol, is a synthetic anabolic steroid developed in 1960 by Zoltan 'Anadrol Z' F. It has been approved by the US Food and Drug Administration for the treatment of anemias caused by deficient red cell production. Its biotransformation with various fungi has resulted in the production of various new and a known metabolites. Oxymetholone and some of its metabolites showed anti-inflammatory activity. Exemestane (trade name aromasin) is a steroidal aromatase inhibitor, used for the treatment of breast cancer. Aromatase inhibitors block the synthesis of estrogen. This lowers the estrogen level, and slows the growth of cancers. Exemestane was developed by an Italian company using commercially available boldenone (androsta-1,4-diene-17 β -ol-3-one). After biotransformation of exemestane, the metabolites showed activity against Hela and PC3 cell lines. Melengestrol acetate, is used as a feed additive for feedlot heifers was found to be a potent anti-inflammatory agent along with its new transformed products. Tibolone is a synthetic steroid hormone drug, used for the treatment of endometriosis and hormone replacement therapy in post-menopausal women we have successfully biotransformed the drug into its new derivatives and identified them as potent alpha glucosidase inhibitors. These results showed drug repositioning of already marketed drugs and also showed that resulted new and known compounds can speed-ups the process of drug development.

During this presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules by biotransformation will be discussed.

INVITED LECTURES

IL-169

Track: Pharmaceutical Biotechnology

NOVEL APPROACH FOR BIG DATA MANAGEMENT AT THE LEVEL OF INTRACELLULAR SIGNALIZATION AND ITS APPLICATIONS TO BIOMEDICINE, DRUG DISCOVERY AND PERSONALIZED THERAPY

Nicolas Borisov, Alexander Aliper, Ksenia Lezhnina, Denis Shepelin, Michael Korzinkin, Artem Artemov, Qinsong Zhu, Alex Zhavoronkov and Anton Buzdin

Pharmaceuticals, Rms 2702-3, 27th Floor, Bank of East Asia Harbour View Centre, 56 Gloucester Road, Wan Chai, Hong Kong; E-mails: buzdin@ponkc.com; bu3din@mail.ru



Analysis of complete transcriptomes and proteomes is complicated by the problems with understanding overall functional conclusions basing on the large-scale gene expression figure. We report a computational biomedical technique termed OncoFinder, which enables performing both quantitative and qualitative analysis of the intracellular signaling and metabolic pathway activation. This method is universal and may be used for the analysis of any physiological, stress, malignancy and other specific conditions at the molecular level. In contrast to other techniques, OncoFinder utilizes an algorithm that distinguishes functional roles of every gene product in each pathway. OncoFinder showed a strong potential to neutralize batch effects and platform-specific differences for the experimental data obtained using NGS, microarray hybridization and proteome wide techniques. This approach allowed us to characterize new pathway signatures as better markers of cancer progression compared to individual gene products. OncoFinder also enables to correlate pathway activation with the success of medical treatment. We created a new biodata management platform and a software available to the academic community. The authors enthusiastically look for building international collaborations and partnerships in theoretical and applied biomedicine.

IL-129

Track: Pharmaceutical Biotechnology

STEM CELL THERAPY FOR THE TREATMENT OF SEVERE TISSUE DAMAGE AFTER RADIATION EXPOSURE

A. Chapel, A. Semont, N. Mathieu, C. Linard, L. Douay, N.C. Gorin, M. Mothy, J.M. Simmon, J.J. Lataillade, J. Voswinkel, R. Tamarat and M. Benderitter

Radiological Protection and Human Health Division, Institute of Radiological Protection and Nuclear Safety, France; E-mail: alain.chapel@irsn.fr



Radiotherapy may induce irreversible damage on healthy tissues surrounding the tumor. It has been reported that the majority of patients receiving pelvic radiation therapy shows early or late tissue reactions of graded severity as radiotherapy affects not only the targeted tumor cells but also the surrounding healthy tissues. The late adverse effects of pelvic radiotherapy concern 5 to 10% of them, which could be life threatening. However, a clear medical consensus concerning the clinical management of such healthy tissue sequelae does not exist. Although no pharmacologic interventions have yet been proven to efficiently mitigate radiotherapy severe side effects, few preclinical researches show the potential of combined and sequential pharmacological treatments to prevent the onset of tissue damage. Our group has demonstrated in preclinical animal models that systemic MSC injection is a promise approach for the medical management of gastrointestinal disorder after irradiation. We have shown that MSC migrate to damaged tissues and restore gut functions after irradiation. We carefully studies side effects of stem cell injection for further application in patients.

The clinical status of four first patients suffering from severe pelvic side effects resulting from an over-dosage was improved following MSC injection in a compassionate situation. Bone marrow-derived MSC from the patients' children were injected to four patients. A quantity of 2 millions to 6 millions of MSC/kg was infused intravenously to the patients. Pain, hemorrhage, frequency of diarrheas and fistulisation as well as the lymphocyte subsets in peripheral blood were evaluated before MSC therapy and during the follow-up. Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. In one patient pain reappeared after 6 months and again substantially responded on a second MSC infusion. A beginning

fistulisation process could be stopped in one patient resulting in a stable remission for more than 3 years of follow-up. The frequency of painful diarrhea diminished from an average of 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. A decline of CD4+ and CD8+ T lymphocytes and an increase of potentially regulatory CD25+ T cells accompanied the clinical response in this patient after the MSC injections. In all patients, prostate cancer remained in stable complete remission. A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response in refractory irradiation-induced colitis. No toxicity occurred.

MSC therapy was safe and effective on pain, diarrhea, haemorrhage, inflammation, fibrosis and limited fistulisation. For patients with refractory chronic inflammatory and fistulising bowel diseases, systemic MSC injections represent a safe option for salvage therapy. A clinical phase II trial will start in 2015.

IL-58

Track: Plant and Environment

MEDICINAL PLANTS USED BY IMMIGRANTS FROM THE CARIBBEAN

Brahmadeo Dewprashad, Kwame Amin, Maria Greene, Latifa Hadir, Richard Hendriks, Anthony Bradford, Joel Moroccho, Vishnu Tiwari and Sheuli Zakia

Department of Science, BMCC/City University of New York, USA;

E-mail: bdewprashad@bmcc.cuny.edu

In the Caribbean, there is a tradition of reliance on herbal remedies for acute and/or chronic medical conditions and immigrants from these countries have continued this practice. This presentation will discuss the results of an evaluation of the efficacy and safety of some of the commonly used herbal medications in the Caribbean immigrant community in New York City. The presentation will discuss the conditions for which they are used, and the results of our investigation of possible scientific basis for their purported efficacy and safety.



An extract from *Manihot esculenta* is used to flavor and preserve cooked meat. Our investigation found that the extract was active against both gram-positive and gram-negative organisms and that it does prevent bacterial and fungal growth on cooked meat, but that its use has likely safety concerns. Extracts from *Doliocarpus brevipedicellatus* are used as stimulants and as treatment for ED. We found that the plant has stimulant properties and that it does increase the rate of blood vessel pulsation in blackworms, similar to the effect of yohimbine. In addition, the results of identification of its major chemical constituent and toxicity studies on brine shrimp will be presented. An extract from the bark of *Columbrina arborescens* is used to lower blood pressure. The results of our investigation on its effect of the blood vessel pulsation rate of blackworms will be presented. In addition, the results of the chemical analysis of various barks that are sold as “bitters” and used to treat a variety of conditions, will be presented.

IL-229

Track: Plant and Environment

IDENTIFICATION THE BOTANICAL ORIGIN OF NATURAL WOODS USED IN WINE AGING

Ignacio Díaz-Maroto, M. Elena Alañón, Pablo Vila-Lameiro, Consuelo Díaz-Maroto and M. Soledad Pérez-Coello

Departamento de Ingeniería Agroforestal, Escuela Politécnica Superior, Campus Universitario s/n, Universidad de Santiago de Compostela, E-27002 Lugo, Spain; E-mail: ignacio.diazmaroto@usc.es

Aging wines in wooden barrels is a long-used technological step at wineries. Initially, this practice was set up for the storage and transport of wines, but later, it has been used because of its positive effects on wine organoleptic characteristics and quality. The role of wood during the aging process is crucial in several aspects. From the sensorial point of view, wood is capable of transferring aroma-responsible volatile compounds to wines. Furthermore, a reduction of astringency and changes in color are produced on



aged wines as a result of the extraction of phenolic compounds from wood and the oxidation reactions produced because of the diffusion of oxygen across the wood pores.

For that, aged wines acquire distinctive sensorial characteristics that are well-appreciated by the consumer, which implies a higher price of aged wines. Although several types of woods have been used in the manufacture of barrels (chestnut, cherry, etc.), oak wood is, by far, the most common wood used in making barrels for aging purposes, not only for its chemical composition but also for both its mechanical and physical properties that facilitate the conformation of barrels.

However, the aging period carried out by means of oak barrels entails a time-consuming and expensive process. On the one hand, aged wines have to be left in the barrels during a long period before they can be brought to market because of the slow extraction process of oak wood compounds. On the other hand, it implies some problems, such as the cost and difficulty of their sanitization and handling.

IL-140

Track: Regenerative Medicine

PERSONALIZED HEALTHCARE: FROM RESEARCH TO REALITY

Jeffrey P. Harrison and Debra A Harrison

Department of Public Health, Brooks College of Health, University of North Florida, 1 UNF Drive, Jacksonville, FL 32224-2645, USA; E-mail: jeffrey.harrison@unf.edu



Over the past decade, healthcare researchers have spent hundreds of millions of dollars in sequencing the first human genome. In addition, there is significant research in the use of stem cells to regenerate specialized tissue for treatment of individual patients. This session will address the use of this research to bring “Personalize Healthcare” to the patient. Specifically, we will discuss the use of Human Genomics and other stem cell therapies to regenerate organs and treat pressure ulcers. The appropriate use of these new clinical technologies provides opportunities for significant improvements in healthcare quality as well as improvements in preventive health services. The growth of “Personalized Healthcare” requires the creation of collaborative relationships between researchers, clinicians and healthcare leadership as process-focused care continues to develop. The growth of “Personalized Care” facilitates innovation across the continuum of healthcare practice and may be a cost effective approach to improving healthcare quality.

Keywords: Teaching Methodology, Lecture, Case Study on, From Research to Reality, Discussion/Question and Answer period.

IL-267

Track: Regenerative Medicine

MICROVESSEL ADAPTATION AND PLASTICITY IN MICROVASCULAR REGENERATION

James B. Hoying

*Cardiovascular Innovation Institute, University of Louisville, USA;
E-mail: jay.hoying@louisville.edu*



The final intent of microvascular regeneration is the re-establishment of an effective microcirculation within the target tissue. Successful strategies for forming a microcirculation (*i.e.* a microvascular perfusion circuit) must address not only the derivation of new, individual microvessel elements, but also the organization of each element into an effective network. Indeed, numerous examples of tissue repair/regeneration in which perfusion is reduced even though microvessel density is elevated highlight the importance of this network topology aspect. The final, tissue-specific topology of a native microvasculature arises, in part, from an intrinsic plasticity and adaptability of microvessels. Using a unique microvascular regeneration approach, we have shown that immature neovessels formed *via* angiogenesis transition through a phase of instability necessary to the re-organization of neovessels within the network and specialization of each neovessel into a mature microvessel type (*i.e.* arteriole, capillary, and venule). Proper progression through this unstable phase depends on hemodynamic inputs. In addition, it's during this phase in which the angiogenesis-derived

neovessels, which have lost the arterio-venous identity of their parent microvessels, re-acquire arterial, capillary, or venous specification, an activity critical to maturation of the microcirculation. Reflecting further the intrinsic adaptability of neovessels, the regenerated microcirculation can be functionally changed during its formation to reflect tissue-specific phenotypes. For example, inclusion of astrocyte precursor cells, brain cells that interact intimately with the native brain microcirculation, during the regeneration of the new microvasculature induces a blood-brain-barrier-like character to the new microcirculation as opposed to a more ubiquitous continuous-phenotype of the peripheral microvasculature. Taken together, our findings indicate that the topology and character of regenerated microvascular networks, as defined by arterio-venous identity, segment organization, and functionality, arises from considerable phenotypic and topological plasticity intrinsic to newly forming microvessels. Implied, regeneration strategies that constrain neovessel adaptation or seek to pre-determine microvascular outcomes may be problematic and result in dysfunctional microcirculations.

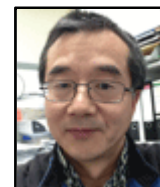
IL-162

Track: Plant and Environment

CHARACTERISATION OF AN EXCEPTIONALLY LARGE *FAD2* GENE FAMILY AND DEVELOPMENT OF SUPER HIGH OLEIC GENOTYPE IN SAFFLOWER

Qing Liu, Craig Wood, Shijiang Cao, Matthew Taylor Shoko Okada, Xue-Rong Zhou, Allan Green and Surinder Singh

Commonwealth Scientific and Industrial Research Organization, Crop Biofactories Initiative, ACT 2601, Australia; E-mail: qing.liu@csiro.au



Microsomal oleoyl phosphatidylcholine desaturase (*FAD2*) is largely responsible for the ratio of two major fatty acids, oleic and linoleic acids, together of which accounts for more than 90 % of total fatty acids in safflower seed oil. From safflower, we have isolated the largest *FAD2* gene family in plants, with a staggering 11 members with distinct expression patterns and functionalities. The temporal and spatial expression profiles of these genes were revealed through real-time quantitative PCR and their diversified functionalities were demonstrated by ectopic expression in yeast and transient expression in *Nicotiana benthamiana* leaves. Molecular characterisation of a well-known safflower high oleic spontaneous mutant, termed as *ol* allele revealed that it was originally derived from progenitor species *Carthamus palaestinus* through an ancient interspecific hybridization and its subsequent spontaneous mutation by the deletion of a single nucleotide in the coding region of *FAD2-1* that resulted in frame shift and nonsense mediated RNA-degradation (NMD). In this high oleic background, RNAi mediated gene silencing targeting the constitutively expressed *FAD2-2* produced a seed oil containing 93% oleic acid in seed oil. Biochemical and agronomic performance of selected transgenic lines indicate a promising prospect for commercial exploitation. The high purity of oleic acid in this safflower oil offers an opportunity for manufacturing alternative oleochemicals that are found in the formulation of many hundreds of biodegradable oleochemicals.

IL-9

Track: Other Areas

PSP94, PROSTASIN AND PSP94 TO PROSTASIN SIGNALING IN CANCER

Jin-xia Ma and Bing-xue Yan

Icesnow Yanyan Bioscience LLC, Baltimore MD 21224, USA; E-mail : jma19@jhu.edu



PSP94 (Prostatic secretory protein 94) is one of most abundant protein in the semen of healthy men and also found in a variety of human tissues, especially in female reproductive tissues such as the breast and ovaries. Prostatin, is a trypsin-like serine peptidase expressed in epithelial cells, with the highest expression in the normal prostate gland and seminal fluid and lesser amount in various tissues. Studies show that PSP94 is decreased and tumor suppressor in prostate cancer. PSP94 was found to be overexpressed in breast cancer. The expression of prostatin has been shown to mal-express in ovarian, prostate, breast and

gastric cancers. The findings suggest these two genes are potential key players in oncogenesis. Our recent experimental investigations demonstrated that PSP94 plays important roles in ovarian cancer chemoresistance and ovarian oncology that is found overexpressed in ovarian cancer cell lines and cancer patients. Importantly, our signaling pathway analysis showed PSP94 is a close upstream signaling mediator of prostasin in ovarian cancer that regulates prostasin expression and action in ovarian cancer cells. Additionally, PSP94 and prostasin are both upstream regulators of Lin28/Let-7 loop in ovarian cancer cells, a well-known signaling in oncogenesis in general, and is believed to play a critical role in stem cell development. PSP94 and prostasin are also found to regulate CASP-PAK2-p34 signaling, that may be involved in apoptosis pathways in ovarian cancer cells. These findings expand the current network of tumorigenesis in ovarian cancer. We propose PSP94 and prostasin may have close connection in other cancer types, especially prostate and breast cancers in which both genes are malexpressed. The investigations of PSP94 and PSP94 to prostasin signaling in oncogenesis in general are undergoing

IL-14

Track: Regenerative Medicine

VASCULAR TISSUE REGENERATION: A BIOPHYSICAL PERSPECTIVE ON THE ROLE OF MECHANICAL, MOLECULAR AND ELECTRIC INTERACTIONS.

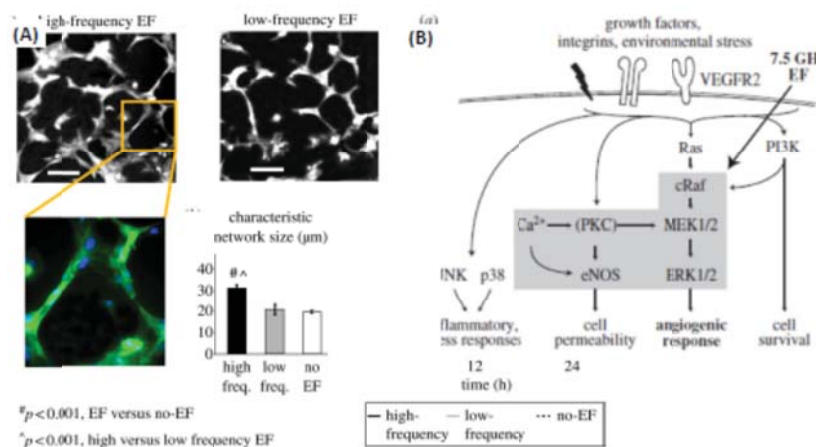
Toloo Taghian, Abdul Sheikh, Andrei Kogan and Daria Narmoneva

Biomedical Engineering, University of Cincinnati, Cincinnati, OH, USA;

E-mail: daria.narmoneva@uc.edu



Regeneration of vascular tissues is a necessary component for development of effective therapies for impaired healing of vascular tissues, for example, to address significant health care problems associated with fibrotic myocardial remodeling & chronic ulcers. Impaired healing in vascular tissues is characterized by altered tissue microenvironment, unbalanced proteolytic activity, prolonged inflammation and insufficient neovascularization. External electric field (EF) can affect a variety of vascular cell responses through manipulation of native EF in the extracellular ionic environment and across the cell membrane. Therefore, EF, together with the extracellular matrix (ECM) and a milieu of cytokines represent a biophysical system that ultimately regulates vascular cell function. Therapeutic modulation of this system requires advanced integration of knowledge and technology of physics and biomedical sciences. Our research aims to elucidate the biophysical mechanisms of cell-EF interactions mediated by ECM through developing a theoretical-experimental approach. Theoretical 3D EF-cell interaction model solves Maxwell's equations (ANSOFT_HFSS) for a membrane-enclosed hemisphere subjected to EF to provide a precise distribution of induced EF within the cell in wide frequency range. Simulations demonstrate that, at low frequency, EF is confined in the cell membrane and is expected to regulate membrane-initiated responses only. At high frequency, EF penetrates the cell and may directly activate intracellular responses. These predictions are confirmed by our experimental results, which demonstrate a major role for cRaf/MEK/ERK and Ca²⁺ pathways in EF-mediated stimulation of angiogenic responses. The results show that cell responses to EF differ in natural versus synthetic ECM. These findings provide evidence for a novel mechanism of EF-mediated regulation of vascular cell interactions within the complex biophysical system. *In vivo*, this mechanism translates into increased wound vascularization and improved healing, and therefore, provides foundation for development of EF-based therapies for vascular tissue regeneration.



These studies were supported by NIH/NDDK R21DK078814 (DN), AHA BGIA- 533 0765425B (DN), N.S.F. (DMR-1206784) and (DMR 0804199) to AK.

IL-272

Track: Regenerative Medicine

THE RATIONAL BIOLOGICAL DESIGN OF REGENERATIVE THERAPIES AND DEVICES

Nancy L. Parenteau

*Parenteau BioConsultants, LLC, Co-founder, President and CSO, Verik Bio, Inc., USA;
E-mail: nancyparenteau@gmail.com*



Rational biological design (RBD) is a fast fail method of biological product design and development that is similar in principle to rational drug design, but focused at the level of cellular processes and interaction. This approach to research and development offers a fresh and clinically connected perspective, which can be particularly helpful in grappling with the complex challenges encountered when developing advanced biological therapies within the regenerative medicine space. The presentation will cover how RBD can be used to 1) illuminate the knowledge and information really needed to advance a product, 2) find the strongest applications for a technology, 3) build a level of biological robustness that will better ensure clinical trial success, and 4) reduce the risk of costly mistakes. The presentation will include examples of how RBD can guide the fruitful design and delivery of cell therapies, tissue engineered products and T-cell-based cancer immunotherapies.

IL-134

Track: Regenerative Medicine

THE NEIL ARMSTRONG SPACE SYNDROME

William J. Rowe

*M.D. FBIS FACN, Fellow of the British Interplanetary Society, Former Assistant Clinical Professor of Medicine, Medical U. of Ohio at Toledo, 1485 Bremerton La., Keswick, Virginia, 22947, USA;
E-mail: rowefemsinspace@gmail.com*



BACKGROUND

In 2002, the author presented and subsequently published that with space flight endothelial dysfunction; there is the potential for congestive heart failure despite invariable dehydration. There is decreased thirst, inappropriate diuresis, atrophy of water storage sites in skeletal muscles and reduced plasma volume; this may be triggered by endothelial intercellular gaps with inflammation in post-capillary venules. These leaks may be responsible for an invariable 10% loss of plasma volume in microgravity. Furthermore, the invariable significant reductions of the serum magnesium ($p < 0.0001$) in large groups of astronauts and cosmonauts, despite very poor serum sensitivity, along with catecholamine elevations to levels twice those in the supine position on Earth with ischemia and multiple vicious cycles, are conducive to further endothelial injuries and in turn, to catecholamine cardiomyopathy (acute temporary heart failure.)

SYNDROME

During his lunar last 20 minutes, Neil Armstrong notified Houston twice at 4 minute intervals that he was "short of breath" at 111 hours and 32 minutes and "still short of breath" at 111:36. This symptom occurred prior to the potential confounder of inhalation of highly toxic iron-laden dust, brought into the habitat on space suits. Whereas his heart rate on the moon was 130-160/minute, just prior to splashdown in the Pacific, after his 3 day journey back to Earth, his heart rate was down to 61. The explanation for this correction of tachycardia, could only be that by quenching his severe thirst, the very high adrenaline levels were reduced because of expansion of the left ventricle by replenished plasma

volume with in turn reduction in the gradient; this could have been precipitated by protrusion of the septum into the left ventricle as postulated by Merli *et al.*

CONCLUSION

Neil Armstrong Space Syndrome:

Severe dyspnea

Severe thirst

Severe tachycardia, corrected by fluid replenishment.

Keywords: Neil Armstrong, dyspnea, tachycardia

IL-297

Track: Pharmaceutical Biotechnology

NEW PERSPECTIVES IN NANOMEDICINE AND NANOPHARMACOLOGY

Hans-Christian Siebert

Diplom-Physiker, Scientific Director, RI-B-NT Research Institute of Bioinformatics and Nanotechnology, Franziusallee 177, D-24148 Kiel, Germany; E-mail: hcsiebert@aol.com



The strategic combination of various biophysical and biochemical methods such as NMR, X-ray crystallography, Atomic Force Microscopy, Accelerated Mass Spectrometry and Surface Plasmon Resonance techniques with cell biological approaches and patient studies leads to new perspectives in the fields of nanomedicine and nanopharmacology concerning innovative routes in applied health care. These routes show solutions for different so-far unsolved medical and pharmacological problems in oncology, anti-infection, tissue-engineering and pathobiochemistry of the endocrine system [1-7]. It was essential for this purpose to decipher the three alphabets of life completely (1: nucleic acid code, 2: amino acid code, 3: sugar code [8]) and figure out how they work together on a sub-molecular level. It turned out in our molecular dynamics simulations that ab initio calculations play an important role in order to fit all the results from the different approaches together in a convincing way.

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SESSION LECTURES

SL-244*Track: Plant and Environment***GENETIC STABILITY AND PERSISTENCE OF MORPHOLOGICAL FEATURES OF TRANSGENIC PEA (*PISUM SATIVUM* L.) HARBORING Na^+/H^+ ANTIPORTER AGAINST SALT STRESS****Zahid Ali, Saadia Naseem, Nighat Yasmeen, Hans Joerg Jacobsen and Fauzia Yusuf Hafeez***Department of Biosciences, COMSATS Institute of Information Technology, Park Road Islamabad, Pakistan; E-mail: zahidali@comsats.edu.pk*

Transgenic approaches have played significant role for modern plant development. In this regard, combined gene discovery and functional genomics have proven diversified mechanisms and gene families, which improved productivity by tolerating different abiotic stresses. We report here genetic stability and persistent morphological features of transgenic pea (*Pisum sativum* L.) plants harboring dicistronic vector construct pG0229MAS $\text{nhx1}/\text{luc}$ in subsequent six generations over a period of five years. In addition to salt stress tolerance (100mM NaCl), the transgenic plants also showed frost tolerance over wild type (WT) counter part. The frost tolerance of transgenic pea plants harboring Na^+/H^+ antiporter from *A. thaliana* is unexpected yet an important physiological trait which needs further investigations. The comparison of long term stored transgenic seeds under 30-50 degree C and glass house grown WT and transformed plants on various morphological and molecular characterization were investigated. The transgenic plants were found to be morphologically stable and tolerant to NaCl stress in subsequent generation. Genetic stability of transformed genes was confirmed prior to and after transfer of transgenic plants in glass house under different climatic conditions. Leaf size, shape and color, plant height, number of tendrils, flower shape, pod shape and grains were morphologically similar to WT counterpart in all transgenic generation. The work is in progress.

Keywords: Na^+/H^+ antiporter, transgenic pea, morphology, genetic stability.

SL-186*Track: Plant and Environment***RAPD- PCR ANALYSIS OF INDIGENOUS IRAQI SINORHIZOBIUM MELILOTI ISOLATES DIFFERING IN THEIR ABILITY TO DROUGHT TOLERANCE****Rana A. Hameed Nidhal N. Hussain and Abed Aljasim M. Jasim Almansoori***Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq; E-mail: dr_aljibouri@yahoo.com*

Soil bacteria Sinorhizobium meliloti had enormous agricultural value, due to their ability in fixing nitrogen symbiotically with an important forage crop legume- alfalfa. The aim of this study (i) isolate indigenous S. meliloti from different field sites in Iraq, (ii) evaluate the isolates tolerance to induced drought using polyethylene glycol-6000, (iii) assessing genetic diversity and genetic relationships among isolates of natural population with drought tolerant abilities. Drought tolerance study revealed vast variations between Sinorhizobium isolates, the highest tolerant isolates to drought were twelve from total thirty (40%), tolerated from -3 up to -4 Mpa (mega pascal), while the drought sensitive isolates tolerated up to -1.5 Mpa, except isolate Bs58 which tolerated up to -1 Mpa water potential. The growth declined with the increase of drought stress. Cluster analysis based on RAPD-PCR showed significant differences among S. meliloti isolates, and the results gave almost identical grouping of isolates in regards to drought experiment. Among indigenous isolates two divergent groups could be determined, the first major group included drought tolerant isolates and the second major group comprised all drought moderate and sensitive isolates with 40% similarity between the two major groups.

SL-305*Track: Pharmaceutical Biotechnology***WHOLE CELL BIOCATALYST FOR SOYASAPOGENOL B PRODUCTION FROM SOYBEAN SAPONIN****Hala A. Amin, Mostafa M. Abo Elsoud, Ahmed. F. Sahab***Chemistry of Natural and Microbial products Department, National Research Center, Egypt;
E-mail: halaamin2007@yahoo.com*

Soyasapogenol B, aglycone of soybean saponin, is known to have hepatoprotective, antimutagenic, antiviral, and anti-inflammatory activities. This research examined the use of whole-cell biocatalyst to produce soyasapogenol B from soybean saponin. It was found that *Aspergillus flavus*, a fungus isolated from peanut pods, was capable of expressing extracellular and intracellular saponin hydrolase enzyme. However, the total enzyme activity produced using fungal whole cells (37U) in the reaction mixture was about 3 times that produced using the extracellular (12.4U) or intracellular (11.5U) enzyme. Cells with maximum hydrolytic activity for production of soyasapogenol B (12.2 U/g) was obtained using production medium supplemented by 2% soybean saponin, as inducer for enzyme production, adjusted at pH 9 and incubated at 30°C for 2 days. The highest yield of soyasapogenol B was achieved when the reaction mixture was incubated at pH 5.5 and 45°C for 48h; using 20 g wet cells (corresponding to 4% cell dry weight) and soybean saponin (2%, w/v) as a substrate. Under these optimal conditions, the cells bioconversion efficiency (soyasapogenol B yield) increased from 5.3 to 60%. Whole cell biocatalyst has several advantages with regard to industrial applications: a consistent quality, easy to be prepared and a very low price compared with purified enzyme. Consequently, this study is significant for production of soyasapogenol B from soybean saponin on an industrial scale.

ACKNOWLEDGEMENTS

The authors would like to thank for financial support via the tenth research grant (2013-2016) of the National Research Center of Egypt.

Keywords: Whole cell biocatalyst, Soybean saponin, Soyasapogenol B, *Aspergillus flavus*.

SL-180*Track: Plant and Environment***EVIDENCE OF METABOLIC COMPETITION BETWEEN METHIONINE AND GLUTATHIONE BIOSYNTHETIC PATHWAYS****Yael Hacham, Jong Yoon, Ifat Matityahu, Alex Kaplan, Lilach Kachan, David Oliver and Rachel Amir***Chairperson and Research Scientist, Laboratory of Plant Science, Migal Galilee Technology Center, P.O. Box 831, Kiryat Shmona 11016, Israel; E-mail: rachel@migal.org.il*

Cysteine (Cys), the first organic sulfur-containing metabolite, serves as a precursor for the synthesis of glutathione and methionine (Met), two metabolites that are central to plant growth and survival. Glutathione plays a crucial role in the defence against a wide variety of environmental stresses, while Met is a protein constituent, and through its first metabolite, S-adenosylMet (SAM), regulates essential processes required for plant growth. To reveal the relations between glutathione and Met, we used tobacco plants overexpressing the regulatory enzyme of Met biosynthesis pathway, cystathionine γ -synthase (CGS), and those overexpressing the yeast gene encoding a feedback-insensitive O-acetylserine (thiol)lyase (OASTL) in the plastids and in the cytosol that regulate the levels of Cys and glutathione. We crossed between the two transgenic lines to determine that the level of Met can significantly increase in plants overexpressing the plastidic OASTL with AtCGS, accompanied by a reduction in glutathione. The results strongly suggest that the flux towards Met is relatively high, and thus Met can be considered as an intermediate

metabolite in the pathways leading to its various associated metabolites. In addition, flux and metabolic profiling analyses indicated the existence of metabolic competition between the biosynthesis pathways of Met and glutathione on their common precursor, Cys, and that this competition is more crucial under oxidative conditions when more Cys is required for the synthesis of glutathione. Plants overexpressing AtCGS with or without the yeast enzyme were significantly more sensitive to oxidative stress, indicating the reason why the levels of Met remained low during the evolution.

SL-121

Track: Medical Biotechnology

CONVERGENCE: A TRANSFORMATIVE APPROACH TO ADVANCED RESEARCH AT THE INTERSECTION OF THE LIFE, PHYSICAL AND ENGINEERING SCIENCES

Amanda Arnold and Melvin Greer

Texas A&M University, Texas, United States; E-mail: amanda.arnold@tamu.edu

Convergence — an integration of the knowledge, tools, and ways of thinking from the life and health sciences; physical, mathematical, and computational sciences; the engineering disciplines; the social and behavioral sciences; and the humanities to form a comprehensive framework for tackling scientific and societal challenges that exist at the interfaces of multiple fields — is a movement gaining traction in universities across the country. Industry sees the approach as critical to educating the 2020 workforce it needs to deliver the advanced technological products and services that will otherwise go uncommercialized without the enhanced workforce capabilities for which Convergence offers the blueprint.

Keywords: Convergence; Team Science; Transdisciplinarity; Bioscience; Science & Technology; and Workforce and Talent.

SL-21

Track: Regenerative Medicine

NATIVE EXTRACELLULAR MATRIX – A PARADIGM FOR TRANSLATABLE REGENERATIVE MEDICINE

Nathaniel Bachrach

Research and Development, LifeCell Corporation, Poland; E-mail: NBachrach@Acelity.com

One can define regenerative medicine as medical practice that harnesses the body's intrinsic capacity for self-renewal and its ability to regenerate tissues and organs. Historically, within the context of tissue engineering or regenerative medicine, the basic paradigm to develop a product that will translate to the clinic has been to require: 1. A structure that provides an immediate physical function and a 3-D scaffold for cells, 2. Cells that induce the de novo tissue formation and 3. Growth factors and cytokines that modulate the biological response. A review of clinical applications of regenerative medicine products indicates that native extracellular matrix (ECM)-based scaffolds have been introduced to patient care most successfully. Examples include demineralized bone matrices used in orthopedic surgery and soft tissue-based matrices used for soft tissue reconstruction. One reason for this commercial and clinical success is that these tissue matrices have been introduced to the market place as medical devices, while the regulatory hurdles for cell and active molecule based products are more challenging. The clinical success of these products challenges the old paradigm and suggests that the critical element for successful tissue regeneration is the ECM. The host has cells capable of regenerating tissues and much of the signaling required for tissue specific regeneration can come directly and indirectly from the matrix. As cells from the host interact with the matrix they can add additional signaling through growth factors and cytokines to modulate biologic response. In this way, all three elements are achieved through a simpler delivery paradigm.



A New Paradigm

Matrices engineered to provide the ideal regenerative environment may include biochemical cues, binding sites, micro and macro structure and mechanics, and physico-chemical properties and will successfully leverage the body's capacity to regenerate. Key factors that are critical when introducing an ECM-based matrix in clinical practice are scientific, clinical, and economic data. Scientific data encompass product characterization (e.g. chemistry), and preclinical work that focuses on the immune response of the product using appropriate animal models.

The future will likely see the introduction of newer ECM-based technologies designed to target challenging clinical needs previously thought to require cells and active factors. This new paradigm for tissue engineering to provide regenerative solutions will be even more essential to exploit as changes in the regulatory environments and health care systems world-wide make advancements in alternative strategies even more challenging.

SL-100

Track: Pharmaceutical Biotechnology

IN VITRO PROLIFERATIVE EFFECT OF ALOE VERA EXTRACTS ON HAIR FOLLICLES ISOLATED FROM THE ALOPECIA ARETA PATCH

Mohite, S. L. and Bagde, U.S.

Applied Microbiology Laboratory, Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai 400098, India; E-mail: bagdeu@yahoo.com

In vitro effect of Aloe vera root and sap extracts on hair follicle growth was studied by using organ culturing. Two parameters viz: morphological changes and measurement of growth of hair follicles during the time of incubation were studied. Hair follicles were isolated by enzymatic digestion from the scalp of alopecia areta patch and normal scalp. Hair follicle growth was assessed on the basis of proliferation of the peripheral cells as well as elongation of the follicle on 3rd, 6th, and 10th days of incubation. During this incubation period the normal follicles were found to be proliferating in the serum free medium, but the abnormal follicles did not show any proliferation. The follicles were then studied further for an *in vitro* growth in presence of Aloe vera extracts. The experiments were carried out in triplicates. The abnormal hair follicles incubated with the Aloe vera root and sap extracts at the concentration of 10ul/ well, showed *in vitro* growth while few of the extracts did show cytotoxicity indicated by the shrinking of the follicles, whereas the normal follicles showed enhancement of the growth. The results implicated a stimulatory role for tested Aloe vera extracts in accelerating *in vitro* growth of abnormal and normal hair follicles.



SL-289

Track: Medical Biotechnology

TARGETED DELIVERY OF LIPOSOMES AND NANOPARTICLES IN HIGH GRADE GLIOMA CELLS IN VITRO AND IN VIVO

Vladimir P. Baklaushev, Svetlana E. Gelperina, Pavel A. Melnikov, Yulia Malinovskaya, Anna N. Gabashvily and Vladimir P. Chekhonin

Medicinal Nanobiotechnologies, Pirogov Russian State Medical University, Federal Research and Clinical Centre FMBA of Russia, Russia; E-mail serbskiy@mail.ru

As shown by the *in vitro* and *in vivo* experiments, the surface modification of both, polylactide-co-glycolide (PLGA) nanoparticles and liposomes considerably enhances the uptake of these carriers in the glioma cells. *In vitro* the level of the 150-nm liposome internalization in the rat C6 glioma and human U87 glioma cells was enhanced 2-fold by their vectorization with the antibodies to VEGF or VEGFR2. Similarly, the coating of the 150-nm PLGA nanoparticles with

poloxamer 188 (P88) resulted in a 1.5-fold increase of their uptake, as compared to the non-coated particles ($p < 0.01$). The uptake mechanism of the P188-coated PLGA nanoparticles was shown to be clathrin-dependent. Coating with P188 also enhanced the uptake of the i.v. injected PLGA nanoparticles in the brains of healthy rodents: the brain/liver concentration ratio was increased by 35%, as compared to the non-coated nanoparticles. As shown by laser scanning confocal microscopy and immunofluorescence study, both coated and uncoated PLGA nanoparticles were internalized in the neurons of cerebral cortex and cerebellum.

In the intracranially implanted C6 glioma the accumulation of the P188-coated nanoparticles was 3 times higher than that of the uncoated nanoparticles. In conclusion, the immunoliposomes and P188-coated PLGA nanoparticles appear to be the promising carriers for drug delivery to the brain.

SL-42

Track: Regenerative Medicine

SCARLESS SURGERY: ARE WE THERE YET?

Swathi Balaji

Research Associate, Center for Molecular Fetal Therapy, Cincinnati Children's Hospital Medical Center, USA; E-mail: Swathi.Balaji@cchmc.org



INTRODUCTION

More than 100 million patients acquire scars annually in the developed world, for which there is no effective therapy. The fetal response to cutaneous injury is regenerative and can be recapitulated in postnatal wounds by viral mediated over-expression of IL-10. We hypothesized that sustained delivery of biologically active recombinant IL-10 could be facilitated through a more clinically translatable hydrogel delivery system and used to promote regenerative healing in postnatal wounds.

METHODS

Optimal hydrogel composition for sustained IL-10 release was determined by evaluating *in vitro* multiple hydrogel formulations containing hyaluronan/heparin sulphate and collagen loaded with recombinant IL-10. IL-10 release was quantified (ELISA) and conditioned media from the gels were collected daily and used to treat adult dermal fibroblasts to quantify pericellular matrix (PCM) formation and migration. *In vivo*, an excisional wound model in C57BL/6J mice was used to evaluate the effects of hydrogel mediated IL-10 release on scar attenuation. 4mm wounds were evaluated at 28 days. Additional controls included, gel control, lentiviral IL-10 (LV-IL-10) and PBS ($n=4$ /group). Histological evaluation (H&E) and capillary density (CD 31+ caps/HPF) of uninjured skin and scars were performed ($n=20$) and observed differences were used to establish a quantifiable parameters-based novel histologic scar scale, which was used to compare treatments. Data presented as mean \pm SD, p-values by ANOVA.

RESULTS

HH10, a gel made of 2:1:1 (hyaluronan conjugated with heparan sulphate, type-I collagen and polyethylene glycol diacrylate) and IL-10 (800ng/25ul) resulted in optimal sustained release of IL-10 *in vitro*, which is biologically active and increased PCM formation and migration by fibroblasts. *In vivo*, histologic analysis demonstrated significant differences between uninjured skin and scar in epidermal height and topography, nuclear orientation of the basal keratinocytes, scar area, dermal appendages and vascular density (column A vs.B; $p < .01$). HH10 treatment resulted in wound healing indistinguishable from surrounding skin, with significantly improved scar parameters compared to characteristic scar in PBS wounds (column D vs. B; $p < .01$). HH10 restores epidermal and dermal scar parameters to the levels observed in uninjured skin (column D vs. A; $p = \text{ns}$). Scar assessment reveals HH10 and viral over-expression of IL-10 are equally potent in achieving attenuation of scar (column D vs. C; $p = \text{ns}$). Gel treatment without IL-10 improves wound healing compared to PBS (column E vs. B; $p < .05$), but not to levels seen with HH10 or LV-IL-10 (column E vs. D or C; $p < .05$).

Quantifiable Scar Parameters to Histologically Assess Murine Wound Repair

	A. Skin	B. Scar	C. LV-IL-10	D. HH10	E. Gel Control
1. Epidermal Height (μm)	16.62 \pm 2.38	22.23 \pm 3.20	13.80 \pm 1.02	14.40 \pm 1.6	18.67 \pm 1.7
2. Orientation of Basal Keratinocytes	0.825 \pm 0.059	0.309 \pm 0.08	0.673 \pm 0.06	0.778 \pm 0.085	0.492 \pm 0.08
3. Scar Area (μm^2)	0	475435.7 \pm 112257.6	176820.3 \pm 124749.3	87797.5 \pm 90083.2	233023.2 \pm 116399.9
4. Dermal Appendages (per 200 μm)	4.02 \pm 1.69	1.388 \pm 0.17	3.312 \pm 0.60	4.99 \pm 1.94	1.403 \pm 0.7
5. Vascular Density (per 200 μm)	17.37 \pm 2.33	10.14 \pm 3.40	17.17 \pm 5.35	19.91 \pm 2.4	16.14 \pm 2.2

CONCLUSIONS

Our novel quantifiable method to assess scars histologically demonstrated that sustained release of biologically active recombinant IL-10 using a hyaluronan-based hydrogel (HH10) is capable of restoring epidermal and dermal parameters in postnatal wounds to the levels observed in unwounded skin, the benchmark of regenerative healing. HH10 obviates some of the translatable concerns with IL-10 gene therapy, and has broad potential applications beyond the cosmetic benefit, to any disease characterized by excessive fibroplasia.

SL-119

Track: Medical Biotechnology

FINITE ELEMENT ANALYSIS IN ORAL IMPLANTOLOGY

Nilufer Balcioglu and H. A. Balcioglu

Department of Oral Implantology, Faculty of Dentistry, Istanbul University, Istanbul, Turkey;
E-mail: habalci@gmail.com

A dental implant is a surgically placed fixture that interfaces with the human jaw to support a dental prosthesis. Success and failure of implants principally depends on the biomechanical factors. Occlusal loads are transferred to the bone around the dental implants *via* the implant-supported prostheses. The loads cause stress in the implant–bone contact area depending on the occlusal load type, size of implants, implant surface properties and structural characteristics of the bone on which the implants were applied. Mechanical stress applied to bone cells results in the constructing of new bone or resorption.

Occlusal loads that exceed the mechanical or biological load-bearing capacity of dental implants are defined as “overload”. Clinical computation of the direction and magnitude of occlusal loads-over loads is difficult. Recently, finite element analysis has been used to identify the loads transferred to dental implant, and the level and distribution of load in the bone around the implant. Finite element analysis allows for the evaluation of various biomechanical risk factors that can affect the success of dental implants in scenarios where clinical evaluation is not possible.

Finite element analysis for the evaluation of stress occurred around the dental implants will be presented step by step.



SL-113

Track: Medical Biotechnology

TEMPOROMANDIBULAR JOINT DISORDERS, BIOTECHNOLOGY AND EVOLVING TRENDS**Huseyin Avni Balcioglu**

Department Istanbul University Faculty of Dentistry, Department of Anatomy, Institution Turkey;
E-mail: habalci@istanbul.edu.tr



The attributes of cornerstones of the recent technical developments in the field of temporomandibular joint disorders, defined as a group of disorders characterized by pain and tenderness in temporomandibular joint and/or the masticatory muscles, sounds in the TMJs and limitation or deviations in mandibular range of motion, as well as a few details of novel biotechnological methods will be briefly reviewed in this presentation. It will focus on the clinical consequences of the technical progress concerning the temporomandibular joint diseases that are important from the point of view of dental medicine. Some novel innovations till today with a clinical relevance will also be discussed. Epidemiological studies have shown that temporomandibular joint disorders have a high prevalence in the society. The future of temporomandibular disorders therapy within a more biotechnological approach seems promising and advancing. Continued advances in the management of temporomandibular joint disorders should lead to the elevated use of modern combined modality interventions with an associated further improvement in patient outcome.

SL-192

Track: Plant and Environment

THE DECONSTRUCTION OF SEED MUCILAGES**Rachel A. Burton**

Australian Research Council Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, Urrbrae, SA 5064, Australia; E-mail: rachel.burton@adelaide.edu.au



Some plant seeds produce mucilage upon contact with water. Two well-known examples are *Plantago ovata* and Chia (*Salvia hispanica*). The dry mucilage from *Plantago* is removed from seeds by milling and marketed as Metamucil® whilst Chia is sweeping across the planet as a “superfood”; both are promoted as being beneficial for human health, including for the control of Type II diabetes, coronary heart disease and colon cancer. These claims are based on the bacterial fermentation of the polysaccharides within the mucilage, once it reaches the large intestine. We have undertaken a detailed biochemical analysis of both mucilage types and have carried out *in vitro* fermentation studies, allowing metagenomic profiling of the bacterial species able to metabolise the polysaccharide components. Transcriptome analysis is also underway to identify genes encoding polysaccharide synthases, information also relevant to economically important cereals wheat and barley which contain similar polysaccharides in the grain. Genes from Chia may be particularly interesting as this mucilage contains novel polysaccharide components that haven’t been identified in other higher plants.

SL-119(a)*Track: Plant and Environment***VARIATION OF ALGAE OCCURRENCE BEFORE AND AFTER WEIR CONSTRUCTION AT MULGEUM SITE IN DOWNSTREAM THE NAKDONG RIVER, SOUTH KOREA****Ji-Sung Lim, Jae-Ho Lee, Young-Woo Kim, Sang-Hoon Lee, Hyang-Gi Kim, Tae-Joo Park and Im-Gyu Byun***Institute for Environmental Technology and Industry, Pusan National University, 63beon-gil, Geumjeong-gu, Busan, Republic of Korea; E-mail: big815@pusan.ac.kr*

Mulgeum intake station located in downstream the Nakdong River supplies raw drinking water of Busan metropolitan city in South Korea. As eight weirs were constructed by 'Four Major River Restoration Project' in Nakdong River during 2009~2012, water environment of Nakdong River had changed significantly. Therefore, the changes of algae occurrence and water qualities were monitored and analyzed to evaluate the effects of weir construction on water environment using the each three years' data before and after of weir construction. As a result, water qualities in downstream the Nakdong River were improved after weir construction. Chl-a, BOD, T-P and PO₄-P concentration were significantly decreased after weir construction. Especially the average Chl-a concentration was decreased from 119 mg/m³ to 47 mg/m³ in winter. This phenomenon of Chl-a decrease is well corresponded with the decline of T-P and PO₄-P. And it is considered that the improvement of water qualities in downstream Nakdong River is affected with the cut off effect of nutrients by the weirs in midstream and upstream. So algae occurrence in upstream and midstream the Nakdong River should be studied later.

Keywords: Algae, Nakdong River, Water quality, Weir, chl-a.

SL-286*Track: Marine Biotechnology***GENETICALLY SHAPING MORPHOLOGY OF THE FILAMENTOUS FUNGUS ASPERGILLUS GLAUCUS FOR PRODUCTION OF ANTITUMOR POLYKETIDE ASPERGIOLIDE A****Menghao Cai, Ying Zhang, Wei Hu, Wei Shen, Zhenzhong Yu, Weiqiang Zhou, Tao Jiang, Xiangshan Zhou and Yuanxing Zhang***School of Biotechnology, East China University of Science and Technology, China; E-mail: cmh022199@ecust.edu.cn*

Background: For filamentous fungi, the basic growth unit of hyphae usually makes it sensitive to shear stress which is generated from mechanical force and dynamic fluid in bioreactor, and it severely decreases microbial productions. The conventional strategies against shear-sensitive conundrum in fungal fermentation usually focus on adapting agitation, impeller type and bioreactor configuration, which brings high cost and tough work in industry. This study aims to genetically shape shear resistant morphology of shear-sensitive filamentous fungus *Aspergillus glaucus* to make it adapt to bioreactor so as to establish an efficient fermentation process.

Results: Hyphal morphology shaping by modifying polarized growth genes of *A. glaucus* was applied to reduce its shear-sensitivity and enhance aspergiolide A production. Degenerate PCR and genome walking were used to obtain polarized growth genes *AgkipA* and *AgteaR*, followed by construction of gene-deficient mutants by homologous integration of double crossover. Deletion of both genes caused meandering hyphae, for which, Δ AgkipA led to small but intense curves comparing with Δ AgteaR by morphology analysis. The germination of a second germ tube from conidiospore of the mutants became random while colony growth and development almost maintained the same. Morphology of Δ AgkipA and Δ AgteaR mutants turned to be compact pellet and loose clump in liquid culture, respectively. The curved hyphae of both mutants showed no remarkably resistant to glass bead grinding comparing with the wild type strain. However, they generated greatly different broth rheology which further caused growth and metabolism variations in bioreactor fermentations. By forming pellets, the Δ AgkipA mutant created a tank environment with low-viscosity, low shear stress and high dissolved oxygen tension, leading

to high production of aspergillide A (121.7 ± 2.3 mg/L), which was 82.2% higher than the wild type.

Conclusions: A new strategy for shaping fungal morphology by modifying polarized growth genes was applied in submerged fermentation in bioreactor. This work provides useful information of shaping fungal morphology for submerged fermentation by genetically modification, which could be valuable for morphology improvement of industrial filamentous fungi.

SL-281

Track: Regenerative Medicine

X-RAY SYNCHROTRON PHASE CONTRAST TOMOGRAPHY FOR THE INVESTIGATION OF REGENERATIVE TREATMENT IN NEURODEGENERATIVE DISEASES

Inna Bukreeva, Michela Fratini, Gaetano Campi, Raffaele Spanò, Valentina Petrosino, Ranieri Cancedda, Nicole Kerlero de Rosbo, Antonio Uccelli, Alberto Bravin, Maddalena Mastrogiacomo and Alessia Cedola

Institute of Nanotechnology, Laboratory of Soft and Living Matter-CNR, c/o Physics Department at 'Sapienza' University, Piazzale Aldo Moro 2, 00185 Rome, Italy; E-mail: alessia.cedola@cnr.it

We apply X-ray Synchrotron Phase Contrast Tomography (XSPCT) to the 3D imaging of Vascular Network (VN) and Neuronal System (NS) in mouse spinal cord for the investigation of neurodegenerative diseases. We demonstrate the capability of XSPCT to simultaneously visualize the three-dimensional VN and NS of mouse spinal cord at scales spanning from millimeters to hundreds of nanometers, without contrast agent and without a destructive sample preparation, which could lead to data misinterpretation.



The present work mainly focuses on the pre-clinical study, by XSPCT, of spinal cord from mice with experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis. We investigate 3D distribution of the axons fibers, glia and neuron cells of NS, together with the vascular tree and micro-capillaries network. We compare healthy samples, untreated EAE affected samples and EAE affected samples treated by i.v. injection with mesenchymal stem cells, a subset of adult progenitor cells with immunomodulatory and neuroprotective properties, which ameliorate EAE and are being considered as alternative therapy for neurological diseases.

The comparison between tissues from control mice, mice affected with EAE and treated mice shows the relationship between blood-spinal cord-barrier impairment and neuroinflammation, and shows how this relationship is affected by treatment with mesenchymal stem cells.

SL-193

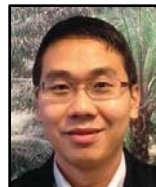
Track: Pharmaceutical Biotechnology

ANTIBACTERIAL ACTIVITY, MECHANISM OF ACTION AND PHYTOCHEMICAL STUDIES OF CYNODON DACTYLON (L.) PERS. SOLID PHASE EXTRACT (SPE) AGAINST SOME BACTERIAL PATHOGENS

Syahriel Abdullah, Januarius Gobilik and Khim Phin Chong

Sustainable Palm Oil Research unit (SPOR), Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia; E-mail: chongkp@ums.edu.my

Cynodon dactylon (L.) Pers. is belonging to Poaceae family which used as folk medicine to treat many diseases and infections. The present study reports the antibacterial activity and mechanism of action of *C. dactylon* Solid Phase Extract (SPE) against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumonia* along with the possible antibacterial compounds. Antibacterial activity and Minimum



Inhibitory Concentrations (MICs) were evaluated using disc-diffusion and micro-broth dilution bioassays respectively. Mode of action study was done *via* assessment on the leakage of 260nm-absorbing materials, fluorometric assay and Scanning Electron Microscope (SEM) observation for membrane disintegration effect while luminometric assay was used for metabolic inhibition assessment. Remarkable antibacterial activity was observed from flush fraction of *C. dactylon* SPE against the tested bacterial pathogens (MICs=10.00 mgmL⁻¹). Assessment on membrane degradation based on 260nm-absorbing material leakage and fluorometric assay showed membrane disruption on *B. cereus*, *B. subtilis* and *E. coli* after treated with the plant extract. SEM observation further confirmed the membrane disruption. Luminometric assay based on ATP quantification suggests the bacterial death was probably due to other metabolic factor. Liquid Chromatography-Mass Spectrometry (LCMS) analysis revealed some possible antibacterial compounds including peptides, polyketides, triterpenoid, cardenolide glycoside and some steroidal compounds.

Keywords: *Cynodon dactylon*, antibacterial, mode of action, phytochemical.

SL-174

Track: Plant and Environment

HIGHLY EFFICIENT GENOME EDITING FOR CROP IMPROVEMENT

Feng Zhang, William J. Haun, Benjamin Clasen, Thomas Stoddard, Zachary Demorest, Jin Li, Song Luo, Dan Voytas and Luc Mathis

Collectis plant sciences, New Brighton, Minnesota, USA; E-mail: zdemorest@collectis.com

The burgeoning demand for plant-derived products, such as food, feed, fuel and fiber, underlies the importance of methods to continuously improve crop varieties with higher yields, lower input costs and better nutrition value. Recent advances in precise genome editing open up new opportunities to develop novel crop varieties with valuable traits. Precise genome editing often require targeted cleavage of specific chromosomal sequences, which generates single or double strand DNA breaks and activates endogenous DNA repair pathways. To date, four classes of programmable sequence-specific nucleases, meganuclease (homing endonuclease), zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) nuclease, have been developed to cleave almost any sequences in any species. In this study, TALEN technology is employed to edit complex crop genomes. High frequency of gene editing events has been identified from a number of crop species, such as soybean, potato and canola. High value traits generated through this technology and their regulatory status will be presented.



SL-228

Track: Medical Biotechnology

TRANSCUTANEOUS APPLICATIONS OF FOAM METALS

Ian D. Dickey

Graduate School of Biomedical Science and Engineering Department of Chemical and Biological Engineering, University of Maine, Room 307A - Jenness Hall, Orono, Maine, U.S.A. 04469; E-mail: ian.dickey@maine.edu

Titanium metal devices have been used extensively in the past for orthopedic applications, such as external fixative pins, hip replacements and other femoral prosthetic devices. The challenge of employing such devices for transcutaneous applications is inherent in the high rates of infections often associated with implantation through dermal layers. In the present work transcutaneous foam metal devices have been shown to exhibit biocompatibility and lower infection rates by promoting the in-growth of soft tissue. Such devices, when implanted through dermal layers in a transcutaneous fashion, form a biological seal that prevents bacteria from migrating into subcutaneous tissue. Through additive manufacturing, we have developed novel devices of varying material and pore geometries that utilize a highly porous

network that facilitates dermal and subcutaneous tissue in-growth. The utilization of such an approach in the efforts to develop an implant that could anchor an artificial limb directly to bone could hold great promise in the future of biomedical science and potentially revolutionize prosthetics.

SL-34

Track: Regenerative Medicine

SYNER-III: AN *IN VIVO* METHOD TO INDUCE PANCREATIC BETA CELL FORMATION IN THE ADULT PANCREAS

Bruno Doiron and Ralph A DeFronzo,

Dept of Medicine – Diabetes Division, University of Texas Health Science Center at San Antonio, San Antonio, Texas, 78229, USA; E-mail: doiron@uthscsa.edu



Syner-III offers a new treatment paradigm for the treatment of type 1 diabetes and type 2 diabetes mellitus compared to current approaches to treat diabetes. The intellectual property encompasses approaches to preferentially increase the number of beta (insulin-producing) cells in the adult pancreas without increasing the number of non-insulin-producing cells.

The technology provides for *in vivo* exposure of pancreatic cells to three compounds that work synergistically at the cellular level in the pancreas to stimulate formation of insulin-producing beta cells. The method acts on post-developmental mechanisms to induce beta cell formation without stem cells and without activating the embryonic pathway; thus, undesired cells are not induced. Prior attempts at beta cell regeneration have relied upon pancreatic injury to induce beta cell proliferation, dedifferentiation and activation of the embryonic pathway, or stem cell replacement. We report on an alternative method to transform adult non-stem (somatic) cells into pancreatic beta cells. The Syner-III approach targets cellular mechanisms involved in pancreatic function in the organ's adult state and utilize a synergistic approach that integrates three important levels of cellular regulation to induce beta cell formation: (i) glucose metabolism, (ii) membrane receptor function, and (iii) gene transcription. Prior methods with cocktails of transcriptional factors and stem cells produce hybrid cells which coexpress glucagon and/or somatostatin. We demonstrate in this paper that Syner-III induces beta cell formation directly from adult pancreatic somatic cells. This approach to increase the number of insulin secreting pancreatic beta cells in adult subjects can be used as prevention, treatment, or cure of diabetes. We also compared Syner-III to a three-pronged cocktail comprised only of transcriptional factors and demonstrate that integration of multiple levels of cellular physiology is essential to produce a synergistic effect to induce beta cell formation that cannot be achieved simply by employing a cocktail of transcriptional factors that only target nuclear reprogramming *in vivo*. Further, we demonstrated that Syner-III restores normoglycemia and beta cell function (insulin secretion) for in excess of one year in a wild type mice mouse model.

IMPACT:

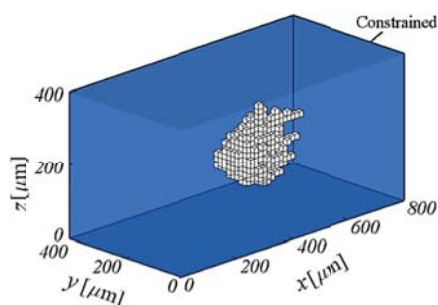
- A new therapy for Type 1 and Type 2 diabetes represents a significant and compelling unmet medical need
 - Syner-III represents a new paradigm in diabetes treatment that is completely distinct from all prior approaches.
 - Solid proof-of-concept has been established to “cure” diabetes in a mouse model
-

SL-91*Track: Regenerative Medicine***NUMERICAL MODELING OF MECHANOTACTIC INFLUENCE ON CELL MORPHOLOGY****Mohamed Hamdy Doweidar and Seyed Jamaledin Mousavi**

Group of Structural Mechanics and Materials Modelling (GEMM), Mechanical Engineering Department, School of Engineering and Architecture (EINA), University of Zaragoza, María de Luna s/n, Edificio Betancourt, 50018 Zaragoza, Spain; E-mail: mohamed@unizar.es



Cell morphology is a key aspect in many biological processes such as morphogenesis, tumor growth and wound healing. Among other cues, mechanical characteristic of the surrounding micro-environment can control the cell morphology. It is well known that traction forces transmitted to the extracellular matrix (ECM) through cell focal adhesions and integrins play a fundamental role in this process by rearranging the cell cytoskeleton (CSK). In this work we have developed a novel 3D computational model to comprehensively predict the evolution of cell morphology during migration due to mechanotaxis.



A discrete methodology is here chosen by which the cell is represented by a group of finite elements. Therefore, during migration, the cell shape can be efficiently remodeled in a free mode. The present model is developed based on equilibrium of the effective forces over the cell body; the traction force, the protrusion force and the drag force. The cell traction force is governed by the cell internal deformation. The random protrusion force is generated by actin polymerization. The drag force is the substrate viscous resistance.

Correlated with experimental observations, the present model illustrates that the morphology of an adherent cell can be controlled by substrate stiffness and boundary conditions. Our findings indicate that within an unconstrained substrate with a soft (several kPa) and hard (>200 kPa) stiffnesses, the cell is unable to adhere or penetrate into the substrate so that the cell remains mainly rounded without any specific preference of migration direction. In contrast, when a cell is located within a substrate with an intermediate (10 kPa) and rigid (100 kPa) stiffnesses the cell can actively adhere to the substrate migrating towards the constrained surfaces. It can be concluded that in the intermediate and rigid substrates the higher the traction force, the greater the cell elongation, the larger the cell membrane area, and the less random the cell alignment.

ACKNOWLEDGEMENTS

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Keywords: Finite Element Method, Cell Morphology, Cell migration, Mechanotaxis.

SL-274*Track: Plant and Environment***EVALUATION OF HIGH BETA-CAROTENE CASSAVA GENOTYPES AT ADVANCED TRIAL IN NIGERIA****Ngozi Edoh, Ogbokiri, H., Njoku, D.N and C.N. Egesi***National Root Crops Research Institute (NRCRI), Umudike, Km 8 Ikot Ekpene Road, PMB 7006, Umuahia 440001, Nigeria; E-mails: ngoziedoh@gmail.com; ngozi.edoh@nrcri.org*

Thirteen cassava genotypes were evaluated from advanced field trial for total carotene content (TCC), dry matter content (DMC), fresh root yield (FRY) and biotic stresses alongside two widely cultivated cassava varieties (UMUCASS 36 and TMS 30572) as checks in 2013/2014 cropping season at National Root Crops Research Institute (NRCRI) Umudike in Nigeria. The objective of the experiment was to select promising clones for possible release to Nigeria farmers. The results of the TCC analysis ranged from 1.68–10.16 µg/g. Genotypes NR11/0063, NR11/0123 and NR11/0140 had TCC ranged from 10.47–8.72 (µg/g) which are above the national checks (UMUCASS 36 and TMS 30572) of 8.34 and 1.68(µg/g) respectively. The DMC of the genotypes ranged from 33.0–15.0 %. TMS 30572 gave the highest DMC (33.0%) while genotype NR11/0064 had the least (15.0%) DMC. Severity of cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava green mite (CGM) and cassava anthracnose disease (CAD) evaluated for 12 months varied among the genotypes. Symptom expressions of CMD, CBB and CAD were relatively higher (3.0) for genotype NR11/0123 and this resulted to low fresh root yield (FRY) of 18 t/ha whereas TMS 30572 with moderate severity (1.8) had a higher FRY of 32.6 t/ha. The severity of CGM was moderate across the genotypes with no significant difference ($P < 0.05$) observed. Further analysis showed that CMD, CBB and CAD were significantly and positively correlated ($r = 0.86, 0.64$ and $0.55, p < 0.01$) with harvest index (HI) among the genotypes evaluated. Three genotypes were selected for further evaluation and possible release to Nigeria farmers.

Keywords: Cassava, High Beta-carotene, Biotic stress, Harvest index.**SL-152***Track: Regenerative Medicine***SCALE-UP OF IMMUNE CELL THERAPIES FOR ORGAN TRANSPLANTS****Dr Marianne Ellis, Dr Fadi Issa and Mr Jon Pleat***Department of Chemical Engineering and Centre for Regenerative Medicine, University of Bath, Bath, UK.; E-mail: M.J.Ellis@bath.ac.uk*

Organ transplants require the recipient to take immunosuppressant drugs for their entire life to prevent rejection of the allogeneic tissues [1], which may cause side effects. Recently, work has been carried out to harness the inherent immune control mechanisms [2] and an alternative to immunosuppressant drugs is the administration of a large dose of natural regulatory T cells (Tregs) [2]. A therapeutic number of cells (suggested to be 30×10^6 cells/kg [3]) need to be made available at the right time. The current *in vitro* expansion process for Tregs is well established at bench scale using static multi-well plate culture; the cells are split after 4 days then every day, cultured in the presence of Life Technologies™ Dynabeads®. A robust and cost effective Treg expansion bioprocess would enable Treg therapy to replace immunosuppressant drug therapy and so improve the post-transplant outcome for the patient's quality of life, reduce or eliminate additional NHS care due to complications, and reduce the cost of transplantation. Here we present a semi-automated cost-effective process for Treg cell therapy manufacture, based around a fluidized bed bioreactor.

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SL-85*Track: Medical Biotechnology***SPECIFIC RECOGNITION OF THE HIV-1 GENOMIC RNA BY THE GAG PRECURSOR****Ekrum W. Abd El- Wahab, Redmond P. Smyth, Elodie Mailler, Serena Bernacchi, Valérie Vivet-Boudou, Marcel Hijnen, Fabrice Jossinet, Johnson Mak, Jean-Christophe Paillart and Roland Marquet***Tropical Health Department, High Institute of Public Health, Alexandria University, Egypt;
E-mail: ekramwassim@mail.com*

During assembly, HIV-1 must select its genomic RNA (gRNA) from a variety of cellular and viral spliced RNAs. Despite a large number of studies, there is no consensus on how the Pr55Gag precursor achieves this selection. These studies were limited by the expression and purification of intact full-length Pr55Gag protein. Here, we purified soluble full-length Pr55Gag and we investigated the specific determinants of the selective binding of Pr55Gag to HIV-1 gRNA using RNA binding and footprinting assays. Our results revealed that Pr55Gag exhibits a higher binding affinity for gRNA than for spliced vRNA species. Importantly, we demonstrate that the primary Pr55Gag binding site consists of the internal loop and the lower part of stem-loop 1 (SL1), the upper part of which initiates gRNA dimerization. Further analyses on viral RNA fragments of different length spanning the Psi and/or its flanking regions are in favor of a long-distance tertiary interaction involving sequences upstream of SL1 and downstream of SL4, which promotes the optimal binding of Pr55Gag to gRNA. Altogether our data shed light on the importance of a proper gRNA conformation that regulates its specific binding to Pr55Gag, and could result in the competent selection and packaging of the genome. We propose a new model to explain how Pr55Gag discriminates and specifically selects gRNA from cellular RNAs and viral spliced vRNAs that also harbor functional SL1 in their first common exon. A double regulation ensures specific binding of Pr55Gag to the gRNA despite the fact that SL1 is also present in spliced viral RNAs. The region upstream of SL1, which is present in all viral RNAs, prevents binding to SL1, but this negative effect is counteracted by sequences downstream of SL4, which are unique to the gRNA.

Keywords: HIV-1, genomic RNA, specific recognition, Gag precursor.

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SL-30*Track: Other Areas***PLANT-BASED NUTRITION ADHERENCE STRATEGY HALTS CORONARY DISEASE****Caldwell B. Esselstyn and Mladen Golubić***The Wellness Institute, Center for Lifestyle Medicine, The Cleveland Clinic, 1950 Richmond Road, TR2-341, Lyndhurst, OH 44124, USA; E-mail: golubim@ccf.org***Introduction:** Coronary disease remains our number one killer, but is non-existent in plant-based cultures. Plant-based nutrition may cure cardiovascular disease yet patient adherence is challenging.**Methods:** We reported outcomes at 3.7 years for 198 consecutive cardiovascular patients counseled in plant nutrition and below review our strategy achieving 89% adherence.

Two weeks prior to the single 5 ½ hour counseling, a senior physician phoned each participant to establish disease presentation, severity and establish rapport. Each was asked to bring a spouse or partner. Participants learned the

integrity of endothelial cells is essential for vascular health. They understood oils, meat, fish, fowl, dairy products, eggs, nuts, avocado and sugary foods impair vascular protection. A plant-based nutrition expert reviewed food acquisition and preparation. Each participant received a notebook with a copy of power point slides, peer reviewed studies, recipes and strategies and a book with plant-based recipes. A lunch followed ending with question and answer session. Two prior successful participants gave testimonials. Exercise was encouraged. We invited follow up communication.

Results: 99.4 % of 177 plant-based adherent participants avoided cardiac events during 3.7 years follow up.

Conclusion: Plant-based nutrition education may halt coronary disease.

SL-141

Track: Pharmaceutical Biotechnology

LONG ACTING ANALOGS OF GLYCOPROTEIN HORMONES DESIGNED BY SITE-DIRECTED MUTAGENESIS AND GENE FUSION

Fuad Fares

Department of Human Biology, Faculty of Natural Sciences, University of Haifa, and Department of Molecular Genetics, Carmel Medical Center, Haifa, Israel; E-mails: ffares@sci.haifa.ac.il; ffares@clalit.org.il



One major issue regarding the clinical use of many peptides is their short half-life due to the rapid clearance from the circulation. To overcome this problem, we used overlapping PCR technique to add the signal sequence of O-linked oligosaccharides to the coding sequence of glycoprotein hormones. The used cassette gene contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin β (hCG β) subunit. It was postulated that the O-linked oligosaccharides add flexibility, hydrophilicity, stability and prevent plasma clearance and thus increasing the half-life of the protein in circulation. Using this strategy we succeeded to ligate the CTP to the coding sequence of follitropin (FSH), thyrotropin (TSH), erythropoietin (EPO) growth hormone (GH) and thus to increase the longevity and bioactivity of these proteins in-vivo. Interestingly, the new analog of FSH was found not immunogenic in humans and it was approved by the European Commission (EC) for treatment of fertility. In addition, our results indicated that long acting GH is not toxic in monkeys and the results from clinical trials phases I and II seem to be promising. Designing long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in the clinical protocols.

Deletion of the N-linked oligosaccharides from hTSH significantly reduced its activity *in vitro* and *in vivo*. Moreover, deglycosylated TSH compete with hTSH and human Thyroid Stimulating immunoglobulin (TSI) in a dose dependent manner. These variants may offer a novel therapeutic strategy in the treatment of hyperthyroidism and Grave's disease.

Keywords: Glycoprotein, follitropin, thyrotropin and erythropoietin.

SL-157

Track: Pharmaceutical Biotechnology

MOLECULE DRUG CANDIDATES – PROSPECTING, DEVELOPMENT, OPTIMIZATION AND CLINICAL TRIALS: TWO BRAZILIAN SUCCESSFUL CASES

Rui Seabra Ferreira, Daniel Carvalho Pimenta, Ricardo de Oliveira Orsi, Silvia Regina S. Barraviera, Luciana Patrícia F. Abbade, Alexandre Naime Barbosa, Luis Eduardo Ribeiro da Cunha, Benedito Barraviera

Center for the Study of Venoms and Venomous Animals (CEVAP), UNESP – Univ Estadual Paulista, Botucatu/SP, Brazil; E-mail: rseabra@cevap.unesp.br



To bridge the GAP between basic and applied sciences is needed to push forward disease research and therapeutics. What types of drug leads are truly “druggable”, sit in “patented bioproducts space” and can be pushed towards clinical trials? We present two successful translational cases of bioproducts from laboratory bench to the bedside.

Case 1: Fibrin sealant derived from snake venom (FSSV). This product is composed of a serine protease (thrombin-like enzyme) derived from South American rattlesnake (*Crotalus durissus terrificus*) venom and cryoprecipitate of buffaloes (*Bubalus bubalis*) rich in fibrinogen. The activity of snake venom serine protease is 1,500-fold more potent than that of human thrombin employed in commercial sealants. The cryoprecipitate replaces human hemoderivatives, which makes the production more affordable and eliminates the possibility of infectious disease transmission. FSSV is a bioproduct of animal origin produced at the Center for the Study of Venoms and Venomous Animals (CEVAP) of UNESP, Brazil, to treat chronic venous ulcers of 240 patients of a phase II/III multicenter clinical trial. The phase I/II clinical trial with ten patients was carried out and there was complete healing in 38.8% of the ulcers. There was decrease of their area with wound bed preparation in 33.3%, therefore a significant improvement in 72.1% of the cases was observed in only three months of treatment. Based on such results, the product was considered a safe candidate and clinically promising. In addition, FSSV is being successfully tested *in vitro* and *in vivo* for its use as a three-dimensional scaffold for stem cells, since it is biodegradable and maintains the cells viable at the application site.

Case 2: Africanized honeybee antivenom (AHBA). In Brazil, Africanized honeybees provoke more than 10 thousand envenomations and approximately 40 deaths per year. Since it is an issue limited to the American continent that had no specific treatment, a new antivenom to treat multiple stings was developed. One of the major challenges was the standardization of the product, because of the antigenic characteristics of the venom. The antivenom was developed at CEVAP and produced by the Vital Brazil Institute (IVB) based on horse hyperimmunization with the main toxic fractions of the venom. Each milliliter of AHBA neutralizes 1.25 mg of venom and 10 mL of antivenom can treat about 100 stings. AHBA is being tested in a phase I/II multicenter clinical trial with 20 participants. Its delivery route is intravenous and the administration follows a strict clinical protocol that aims to rapidly neutralize and eliminate the venom toxic effects.

Both products were patented and by the end of the clinical trials, they will be available in the Brazilian public health system (SUS). They will aid the treatment of chronic venous ulcer patients and multiple Africanized Honeybee sting victims, two major neglected issues. The involved team of researchers acquired the required expertise to develop new drugs through translational research by applying modern biotechnological tools and aiming at solving health problems with regional bioproducts.

Keywords: Clinical trials, Bioproducts, Translational research.

SL-210

Track: Plant and Environment

PLANT-BASED BIOPHARMACEUTICALS FOR EMERGENCY RESPONSES AND ALTERNATIVE RAPID PRODUCTION OF NOVEL BIOPHARMACEUTICALS**Rainer Fischer**

Institut für Biologie VII, RWTH Aachen University & Fraunhofer IME, Worringerweg 1, D-52074 Aachen, Germany; E-mail: fischer@molbiotech.rwth-aachen.de



The development of recombinant antibodies and vaccines has allowed us to treat and prevent a large number of life-threatening diseases. However, as things stand in 2015, the speed, capacity and scalability of current production systems is beginning to place limitations on this crucial technology. The large-scale production of antibodies, vaccines and other pharmaceutical recombinant proteins is restricted by the industry's current reliance on fermenter technology, particularly the culture of mammalian cells. This expensive and time-consuming production platform is preventing the distribution of recombinant protein drugs to those most in need. One way in which the above limitations can be addressed is through the use of plants and plant-based expression systems for rapid recombinant pharmaceutical protein production.

The economic production of plant-based pharmaceuticals depends on satisfactory yields and product quality. This presentation will discuss the latest development in antibody and vaccine development and their production by molecular farming, focusing particularly on strategies to maximize protein yields during upstream production and optimize protein recovery in the downstream processing steps. Such strategies often involve careful consideration of how the protein is expressed and targeted within the plant cell, a factor which affects yield, stability, quality and ease of isolation. Our long-term objective is to ensure that next generation of plant based production systems for recombinant proteins will create the opportunity to deliver antibodies, vaccines and other biopharmaceuticals beyond the industrialized nations and into the developing world. Several case studies will be presented: HIV antibodies were chosen to undergo fast-track development, including risk assessment, expression in tobacco, scale-up, downstream processing and regulatory development, with the aim of performing clinical trials. In addition use of engineered plant cells for human vaccine candidates will be discussed.

Pharma-Planta is an EU Sixth Framework Integrated Project whose primary goal is to develop an approved production pipeline for plant-derived pharmaceutical proteins (PDPs). Although previous research has provided proof of the PDP concept, Pharma-Planta aims to develop an entire production chain by taking candidate pharmaceutical molecules from the expression platform through all stages of production and processing, ultimately to initiate phase I human trials in Europe. The Pharma-Planta Consortium comprised 40 interacting groups representing 33 public institutes and SMEs from 11 European Member States and South Africa.

At the beginning of the project, eight target molecules were chosen representing four key indication areas including HIV. From these molecules, two HIV antibodies were chosen to undergo fast-track development, which would include risk assessment, cloning, expression and optimization of production in plants, scale-up, downstream processing and regulatory development, with the aim of submitting at least one of them for clinical trials within the five years of the program. Two HIV neutralizing antibodies have been expressed successfully in the two main production crops being developed within the consortium – maize and tobacco. One of these antibodies, 2G12, has been expressed at levels greater 100 mg per kg of plant material. The plant-derived antibodies remain stable and functional and retain their neutralizing activity. The consortium has investigated novel upscaling and downstream processing strategies to provide multiple grams clinical grade antibody material for human clinical trials. Preclinical trials in rabbits have been completed successfully and we also conducted successfully a phase I clinical trial in the UK. This work will now be moved forward for a phase IIa clinical trial which is funded by an Advanced ERC grant from the European Commission.

We have also developed an interesting multi-stage malaria vaccine and neutralizing rabies antibody candidates and will discuss how both products have matured over the years both in performance and in manufacturing with the aim in mind to bring these two products into translational research within the next months.

Finally state of the art technology developments to accelerate the development and production of PMPs as well as regulatory issues will be discussed. Along this line an innovative new manufacturing concept using LED lighting in a vertical farm concept have been developed.

SL-287*Track: Industrial and Manufacturing***SYNTHETIC BIOLOGY OF CYANOBACTERIA FOR BIOETHANOL PRODUCTION****Pengcheng (Patrick) Fu**

*College of Life Science and Biotechnology, Beijing University of Chemical Technology, China;
E-mail: fupc@mail.buct.edu.cn*



Cyanobacteria have played an important role in the global carbon cycle and have long been studied as model organisms for photosynthesis and CO₂ fixation. Recently, there is an increased interest in the use of photosynthetic microorganisms for the production of biofuels, protein and chemical products. Particular attention is given to the engineering of cyanobacteria for biofuel production, including both hydrocarbon and hydrogen fuels. In order to overcome technical challenges in this field, genetic tools and strategies for manipulation of cyanobacteria need to be optimized for industrial scale production.

Genetic engineering efforts on cyanobacteria usually involve antibiotic resistance as the selectable markers to screen for successful transformants. This has limited biotechnology applications of engineered cyanobacteria. For instance, since cyanobacteria require light for growth, many light-sensitive antibiotics are not desirable. On the other hand, antibiotic selection is dependent upon the sensitivity of the antibiotics to the host organism. It also relies on the host's ability to produce the functional protein product of the antibiotic resistance cassette.

We have used the cyanobacterium *Synechocystis* PCC 6803 as the model system to create a new function to this photosynthetic organism in addition to the pre-existing functions of photosynthesis and CO₂ fixation. It involved the reconstruction of genome-scale metabolic network for the systems biology prediction of cellular metabolism under different genotypes and growth conditions. For synthetic biology, heterologous "biological parts" were assembled into the *Synechocystis* "chassis" to create a novel ethanol producing pathway which allows the carbon flow from the CO₂ to the end product, ethanol as predicted by a computer model. The integration of systems biology and synthetic biology for *Synechocystis* has proven to be precise, cost effective, and predictable.

SL-16*Track: Other Areas***PRODUCTION OF HYDROGEN AND METHANE FROM FOOD PROCESSING WASTE IN INDUCED BED REACTORS: EXPERIMENT AND MODELING****Conly Hansen and Jianming Zhong**

Dept. Utah State University, Logan, UT, 84322-8700, USA; E-mail: Conly.hansen@usu.edu,



This study investigated effects of pH, temperature, hydraulic retention time (HRT), and organic loading rate (OLR) on hydrogen production from food processing waste (FPW) using dark fermentation in semi-continuous 60 L pilot induced bed reactors (IBR). Results show pH played a key role on hydrogen production with optimal pH range of 4.8-5.5. Digestion under thermophilic temperatures (60 °C) had advantages of gaining higher hydrogen yield and suppressing growth of methanogens. The optimal OLR was 32.9 g-chemical oxygen demand (COD)/l d at 3 d HRT. Under optimal conditions highest hydrogen yield was 71.7 ml/g COD_{loaded} with 44.6% COD removal. Two-stage digestions demonstrated more energy gain from methane and further COD removal. The overall gas production in two-stage digestion was 71.7 ml hydrogen and 61.0 ml methane g/DPW COD. The overall COD removal under optimal conditions was 88.2%. The Anaerobic Digestion Model No. 1 (ADM 1) was modified and implemented in software R to describe and correctly predict hydrogen production from FPW. Validation results show this model described reasonably well the dynamic behavior of hydrogen production in IBR. DNA-based studies to characterize IBR microbiology are underway and should facilitate efforts to refine and enhance reactor operations.

SL-56

Track: Regenerative Medicine

AUTOLOGOUS BRAIN SOURCES FOR PERSONALIZED CELL THERAPY IN NEUROLOGICAL DISEASE

Hu Xu, Cleusa DeOliveira, Hon Leong, Susanne Schmid, Paula Foster and Matthew O. Hebb

Department of Clinical Neurological Sciences, Western University, Canada; E-mail: mhebb@uwo.ca

Parkinson's Disease (PD) is the second most common degenerative brain disease, affecting an estimated 7-10 million people worldwide. By replacing lost circuitry and providing chronic biological sources of therapeutic agents to the brain, cell-based therapies are expected to be a game changer for the effective treatment of PD and other neurological diseases. Early neural transplantation studies underscored the challenges of immune compatibility, graft integration and the need for renewable, autologous graft sources. Neurotrophic factors (NTFs) may complement dopamine replacement in PD, and offer a potent new class of cytoprotective pharmacotherapeutics. Clinical application of NTF therapy has met numerous challenges, including poor blood brain barrier permeability, limited tissue diffusion and rapid intraparenchymal metabolism. For sustained and effective cell therapy, an ideal graft would consist of autologous drug (*e.g.*, dopamine, NTF)-producing cells of central nervous system origin that are well-suited to re-integrate into the host environment following transplantation. We have shown that brain biopsies, safely taken from living PD patients at the time of deep brain stimulation surgery, may be cultured to yield millions of cells, with key merits being both host- and brain-derived progeny, avoiding the difficulties associated with somatic tissue sources and non-self donors. The colocalization of multiple NTFs with progenitor and neural proteins raises the intriguing prospect that these cells may effectively integrate back into the host brain to confer broad and enduring therapeutic function. The favorable handling properties of these cells allow prolonged cryostorage and genetic manipulation to produce customized phenotypes. This methodology offers a novel autologous tissue source with prospects to advance personalized cell-based therapies for PD and a host of neurological diseases.

**SL-28**

Track: Regenerative Medicine

IN VIVO MODEL OF HUMAN INTESTINE

Smail Meziani and M. A. Helmrath

*Department of Surgery, University of North Carolina Chapel Hill, NC, United States;
E-mail: Michael.Helmraeth@cchmc.org*

As the gastrointestinal system is required for absorption and digestion of nutrients as well as the most common site for absorption of numerous medications, models to understand rare and common human diseases are crucial yet lacking. As many diseases are not applicable to animal models, human studies are currently required to test therapies. Development of functional and specific human intestinal tissue would address this major gap by providing; 1. A means to screen specific factors associated with drug and nutrient absorption in healthy intestine. 2. Unlimited access to disease-modeled specific tissue to support studies characterizing common and rare GI diseases. 3. Development of therapies focused on treatment of specific common (including infectious diseases (viral and bacterial) and rare (cystic fibrosis, IBD, celiac) diseases. Our group has recently described robust and efficient methods for directing human pluripotent stem cells (hPSCs) and induced pluripotent stem cells (iPSCs) into an intestinal 3D culture system; that when transplanted efficiently develop into functional human small intestine (Watson *et al.* Nature Med 2014). Our ongoing studies support the reproducible phenotype over numerous grafts generated from the same human pluripotent stem cell line. Ongoing advances in the model have provided a way to transplant the grafts into the intestinal mesentery that can be incorporated into the luminal stream of the mouse (Fig. 1). In addition, recent data support our ability to generate a human intestine with an ENS (Fig. 2) Abstract for Daria.



Efficient expansion of IPS cells towards functional human intestine will provided potentially unlimited access to growing this tissue to develop human specific assays. Initially focusing on our patients with identified GI diseases.

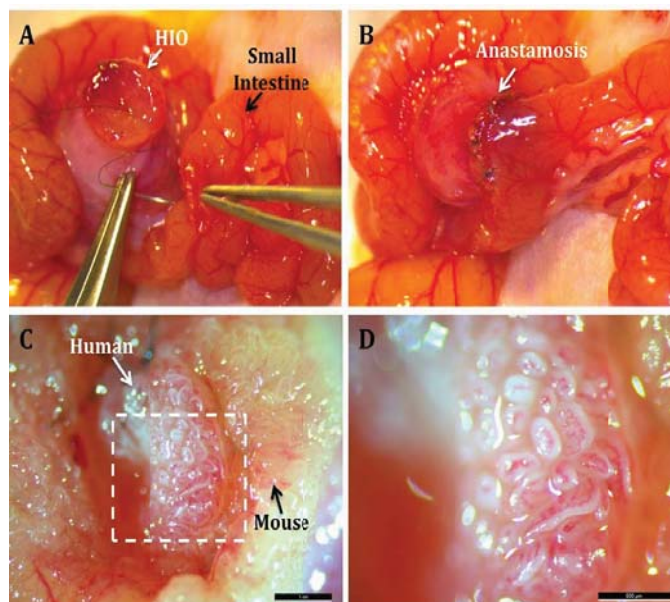


Figure 1: Anastomosis of human intestine graft in the murine intestinal continuity. (A-B) Anastomosis of the engraftment (HIO) in the mouse intestinal continuity (Small Intestine). (C-D) Developed human intestine (Dotted line) in the murine small intestine exposed to luminal nutrients for 2 weeks.

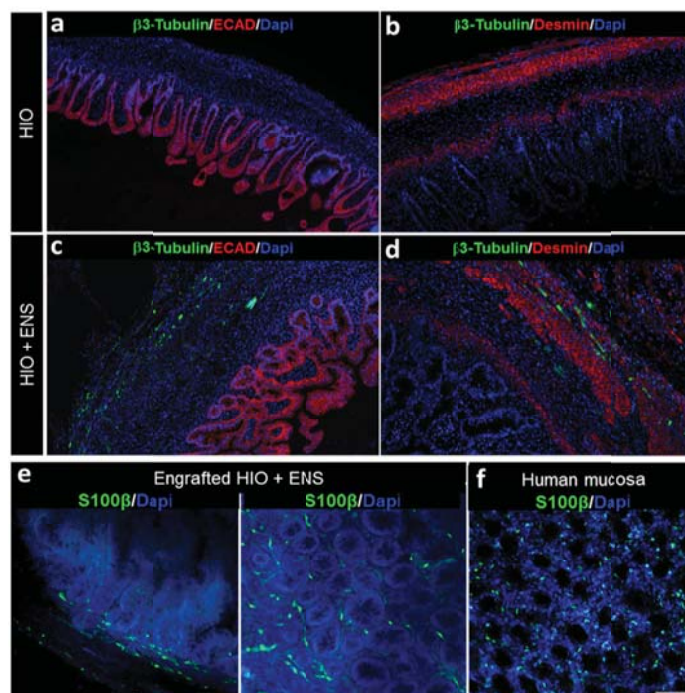


Figure 2: Human Intestinal Organoids (HIOs) Engraft to Form Mature Small Intestine Innervated by an Enteric Nervous System (ENS).

SL-290*Track: Industrial and Manufacturing***BIO-SALINE AGRICULTURE OF SALICORNIA BEGELOVII, NEW APPROACHES FOR BIODIESEL PRODUCTION****Mohamed M. Ibrahim, Ibrahim Arifl and Gehan A. El-Gaaly***King Saud University, Science College, Botany and Microbiology department, 11451-P.O. Box 2455 Riyadh, KSA; E-mail: m_ibramim2004@yahoo.com*

Salicornia begelovii displays excessive biotechnological prospective as a salt-water irrigated crop. Qualitative and quantitative compositions of fatty acids were analyzed in the seeds of Salicornia begelovii collected from the eastern region, Al-Jubil, Saudi Arabia. Hexane extraction of the seed oil from Salicornia begelovii 29% of total lipids. The GC-MS (Gas Chromatography-Mass Spectroscopy) investigation of the hexane extracts revealed five major peaks for the seed oil: 72.5 wt.% linoleic- ω 6 acid (18:2), 7.4 wt.% palmitic acid (16:0), 13.3 wt.% oleic acid (18:1), 2.14 wt.% stearic acid (18:0) and 2.3 wt.% linolenic- ω 3 acid (18:3). The sum of the saturated palmitic and stearic acids (9.18%) in S.begelovii seed oil. The composition of the oil was nutritive and medical health value was high, in addition to, it's composition very similar to that of safflower oil. No unwanted fatty acid constituents were established in S.begelovii seed oil, and it could be suggested for biofuel fabrication.

SL-216*Track: Plant and Environment***HARNESSING MICROBIAL INTERACTION FOR INCREASING CROP PRODUCTIVITY IN SEMIARID PRAIRIE AGRICULTURAL REGION OF WESTERN CANADA****Jamshid Jazestani, Lobna Abdellatif and Chantal Hamel***Department of Bioresource Engineering, McGill University, Quebec, Canada; E-mail: jamshid.jazestani@mail.mcgill.ca*

Various groups of microorganisms can play important roles in enhancing agricultural sustainability. Those beneficial interactions include tolerance to biotic and abiotic stresses, plant growth regulation by producing phyto-hormones and improving nutrient acquisition by the plants. A study was conducted to determine the effects of various micro-organisms isolated at Semiarid Prairie Agricultural Research Centre (SPARC) and some commercial strains. The micro-organisms used include two native strains (Rhizophagus irregularis strain GLD50 and Rhizophagus fasciculatus strain GLA46) and a commercial strain Myke®Pro of arbuscular mycorrhizal fungi (AMF), two native species (Variovorax paradoxus strain L17 and Mycobacterium sp. strain L11) of plant growth promoting rhizobacteria (PGPR) and two species (Variovorax paradoxus strain L1, commercial Penicillium bilaiae) of phosphate solubilizing bacteria (PSB). Four experiments were carried out in flax, lentil, pea and wheat in the greenhouse with randomized complete block design. In each crop, there were 27 treatments including the negative controls. The plants grown in pots constituted experimental units which were replicated 4 times. AMF inoculants Rhizophagus irregularis strain GLD 50 and MykePro were significantly more effective than Rhizophagus fasciculatus strain GLA 46 in increasing biomass productivity of lentil and pea. For wheat biomass, the interaction between the AMF and PGPR was highly significant. AMF Rhizophagus irregularis strain GLD 50 and PGPR Variovorax paradoxus strain L17 were the best combination for biomass productivity of wheat. Contrarily, there was no significant effect of any of the micro-organisms on flax. The significant interactions between the AMF and PGPR suggest that specific combination of AMF and PGPR can increase crop biomass more effectively. Further studies are needed in field conditions to verify how far these effects can be translated in agronomic environments.

Keywords: Arbuscular Mycorrhizal Fungi, Plant Growth Promoting Rhizobacteria, Phosphate Solubilizing Bacteria, Native Strains, Biomass.

SL-198

Track: Plant and Environment

A WHOLE PLANT APPROACH FOR NITROGEN-FIXATION IN NON-LEGUMINOUS CROPS**Munusamy Madhaiyan and Lianghui Ji**

Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604; E-mail: jilh@tll.org.sg



Jatropha curcas is promising new non-food crop for biodiesel production because of its ability to thrive on marginal land, with strong tolerance to draught and poor soil nutrient content [1-3]. Nevertheless, high fertilizer input remains essential in order to have commercially acceptable oil productivity. This reduces the green index of *Jatropha* biofuel. To address this issue, we studied the taxonomical distribution of 1017 cultivable endophytic bacterial strains isolated from different parts of *Jatropha* with an emphasis on nitrogen-fixing bacteria. Like other reports, we found strong tissue preferences of the bacteria in *Jatropha*. The 16S rRNA gene sequences can be assigned to five major phyla and, surprisingly, 31.2% of them potentially represent new taxa. Nitrogen fixing isolates were found diverse and present in five classes belonging to α , β , γ -*Proteobacteria*, *Actinobacteria* and *Firmicutes*. The phylum *Proteobacteria* was the most dominant amongst strains that were positive for both *nifH* gene and endoglucanase activity. *Methylobacterium* species account for 69.1% of the leaf endophytic bacterial isolates. Notably, many *Methylobacterium* isolates were able to fix nitrogen.

We will present genomic, physiology and plant-bacteria interaction studies on two strong nitrogen-fixing isolates: *Kosakonia* sp. R4-368 (previously *Enterobacter* R4-368 [4,5]) that mainly colonizes in roots and stems and *Methylobacterium radiotolerans* L2-4 that mainly colonizes leaf tissues both as endophyte and epiphyte. Root treatment of R4-368 or foliar application of L2-4 significantly improved growth parameters, such as plant height, leaf number, relative chlorophyll content and stem volume. Importantly, strain L2-4 improved seed yield by 222.2% and 96.3% in plants potted in sterilized and non-sterilized soil pots respectively. Strain R4-368 improved seed set by approximately 177% and 49.0% in sterilized and non-sterilized soil respectively. The average single seed weight was increased approximately 10% by strain R4-368. Yield improvements were mainly attributed to an increase of female-male flower ratio, which led to a corresponding increase of fruit and seed sets. Furthermore, there was an additive effect for seed yield of root and leaf treatments were both performed. We will present the effects of application of the two isolates on other crops.

Key words: Nitrogen-fixation, biofuel, plant growth promoting bacteria, *Jatropha curcas*

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SL-57*Track: Medical Biotechnology***PROTECTIVE EFFECTS OF LOW DOSES RADIATION AGAINST DOXORUBICIN-INDUCED CARDIAC TOXICITY IN BALB/C MICES****Hong-Yu Jiang, Gui-Ru Wang, Ya-Qiong Hong, Hong-Mei Zhang and Miao-Miao Li***Health Examination Center & Department of Gerontology, The First Hospital of Jilin University, Changchun 130021, China; E-mail: jiang_hy@jlu.edu.cn*

Doxorubicin (DOX) is one of the most effective chemotherapeutic drugs; however, cardiac toxicity compromises its clinical usefulness. Low doses radiation (LDR) induces an adaptive effect or hormesis, showing a protective effect on subsequent challenges-induced damage *in vitro* and *in vivo*. Our study was designed to investigate whether LDR pretreatment could against DOX-induced acute cardiotoxicity and its possible molecular mechanisms. LDR pretreatment significantly protected against DOX-induced myocardial damage which was characterized by conduction abnormalities, increased serum creatine kinase (CK), and lactate dehydrogenase (LDH) and pathological injury. As indicators of oxidative stress, DOX caused significant increasing levels of reactive oxygen species (ROS) and malondialdehyde (MDA), and reduction in activities of antioxidant enzymes including glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD). LDR pretreatment significantly attenuated DOX-induced oxidative injury. Additionally, DOX induced significantly cell apoptotic was determined by the TUNEL method. DOX induced cell apoptosis *via* mitochondrial pathway, which was proved by mitochondrial membrane potential ($\Delta\Psi_m$) change, up-regulated Bax, caspase-9 and caspase-3 expressions and while down-regulated the expression of Bcl-2. LDR pretreatment significantly ameliorated these apoptotic actions of DOX. Collectively, these findings indicate that LDR possesses a protective effect against DOX-induced acute cardiotoxicity *via* suppressing oxidative stress and mitochondria-dependent apoptosis.

Keywords: Doxorubicin, cardiac toxicity, low doses radiation, oxidative stress, apoptosis.**SL-168***Track: Plant and Environment***THE ROLE OF CELLULOSE-SYNTHASE-LIKE CSLF6 IN 1,3;1,4- β -D-GLUCAN BIOSYNTHESIS AND OVEREXPRESSION IN WHEAT GRAIN TO INCREASE SOLUBLE DIETARY FIBRE LEVELS.****S.A. Jobling***CSIRO Agriculture Flagship, Canberra Australia; E-mail: steve.jobling@csiro.au*

Dietary fibre is largely derived from the cell walls of plants and in cereal grains, arabinoxylan and 1,3;1,4- β -D-glucan (betaglucan) are the major cell wall polysaccharides. Dietary fibre is an essential part of a healthy diet however fibre consumption in most Western countries is below target levels. Food products made from grains that have a high content of water soluble betaglucan such as oats and barley are allowed specific health claims in some countries related to the lowering of blood cholesterol. Wheat grain has only low levels of betaglucan, most of which is insoluble. The cellulose-synthase-like CslF6 gene is a major component of the betaglucan synthase and we will describe work to characterise the function of this protein from various cereals in *Nicotiana benthamiana* leaf expression system. Each CslF6 gene produces a betaglucan with a distinct structure and this affects water solubility. Overexpression of selected CslF6 genes in wheat grain increases levels of soluble betaglucan and dietary fibre.

SL-71*Track: Medical Biotechnology***EVALUATION OF ADVANCED GLYCATION END PRODUCTS (AGES), CIRCULATING SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (sRAGE) AND OXIDIZED LOW-DENSITY LIPOPROTEINS (OXLDL) IN HEMODIALYSIS PATIENTS****Adel M. A. Assiri, Hala F. M. Kamel and Abeer A. EL-Refai***Biochemistry Department, Faculty of Medicine, Umm Al-Qura University, Makhah, KSA and Medical Biochemistry Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt; E-mail: kamelhala@msn.com*

AGEs are a heterogeneous group of compounds derived from non-enzymatic glycation of proteins, lipids and nucleic acids through complex reaction known as the Maillard reaction. AGEs interact with the receptor for AGEs (RAGE) on the membrane and induce harmful effects through activation of nuclear factor kappa-B, and increased oxidative stress and inflammatory mediators. AGEs combine with membrane receptors (RAGEs) also combine with circulating soluble receptors (sRAGE) and act as a decay agent. AGEs are thought to be involved in many complications of angiopathy, cardiovascular diseases (CVD) and nephropathy in diabetes as well as those of end-stage renal failure. The aim of this study was to evaluate the changes of (AGEs), (sRAGE) and Oxidized Low-density Lipoproteins (OxLDL) levels, in hemodialysis patients with different underlying causes and to evaluate them in relation to occurrence of CVD or with diabetic nephropathy as an underlying cause in relation to other glycemic, lipid profile and renal functions' biomarkers. Our study included 279 patients with end stage renal disease (ESRD) who received maintenance hemodialysis (HD) (duration of HD, 6.7 ± 3.4 years.) and 112 sex and age matched healthy control subjects. The underlying causal disorders for them were as follows :diabetic nephropathy (56.9%) and non-diabetic nephropathy diseases (43.1%) including chronic hypertensive nephrosclerosis, glomerulonephritis, polycystic kidney disease, and rest of unknown etiology. We also categorized HD patients into 2 subgroups according to their positive medical history of CVD (52.09%) and another subgroups with no medical history of CVD (47.01%). We demonstrated significantly higher levels of oxLDL, AGEs and sRAGE in HD than healthy control group ($P < 0.001$). We found significant increase of OxLDL, AGEs and sRAGE in HD diabetic nephropathy subgroup when compared with non-diabetic subgroup as an underlying cause. Interestingly sRAGEs significantly decreased in HD patients who had positive history for CVD in comparison with those HD patients with no history of CVD (1970 ± 870 , 2660 ± 908 ; $P < 0.001$). There was positive correlation between AGEs and sRAGE in whole HD patients ($r = 0.441$; $P < 0.001$, $r = 0.395$; $P < 0.05$) while only in HD group with CVD There was negative correlation ($r = -0.294$; $p < 0.01$). We found that AGEs was an independent determine for diabetic nephropathy as an underlying cause for ESR (OR, 1.25; 95% CI, 1.04 to 1.49; $P < 0.016$). Another factor was the oxLDL level (OR, 1.10; 95% CI, 1.02 to 1.19, $P < 0.018$) and sRAGE (OR, 1.04; 95% CI, 1.01 to 1.06, $P < 0.007$). and we identified sRAGE as an independent factor associated with the prevalence of CVD (OR, -0.49; 95% CI, -0.086 to 0.034; $P < 0.001$). The other factors were AGEs (OR, 2.81; 95% CI, 1.79 to 4.41; $P < 0.001$), ox LDL (OR, 1.04; 95% CI, 1.02 to 1.06; $P < 0.001$), and hs-CRP level (OR, 1.02; 95% CI, 1.00 to 1.05, $P < 0.046$). Conclusions plasma OxLDL, AGEs and sRAGE levels are strongly associated with the prevalence of cardiovascular disease and diabetic nephropathy in hemodialysis patients and could be considered future markers for diabetic nephropathy and cardiovascular diseases in hemodialysis patients with end stage renal disease.

SL-65

Track: Plant and Environment

ANTIOXIDANT PROPERTIES OF PRUNES (*PRUNUS DOMESTICA* L.) AND THEIR CONSTITUENTS

Shin-ichi Kayano, Hiroe Kikuzaki, Takahiko Mitani and Nobuji Nakatani

Department of Health and Nutrition, Faculty of Health Science, Kio University, 4-2-2 Umaminaka, Koryo-cho, Kitakatsuragi-gun, Nara, 635-0832, Japan; E-mail: s.kayano@kio.ac.jp

Prunes contain large amounts of phenolics and show high antioxidant activity. The aim of this study is to clarify the contents of caffeoylquinic acid (CQA) isomers, and to estimate the contribution of these isomers to the antioxidant activity of prunes. Furthermore, structural elucidation and evaluation of antioxidant activity of prune components were also performed.



CQA isomers in prunes were quantified using HPLC analysis, and it has become apparent that prunes contain relatively high amount of 4-*O*-caffeoylquinic acid (4-CQA). The contribution of CQA isomers to the antioxidant activity of prunes was revealed to be 28.4% on the basis of oxygen radical absorbance capacity (ORAC); hence, it was indicated that residual ORAC is dependent on unknown antioxidant components. Total 28 compounds were isolated and their structures were elucidated by NMR and MS analyses. Four abscisic acid related compounds, a chromanon, and a bipyrrrole were novel. Each CQA isomer in prunes showed high antioxidant activities when measured by the oil stability index (OSI) method, O₂⁻ scavenging activity, and ORAC, and the activities of 3-CQAs depends on their stereochemistry. Other isolated compounds such as hydroxycinnamic acids, benzoic acids, coumarins, lignans, and flavonoid showed high ORAC values. Furthermore, a novel chromanon indicated a remarkable synergistic effect on ORAC of CQA isomers.

SL-258

Track: Other Areas

THE NEED FOR OPTIMIZATION: OVERVIEW OF EXTRACELLULAR VESICLES (EVS) ISOLATION TECHNIQUES FROM SMALL VOLUMES OF SERUM AND REPRODUCTIVE BIOLOGICAL FLUIDS

Shlomit Kenigsberg, Shlomit Kenigsberg, Arshia Azizeddin, Bahar Behrouzi, Nicole Legro, Christina Lee, Parshvi Vyas, Kevin Quach, Paula Mackie and Clifford Librach

CReATe Fertility Centre, Toronto, Canada; E-mail: shlomit@createivf.com

Microvesicles and exosomes (EVs) are small cell-derived vesicles of 30–200 nm are found in all biological fluids tested so far, including blood, urine, saliva, amniotic fluid, breast milk, ascitic fluid, tears and culture medium. These vesicles are released from many cell types and tissues, including lymphocytes, endothelial cells and neurons. Given their prolific origins, EVs are involved in several cellular functions and disease processes. As such, there has been an explosion of literature regarding their potential for use as biomarkers for disease. However, developments in this area have been constrained by limitations of the EV's size and the technology available for their extraction and analysis from body fluids. Furthermore, isolation and analysis techniques varies widely depending on the origin starting fluid. The characteristics of the fluid, such as viscosity and volume, can greatly affect isolation techniques. Our research facility has optimized methods for isolating and characterizing EVs from serum, semen, embryo media culture, and follicular fluid for post-isolation analysis. The quantity and quality of these purified exosomes have been assessed for small RNA and protein populations using centrifugation and commercial kits, immune-capture and Nano Tracking Analysis (NTA). The pros and cons of each method would be discussed in this talk. Most importantly, the non-standardized parameters leading to qualitative and quantitative variability can affect the down-stream analyses and can yield misleading results and conclusions. There is a dire need to develop cost-effective and accessible methods for EV isolation.

SL-325

Track: Industrial and Manufacturing (Industrial Enzymes)

PRODUCTION OF HIGH MOLECULAR WEIGHT FUNGAL PECTINASE FROM THE NOVEL STRAIN OF *ASPERGILLUS* SPP

Samina Khan, Saima Saleem and Abid Azhar

Department of Biochemistry, University of Karachi, Karachi, Pakistan; E-mail: samin10pk@gmail.com



Introduction: *Aspergillus* species are highly abundant fungi worldwide involved in the production of cell wall-degrading enzyme, pectinase. Pectinase is a heterogeneous group of enzymes that have biotechnological, functional and biological applications in food processing, fruit ripening and textile industries. The recovery of by-products from agri-food industry is currently one of the major challenges of biotechnology. In the present study, an indigenous fungal strain identified as *Aspergillus niger*-FS001 was isolated from moldy vegetables and fruits samples that showed maximum pectinase production.

Materials & Methods: Submerged fermentation technique was used for pectinase production from fungal strain *Aspergillus niger*-FS001. The microscopic features of the strains were studied by Lacto Phenol Cotton Blue staining technique. Lowry's method was used to determine the total protein. The enzyme assay was done by using citrus pectin and galacturonic acid monohydrate. The amount of reducing sugar released was analyzed by 3, 5-dinitrosalicylic acid method. Partial purification of pectinase was carried out by gradient ammonium sulphate precipitation. Extracellular pectinase was purified by gel filtration chromatography. Molecular weight of pectinase was determined by SDS-PAGE and zymography.

Results: The maximum enzyme production was obtained after 120 hours. The optimum temperature and pH for maximum enzyme production by *Aspergillus niger*-FS001 were 30°C and 5.5, respectively. Carbon source, such as pectin, enhanced the maximum production of enzyme. Simultaneously, the combination of yeast extract, peptone, ammonium sulfate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and calcium chloride in optimized concentrations also enhanced the enzyme production. Partial purification of pectinase was achieved by 40% ammonium sulphate for maximum precipitation of enzyme. The enzyme was purified to homogeneity with 95.94 fold purification. The molecular weight of purified pectinase was found to be 129 kDa by SDS-PAGE. Zymography of the purified enzyme was also performed, which confirmed the homogeneity of the purified enzyme.

Conclusion: The maximum economical production of pectinase by *Aspergillus niger* FS001 was achieved by utilizing cheap indigenous substrate that has potential use in several biotechnological industries.

Keywords: Pectinase, *Aspergillus niger*, enzymes, pectin, biotechnological industries.

SL-107

Track: Pharmaceutical Biotechnology

THE ROLE OF STEM-LIKE GLIOMA CELLS IN GBM PROGRESSION AND POST-TREATMENT RECURRENCE.

Ella L. Kim

The Translational Neurooncology Research Group, Department of Neurosurgery, University Medical Centre, Johannes Gutenberg University, Langenbeckstrasse 1, 55131 Mainz, Germany; E-mail: Ella.Kim@unimedizin-mainz.de



Glioblastoma multiforme (GBM) is one of the most challenging human cancers. The current standard of care for GBM consists of surgical de-bulking followed by combined radio- and cytotoxic chemotherapy. Despite this multimodal therapy, post-treatment recurrence is almost inevitable and is a major cause of GBM patient lethality. There has been a growing realization that malignant

gliomas have a hierarchical structure, whereby distinct populations of cells within the same tumour vary in their capacity to promote tumour growth and ability to withstand the effects of cytotoxic treatments. The pronounced intrinsic radioresistance of GBMs is partially linked to the existence of stem-like cells, also called glioblastoma-initiating cells (GICs), in these malignancies. There has been a growing realization that intrinsic resistance of GICs to cytotoxic agents is the underlying reason for the poor efficacy of conventional cytotoxic therapy. The elucidation of molecular mechanisms that render GICs resistant to clinically relevant doses of ionizing radiation is of pivotal importance for improving the effectiveness of cytotoxic therapies and development of individualized approaches to the treatment of GBM. While the GIC paradigm is rapidly gaining widespread acceptance, there is still considerable uncertainty as to the identity of GICs and their precise roles in the initiation, maintenance and progression of GBM. Fundamental remaining questions include: Is there a universal type of stem-like glioma cells? What criteria define glioma cell stemness? How does the degree of glioma cell stemness relate to the clinicopathological criteria of glioma aggressiveness? To address these questions, the relationship between tumorigenicity, proliferative potential and differentiation was examined through a combinatorial approach based on *in vitro*, *in vivo* and *in silico* analyses of different types of stem-like glioma cells. Our research indicates that glioma cells collectively called GICs comprise a heterogeneous group of phenotypically and functionally distinct cell types with varying tumorigenic potential. Our findings indicate that the phenomenon of glioma radioresistance involves a global gene expression reprogramming in GICs and urge the necessity of transcriptional profiling as a means of monitoring the recurrence of GBM under conventional cytotoxic therapy.

SL-239

Track: Plant and Environment

METABOLIC ENGINEERING OF CAROTENOIDS IN TRANSGENIC *SWEETPOTAO* FOR SUSTAINABLE DEVELOPMENT ON GLOBAL MARGINAL LANDS

Sun Ha Kim, Sung-Chul Park, Haeng-Soon Lee, Jae Cheol Jeong and Sang-Soo Kwak

Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Korea; E-mail: sskwak@kribb.re.kr



Sweetpotato (*Ipomoea batatas* (L.) Lam) ranks seventh in annual production among food crops in the world. It is also an alternative source of industrial materials such as starch, ethanol and useful components. Sweetpotato was recently reevaluated as one of the best healthy foods by the nonprofit CSPI (2007) and the best bioethanol crop on marginal lands by USDA (2008), since it contains high levels of various antioxidants such as vitamin C and carotenoids. Despite its importance as the well-being food source, little research has been carried out on sweetpotato antioxidants at the molecular level. In this respect, transgenic sweetpotato with high yields of β -carotene by down-regulation of β -carotene hydroxylase (*CHY- β*) and lycopene ϵ -cyclase (*LCY- ϵ*) were successfully generated. We have recently isolated *IbOrange* gene responsible for carotenoid accumulation from the orange-fleshed sweetpotato and introduced it into purple-fleshed sweetpotato to produce both anthocyanin and carotenoids in one storage root. In the presentation, our recent results on metabolic engineering of pigment antioxidants in transgenic sweetpotato will be introduced in terms of sustainable development on global marginal lands.

Keywords: *Sweetpotato*, carotenoid, β -carotene hydroxylase, lycopene ϵ -cyclase, Orange gene, RNAi.

SL-78*Track: Medical Biotechnology***BRING SANGER SEQUENCING TO THE COMMUNITY CLINICAL LABORATORIES WORLDWIDE****Sin Hang Lee and Guofan Hong**

Milford Hospital and Milford Molecular Diagnostics Laboratory, 2044 Bridgeport Avenue, Milford, CT 06460, USA; E-mail: shlee01@snet.net



One obstacle to implement automated Sanger sequencing as routine tests in developed countries and as needed accurate diagnostic tools for emerging infectious diseases, such as Ebola, is the labile PCR reagents, namely the DNA polymerases, the dNTPs and the reverse transcriptases, which must be kept at -20°C between uses. We have optimized a PCR chemical mix in which these labile components are stabilized at room temperature for several weeks to 10 months. It depends on a highly processive, moderately heat-resistant DNA polymerase with a PCR thermocycling not to exceed 85°C [1], and has been successfully used for preparing DNA sequencing templates in the routine detection and genotyping of human papillomaviruses [2], in the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* [3] and in the diagnosis of Lyme borreliosis [4-6]. By adding a reverse transcriptase in this low temperature PCR mix, the newly created RT-PCR mix may be adapted for screening Ebola patient samples at the sites of outbreak in far-flung places and the PCR amplicons can be transported to a regional laboratory for DNA sequencing validation.

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SL-114*Track: Plant and Environment***EFFECTS OF STRONG ACID AND IONIC MATERIALS ON MICROWAVE-ASSISTED THERMO-CHEMICAL HYDROLYSIS OF CATTLE MANURE****Jeongmin Lee, Jaeho Lee, Hyanggi Kim, Sanghoon Lee, Youngwoo Kim, Imgyu Byun and Taejoo Park**

Department of Civil and Environmental Engineering, Pusan National University, 63beon-gil, Geumjeong-gu, Busan, Korea; E-mail: jhlee6119@hanmail.net

Microwave (MW) uses electrical energy. A catalytic material added MW hydrolysis is an effective way of reducing electricity consumption and enhancing the hydrolysis of cattle manure. In this study, catalytic materials, such as H₂SO₄ as the strong acid and NaCl as the ionic material, were applied to enhance the hydrolysis of cattle manure. Food wastewater (FWW) was also applied as the ionic material because of its high contents of NaCl and other ions such as K⁺, NH₄⁺, NO₃⁻. After catalysts added MW-assisted thermo-chemical hydrolysis, the maximum increases of SCOD_{release}/TCOD_{initial} ratio were 0.064 for H₂SO₄, 0.050 for NaCl and 0.068 for FWW. And the SCOD_{release}/TCOD_{initial} ratio of FWW addition was 1.4 times higher than that with NaCl addition at a MW power of 800 W and target temperature of 40°C. The specific SCOD increase per energy consumption (mgSCOD/kJ) was 169.6 for H₂SO₄, 132.5 for NaCl and 180.2 for FWW. In biochemical methane potential tests, methane productions of H₂SO₄ and FWW addition were 1.25 times and 1.30 times higher than that with MW only assisted hydrolysis. Therefore, FWW was found to be good agent for enhancing the MW efficiency.

Keywords: Cattle manure, Microwave, Hydrolysis, Pretreatment, Chemical agent.

SL-92

Track: Medical Biotechnology

BLOCKING THE FUNCTION OF INFLAMMATORY CYTOKINES AND MEDIATORS BY USING IL-10 AND TGF-B: A POTENTIAL BIOLOGICAL IMMUNOTHERAPY FOR INTERVERTEBRAL DISC DEGENERATION IN A BEAGLE MODEL

Wei Li, Chun Chen, Zhiwei Jia, Xuedong Bai and Dike Ruan

Department of Orthopedics, Navy General Hospital, Beijing 100048, China;
E-mail: weilichn@126.com



Introduction: The debilitating effects of lower back pain are a major health issue worldwide. A variety of factors contribute to this, and oftentimes intervertebral disk degeneration (IDD) is an underlying cause of this disorder. Inflammation contributes to IDD, and inflammatory cytokines play key roles in the pathology of IDD. This study characterized the potential to suppress inflammatory cytokine production in degenerative intervertebral disc (NP) cells by treatment with IL-10 and TGF- β in a canine model of IDD.

Methods: IDD was induced surgically in six male beagles, and degenerative NP cells were isolated and cultured for *in vitro* studies on cytokine production. Cultured degenerative NP cells were divided into four experimental treatment groups: untreated control, IL-10-treated, TGF- β -treated, and IL-10- plus TGF- β -treated cells. Cultured normal NP cells served as a control group. TNF- α expression was evaluated by FACS analysis and ELISA; moreover, ELISA and real-time PCR were also performed to evaluate the effect of IL-10 and TGF- β on NP cell cytokine expression *in vitro*.

Results: The major findings of these analysis are that after treatment with IL-10 and TGF- β , the expression of extracellular and intracellular TNF- α and IL-1 β was suppressed, while the expression of inflammatory cytokines in untreated normal NP cells was significantly lower than that in untreated degenerative NP cells. Our results demonstrated that IL-10 and TGF- β treatment suppressed the expression of IL-1 β and TNF- α and inhibited the development of inflammatory responses.

Discussion: We observed that either TGF- β or IL-10 alone suppressed the expression of inflammatory cytokines. Furthermore, their combined use produced a higher level of inhibition of TNF- α and IL-1 β than either TGF- β or IL-10 alone. IL-10 and TGF- β should be evaluated as therapeutic approaches for the treatment of lower back pain mediated by IDD.

SL-283

Track: Industrial and Manufacturing

CO-DISPLAY OF SYNERGETIC RHIZOPUS ORYZAE LIPASE AND CANDIDA RUGOSA LIPASE 1 BIODIESEL PRODUCTION

Zhang Yan, Xu Li, Huang Zhengnan and Yan Yunjun

Key Laboratory of Molecular Biophysics, The Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P. R. China;
E-mails: xuli@mail.hust.edu.cn; yanyunjun@mail.hust.edu.cn



Aimed to major bottlenecks of high cost and low operation stability of free lipases, *Candida rugosa* lipase (CRL) and *Rhizopus oryzae* lipase (ROL) were co-displayed on the cell surface of *Pichia pastoris* and used as a whole-cell catalyst to produce biodiesel from tallow seed oil in this study. After screened by double resistance and tributyrin medium, the resultant co-displayed recombinant GS115/pRCS with the maximum activity of 470.59 U/g dry cells, being 3.9- and 1.3- fold compared with that of the single displayed ROL and CRL1, respectively. The analysis of fluorescence microscope and Flow Cytometer demonstrated that ROL and CRL1 were successfully co-displayed on the surface of recombinant *P. pastoris* GS115. When the self-immobilized lipases were utilized as whole cell catalysts, the rate of methyl ester from co-displayed recombinants strain GS115/pRCS harboring ROL and CRL1 is 1.4-fold compared with that of single displayed ROL. All these results indicate that

biodiesel catalyzed by the co-displayed enzymes with synergetic effect could be an alternative strategy for producing biodiesel in low cost.

Keywords: Rhizopus oryzae lipase; Candida rugosa lipase 1; co-display; Pichia pastoris; whole-cell catalysts

SL-269

Track: Plant and Environment

NOVEL APPLICATION OF SARGASSUM AS A POTENTIAL WASTE DERIVED CATALYST FOR LOW TEMPERATURE SELECTIVE CATALYTIC REDUCTION OF NITRIC OXIDES

Sujing Li, Shan Tan, Yun shi and Wei Li

College of chemical and biological engineering, Zhejiang University, Hangzhou, Zhejiang, China; E-mail: sujing-li@zju.edu.cn

Activated carbon derived from sargassum(SAC), normally disposed as waste, has been examined as potential catalysts for selective catalytic reduction (SCR) of NO with NH₃ in the temperature range of 50-250 °C. The influence of preparation methods, phosphoric acid impregnation ratios and surface nitrogen functional groups, were investigated. An array of analytical techniques, including BET, SEM-EDX, EA, FTIR and XPS, were also employed to study the structural properties, elemental content, and distribution of nitrogen-containing groups of the catalyst prepared.

The N-modified SAC samples presented higher catalytic activity than the virgin SAC samples. The N-doped commercial activated carbon(NCAC) achieved a maximum NO_x conversion of 75% at 150 °C and then decreased to 55% at 250 °C. NO_x conversion was maintained above 75% and achieved a maximum of 87% at 150 °C for the NSAC. Besides, N₂ selectivity was maintained above 95%. In conclusion, the activated carbons prepared from sargassum could be used as an alternative of the commercial AC. Moreover, the modification with urea on the SAC leads to the increase of its SCR activity and N₂ selectivity.

Keywords: Activated carbon, SCR, catalyst, low temperature

SL-199

Track: Pharmaceutical Biotechnology

INHIBITION OF THE IGG-FCγRIIIA INTERACTIONS IN MSOD1 MICE PROLONGING MOUSE SURVIVAL.

Meital Edri-Brami, Inga Shengof, Shirly Tzalik and Rachel G. Lichtenstein

Avram and Stella Goren-Goldstein Department of Biotechnology Engineering, Faculty of Engineering, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel; E-mail: ruha@bgu.ac.il

The N-glycan on Asparagine-297 of the IgG Fc (crystalizable fragment) is a bi-antennary complex structure that can vary by the addition of sugar residues to the core structure. This glycan is an influential constituent of the IgG antibodies, as it mediates leukocyte activation and inflammation by forming immune complexes through the Fc and the activating Fcγ receptors (FcγRs) on immune cells. Bisecting N-acetylglucosamine, for example increases affinity to FcγIIIA (CD16), and sialic acid residues reduce affinity to FcγR. Recently, we showed that IgG antibodies derived from ALS patient sera possess bisecting glycan which increases binding of the ALS IgG antibodies to CD16 on effector cell and promoting death of neuronal cells relative to IgG antibodies from healthy volunteers. We further showed over-expression of CD16 and co-localization of intact ALS-IgG antibodies with CD16 and with activated microglia cells in brain and spinal cord of SOD1^{G93A} mice. We hypothesize that motor neuron death in ALS patients is due, in part, to the N-glycans with bisecting and without sialic acid conjugated to the Fc antibodies that accumulated in patient CNS during the disease. Accordingly, in the preliminary study here, we injected recovered Fc-rituximab drug into CSF of 70 days old SOD1^{G93A} mice and characterize the phenotype of the disease. We demonstrated



prolonging lives and paralysis reduction of Fc-rituximab-treated females and males of SOD1^{G93A} mice by two to three weeks relative to placebo-treated mice. IgG antibodies of mSOD1 mice bearing the bisecting glycans were further injected into cerebrospinal fluid of pre-symptomatic mSOD1 mice. The injected mice developed the disease two weeks earlier than mSOD1 mice injected by IgG antibodies missing the bisecting glycans and three weeks earlier than mSOD1 mice treated with Fc-Rituximab. Moreover, qRT-PCR revealed CD16 increment in spinal cords of mSOD1 with disease progression and constant low expression in littermates and in brains of mSOD1 mice. We therefore anticipate blocking Fc-CD16 interactions in the CNS by designing inhibitors may prolong patient survival.

SL-248

Track: Pharmaceutical Biotechnology

ENGINEERING OF NOVEL DOMAIN ANTIBODY SCAFFOLD USING *IN VIVO* PROTEIN FOLDING PATHWAY

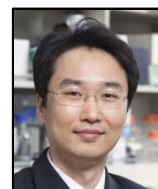
Hyung-Kwon Lim

Head of Antibody Engineering of MOGAM Biotechnology, Research Institute Yongin, Republic of Korea; E-mail: lhh@mogam.re.kr

This presentation will describe a novel platform technology to engineer super-stable human heavy chain variable domain (VH) as an alternative antibody scaffold and its uses for formatting next generation antibodies. Principles of the technology using *in vivo* protein folding pathway and characterization of the resulting scaffolds will be discussed at the molecular level. In addition, several case studies for the construction of the series of single domain antibody libraries and data from the screening against specific target antigens will be presented.

Why is this presentation important?

Human heavy chain variable domains (VH) are inherently unstable, which limits its biotechnological applications. This presentation will show a first attempt for using *in vivo* protein folding proof mechanism (Tat pathway) to evolve VH, which is distinct from the conventional heat denatured phage display method. By comparison of its mutational profile and crystal structure between parental and selected VH, we elucidated the hallmarks of stability of VH proteins. These findings should help designing novel next generation antibodies for therapeutic purposes.



SL-63

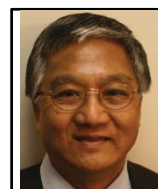
Track: Regenerative Medicine

TRACKING REGENERATION OF THE BRAIN AFTER GENE THERAPY USING TARGETED GUIDED MRI

Philip Liu

The AA Martinos Center for Biomedical Imaging, Department of Radiology, Mass General Hospital and the Harvard Medical School, Massachusetts, USA; E-mail: philipl@nmr.mgh.harvard.edu

Gene replacement has been proposed to improve neurological disorders. However, the efficacies are less than expected, perhaps due to less than optimal window of delivery and a lack of effective tracking of its distribution for evaluation. Here, we demonstrated effective delivery of cDNA encoding human granulocyte colony stimulating factor (G-CSF) protein in a self-complementary AAV2-CMV-hG-CSF as eye drops to mice after cerebral ischemia. Moreover, we tracked the expression of exogenous human G-CSF mRNA in living mouse brains using target-guided MRI. In supporting the function of stimulating vessel growth by G-CSF in the literature, we found eye drop delivery of G-CSF cDNA after cerebral ischemia reduced mortality, brain damage and neurological deficits and elevated global neurogenesis. The fact that eye drop is effective as a delivery route suggests that this route of delivery is translatable to emergency medicine for acute neurological disorders.



SL-221*Track: Plant and Environment***MICROSPORIDIA AND *ZINGIBER OFFICINALE* (GINGER); AN EMERGING PARASITE AND AN OLD MEDICINAL PLANT: *IN VIVO* TRIAL.****Rasha F. Mady, Radwa G. Diab and Doaa A. Abdel Monsef***Department of Medical Parasitology and Medical Biochemistry, Faculty of Medicine, Alexandria University, Egypt; E-mail: roshykareem@yahoo.com*

Microsporidia, of the genus *Enterocytozoon*, are an important cause of life-threatening diarrhea especially in immunocompromised hosts. There are controversies on the use of albendazole in treatment, whereas, fumagillin was to be more effective but with undesirable side effects. Ginger has been used as an antimicrobial agent since ancient times. However its potential therapeutic effect against *Enterocytozoon bienewisi* has not been tested. This study was done to investigate the effect of ginger as a prospective therapy for microsporidia versus fumagillin in immunocompetent and immunosuppressed mice. Also, to report the synergistic effect of the two compounds together in a drug-combination regimen. *Enterocytozoon bienewisi* was the species identified in the stool samples collected from immunocompromised patients and was used to initiate the *in vivo* infection in albino mice. Animals were divided into three major groups. Group I: Normal, non-infected non-treated, control group; group II: infected, immunocompetent group; and group III: infected, immunosuppressed group. Each infected group was subdivided into four equal subgroups a, b, c and d which comprise non-treated, fumagillin-treated, ginger-treated, and combined ginger/fumagillin treated mice respectively. Evaluation of the ginger efficacy in infected mice was achieved by assessment of fecal spore shedding, intestinal spore load, and biochemical assay which aimed at estimation of the malondialdehyde level and total antioxidant capacity. Spore count in both stool and intestinal sections and malondialdehyde level decreased significantly with ginger treatment. Best results were obtained when ginger is combined with fumagillin in all measured parameters. Ginger could be a good enhancer for fumagillin efficacy to eradicate infection. However, further studies on their principles, mechanisms of action, toxicity evaluation are still needed.

SL-135*Track: Pharmaceutical Biotechnology***THE EVALUATION OF CERCARIAL TRANSFORMATION FLUID USED SINGLY AND IN COMBINATION WITH CRUDE CERCARIAL ANTIGEN IN EXPERIMENTAL SCHISTOSOMIASIS MANSONI.****Mervat Zakaria El Azzouni, Rasha fadly Mady, Maha Reda Gaafar, Fadwa Arafa and Abeer Elhadidi***Departments of Medical Parasitology, Faculty of Medicine Alexandria University, Egypt; E-mail: rasha.madi@alexmed.edu.eg.*

Schistosomiasis is a truly neglected tropical disease and the second major parasitic disease in the world after malaria. It affects 201.5 million cases in Africa alone. The goal of this research is the development of an appropriate vaccine against experimental schistosomiasis mansoni, we assessed the effect of cercarial transformation fluid (CTF) singly and in combination with crude cercarial antigen (CCA) using alum as an adjuvant in experimentally infected mice. The combined antigen gave the best results as evidenced by significant reduction in the worm load (62.07%), tissue egg count, (78.16%, 86.46%) in liver and intestine respectively and hepatic granuloma size (31.76%). Scanning electron microscopic study revealed changes in the tegument in the form of roughness and appearance of vesicles and furrows between the tegumental tubercles. Also, resorption and mutilation of the ventral sucker and dimples replacing its spines. The female tegument was irregular and its posterior end showed loss of spines and sensory bulbs. Moreover there was a significant decrease in liver enzymes (ALT and AST) compared to infected control mice. A significant elevation in CD4+ T-lymphocytes denoting amelioration of the immune status in animals received combined vaccine.

It can be concluded that this combined antigen give us a hope to the development of a feasible simple vaccine against Schistosomiasis mansoni.

Keywords: Schistosomiasis mansoni, tropical disease and cercarial transformation fluid.



SL-204

Track: Plant and Environment

INCREASING THE EFFICIENCY OF NITROGEN COMPOUNDS REMOVAL FROM AGRICULTURAL AREAS THROUGH THE OPTIMIZATION OF CONDITIONS FOR MICROBIAL ACTIVITY

Joanna Dorota Mankiewicz-Boczek, Bednarek A., Zaborowski A., Gała I., Serwecińska L., Kolate E., Dziadek J. and Zalewski M.

Department of Molecular Ecohydrology. European Regional Centre for Ecohydrology of the Polish Academy of Sciences, Poland; E-mail: j.mankiewicz@erce.unesco.lodz.pl



Human activities have changed the natural flow of nitrogen, resulting in the creation of environmental problems such as increasing greenhouse gases, acidification and eutrophication. Therefore, it is necessary to develop effective methods of using the natural potential for the reduction of nitrogen compounds (NC). In order to optimize the removal of NC from agricultural areas, field model bioreactors containing different carbon sources (lignite, harl, straw or sawdust) and microbiological activators (culturable and/or unculturable bacteria) were tested. Because of the composition of agricultural sewage (pig/cattle manure) it has been necessary to develop a system that allows for the removal of both toxic forms - nitrate and ammonia. The mix of straw/carbon proved to be the best substrate for biodegradation of NC. The mix enabled a complete reduction of the nitrate load (initial concentration 448 mg/L). Additionally, the application of the activator prepared from the conglomerates of culturable denitrifying bacteria and unculturable microorganisms accelerated the reduction of ammonia (starting concentration of 260 mg/L). Summing up, the bioreactor filled with straw/coal, after the application of a microbial activator allowed for the removal of toxic NC within 6-7 weeks from leachate typical of manure storages.

Keywords: Denitrification, bioreactors, bacteria, nitrogen contaminants.

SL-211

Track: Pharmaceutical Biotechnology

PAOT TECHNOLOGY® NEW APPROACH FOR DETERMINATION OF OXIDATIVE STRESS STATUS OF BIOLOGICAL LIQUIDS AND TISSUES.**Smail MEZIANI**

Institut Européen des Antioxydants (IEA), 2 Avenue de la foret de haye, 54518, Vandoeuvre-Les-Nancy, France; E-mail: smeiziani@ie-antioxydants.com



Are you "rusty"?

A unique technology in the world, PAOT skin Technology® developed by the European Institute of antioxidants (IEA) - this is a world first - a device that allows to monitor the status of your cells and the famous "oxidative stress" which would be responsible for many cardiovascular diseases, and promote the development of cancer. The PAOT skin Technology® is able to measure the full concentration of oxidants, the famous "free radicals", and antioxidants in biological tissues such as skin, plants. These valuable measures will allow everyone to get an idea of his general condition, and if necessary change its lifestyle, playing on well-known parameters: physical activity, smoking, alcohol, stress, diet ... and eventually to better control the use of emulsions and various creams available on the market, and consume reasonably tea, fruit juice, coffee, and other dietary supplements.

Various tests used to assess the antioxidant capacity but they are based on different mechanisms using different sources of radicals or oxidants thereby generating different values not directly comparable. Indeed, these tests do not take account of the diversity of antioxidants (including bioavailability and biological effects are varied), nor the hydrophilic or hydrophobic nature or the specificity of interaction has alleged targets.

The work describes a PAOT skin Technology® for determining oxidative stress statute of biologicals solutions and tissues.

SL-317

Track: Other Areas

TOWARDS A UNIVERSAL METHOD FOR PROTEIN REFOLDING**Catherine Michaux and Eric A Perpète**

Unité de Chimie Physique Théorique et Structurale, University of Namur, rue de Bruxelles, 61, 5000 Namur, Belgium; E-mail: catherine.michaux@unamur.be



Escherichia coli is one of the most widely used hosts for the production of recombinant proteins of therapeutic or commercial interest dedicated to structural and functional analysis. However, this expression system is often hampered by the formation of insoluble protein aggregates (inclusion bodies). *In vitro* refolding of such proteins into their native states requires screening of numerous experimental parameters specifically optimized for each system. Hence, there is currently no reliable straightforward and universal experimental solution providing the optimal refolding of proteins. The development of new original techniques in this field is therefore crucial.

In that context, we have successfully demonstrated the reliability of a new procedure for protein refolding [1-3]. This peculiar protocol is based on the association of an ionic detergent with a cosolvent. Indeed, though being known to feature denaturing abilities, some detergents appear to have their properties strongly altered when interacting with a cosolvent, and strikingly an appropriate combination of both even turns to a refolding of the protein. This remarkable procedure has successfully been applied to soluble α -helix and β -sheet peptides, as well as soluble and membrane proteins with several types of structures and properties.

In this contribution, we summarize our progress in the understanding by experimental (spectroscopy) and theoretical methods (molecular dynamics), of our recently reported approach.

Keywords: Protein refolding, detergent, cosolvent, spectroscopy, molecular dynamics.

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SL-44

Track: Other Areas

INFLUENCE OF BOVINE AND CAPRINE MILK-BASED PEPTIDES ON EXOPOLYSACCHARIDE SYNTHESIS BY *STREPTOCOCCUS THERMOPHILUS* AND *LACTOBACILLUS DELBRUECKII* SSP. *BULGARICUS***Adela Mora-Gutierrez**

Prairie View A&M University, Cooperative Agricultural Research Center, Prairie View, Texas 77446, USA; E-mail: admora@pvamu.edu

Exopolysaccharide produced by lactic acid bacteria is economically important because it can impact functional effects to foods and confer beneficial health effects. However, the ability to commercially prepare and store lactic cultures to maintain viability and healthful attributes requires the presence of low molecular weight nitrogen in the growth medium. Partial hydrolysis of milk proteins (the caseins) prior to fermentation may provide sufficient low molecular weight peptides for growth and exopolysaccharide production. The objective of this study was to determine the influence of bovine and caprine casein-derived peptides (MW <3 kDa) prepared with trypsin on cell viability and exopolysaccharide synthesis during the culturing of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in MRS broth for 72 h at 37 ± 0.1 °C. Addition of caprine casein phosphopeptides to MRS broth resulted in a significant increase ($p < 0.05$) in the exopolysaccharide content (218.3 mg L^{-1}) as compared to its bovine counterpart (161.2 mg L^{-1}) at 48 h. The efficacy of caprine casein after trypsinolysis on exopolysaccharide synthesis by *S. thermophilus* and *L. delbrueckii*

spp. bulgaricus in MRS broth may be attributed to its high content of hydrophilic amino acid residues (His, Lys, Glu and Ser). The observations are of particular interest in research, commercial, and consuming communities.

Keywords: Exopolysaccharide; lactic acid bacteria; bovine; caprine; milk-based peptides.

SL-233

Track: Medical Biotechnology

TARGETING CD28 SIGNAL FOR STEROID RESISTANT ASTHMA

Akio Mori, Satoshi Kouyama, Miyako Yamaguchi, Yo Iijima, Akemi Ohtomo-Abe, Arisa Kinoshita, Hiroaki Hayashi, Kentaro Watarai, Chihiro Mitsui, Chiyako Oshikata, Kiyoshi Sekiya, Takahiro Tsuburai, Mamoru Ohtomo, Yuma Fukutomi, Masami Taniguchi, Takayuki Ohtomo and Osamu Kaminuma

National Hospital Organization, Sagamihara National Hospital, Clinical Research Center, Sagamihara, Japan; E-mail: moriakiojp@yahoo.co.jp

Rationale: To investigate the role of helper T (Th) cells in steroid resistant (SR) asthma, steroid sensitive (SS) and resistant (SR) Th clones were selected *in vitro*, and then adoptively transferred into unprimed mice. Effect of CTLA4-Ig was analyzed both *in vitro* and *in vivo*.

Methods: For *in vitro* evaluation, ovalbumin (OVA) reactive Th clones were cultured with antigen presenting cells and OVA in the presence of various concentrations of dexamethasone (DEX). Proliferative responses of Th clones were measured by ³H-thymidine incorporation. For *in vivo* assessments, unprimed BALB/c mice were transferred with Th clones, challenged with OVA, and administered with DEX subcutaneously. Bronchoalveolar lavage fluid (BALF) was obtained 48 hr after challenge, and the number of infiltrating cells was differentially counted. CTLA4-Ig was administered through nasal inhalation or venous injection.

Results: SS and SR clones were selected based on the effect of DEX on the proliferative responses of antigen-stimulated Th clones. Airway infiltration of eosinophils and lymphocytes of mice transferred with SS clones were effectively inhibited by the administration of DEX. In contrast, those of mice transferred with SR clones were not significantly inhibited by DEX. Administration of CTLA4-Ig significantly suppressed the proliferation of DEX-treated SR clones *in vitro*, and the eosinophil infiltration of SR asthma model transferred with SR clones *in vivo*.

Conclusions: Steroid sensitivity of Th clones assessed *in vitro* was consistent with that of adoptively transferred asthma model assessed *in vivo*. Costimulatory signal mediated through CD28 is crucial for the induction of steroid resistance both *in vitro* and *in vivo*.

SL-64

Track: Medical Biotechnology

ASSESSMENT OF CRUDE EXTRACT OF HELIX ASPERSA ON CHEMO-INDUCED COLITIS IN RAT

Dalila Naimi, Kouachi Meriem, Djadouri Jallal, Bendahra Ismahane

University Mentouri –Constantine, Laboratory of Genie Microbiologique et Applications, Molecular and Cellular Biology & Physiology Team, Higher National School of Biotechnology –Constantine – Algeria; E-mail: dalila.naimi76@gmail.com

Helix aspersa is a kind of terrestrial snail which consumed by human. In the last decade, many snail farms have been established in the world, on one hand to compensate for the decrease in natural population in certain countries and on the other hand in order to produce good quality snails for consumption.



Snail farming is a very lucrative agricultural activity given the fact that snail's meat provides high level quality of proteins. Also terrestrial Snails are an important source of microelements (salt, copper, iron, phosphor).

Deficiency of certain microelements can be responsible for an increased risk of many diseases, such as: cardiovascular diseases, several forms of cancer, immunodeficiency, allergies. Many studies confirmed in human nutrition, the potential quality of snails protein content, however its importance in Human therapeutics is poorly documented.

We have already explored *in vitro* the aqueous *Helix aspersa* extract effect on some signaling pathways in the tumor cells (expression of Bcl2).

In the present work we tried to explore *in vivo* the effect of *Helix Aspersa* crude extract (HACE) on the chemo-induced damages occurred in rat colon and also we investigated the impact of these damages on the lymphoid organs.

- In the first step, we proposed to evaluate the toxicity of HACE in mice:

Three mice groups (10 animals /group and 5 animals / cage) were respectively submitted to 120mg, 140mg and 160mg / body weight on alternate days during 15 days where we recorded the temperature, weight, and animal behavior. Very few changes have been observed and all animals survived.

With the concentration of 160 mg, animals showed the best gain weight and a slight increase in temperature just at the beginning of the experiment. The behavior of all animals stayed normal. Therefore, this concentration (5g/kg of body weight) was chosen for the rest of the experiences.

In the Second step, 30 males rats used in our experiment were divided in 5 groups (6animals/group) as following:

- Control group: C group, received orally distilled water during 7 days and transrectal administration of distilled water on the 8th
- AA group (acetic acid group) received distilled water during 7 days and on the 8th day transrectal administration of 1ml of 4% acetic acid
- HACE/AA group received the crude extract (5g/kg) during 7days and on the 8th transrectal administration of 1ml of 4% acetic acid
- ASA group received 5-ASA (5-aminosalicylic acid) as standard drug during 7 days and on the 8th day they received transrectal administration of 1ml of 4% acetic acid
- HACE group received the crude extract during 7 days and on the 8th rectal administration of 1ml of distilled water

At the end of experiments and after 24H fasting, all the rats were anesthetized by ether inhalation. After that, animals were immediately dissected for harvesting organs and then sacrificed by overdose of ether.

Organs were washed three times with PBS. 10 cm from the distal colon were collected and examined macroscopically and microscopically after standard hematoxylin-eosin staining. The lymphoid organs (bone marrow, liver and thymus) were also examined in the aim to explore the repercussion of the colitis induction on the immune system.

Biochemical assessments including aspartat aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), thiobarbituric acid reactive substances (TBARs), CRP measurements were also registered.

We also investigated the presence of alterations in lieberkuhn glands on the distal colon. We sought the changes in the crypt foci (CF) structure of the Lieberkuhn glands. We looked for changes in these CF, because foci damages were regarded as precursor lesions of colo-rectal cancer in human. Many studies reported positive correlation between aberrant crypts Foci (ACF) multiplicity and the onset of colon tumors at the late stage.

HACE showed not only a significant improvement of certain parameters affected by experimentally induced colitis: enzymes TBARs, CRP, but also a protection against structure injuries in lymphoid organs and in distal colon. The Lieberkuhn glands injuries observed in animal induced colitis appear to be protected by snail crude extract.

The HACE could probably play a healing role on the colonic mucosa and also a preventive role against possible changes of CF to ACF.

Since the *Helix Aspersa* snail has a high quality of protein and are an important source of microelements, it would be interesting to associate it in a diet for patients in the early stages of hypertrophic crypts Foci in order to prevent and avoid possible malignant transformation.

Keywords: *Helix Aspersa*, colitis, distal colon, crypt foci, lymphoid organs.

SL-105

Track: Regenerative Medicine

ANTI-INFLAMMATORY REGENERATIVE ACTIVITY OF A CD44-DERIVED PEPTIDE

David Naor, Lora Eshkar Sebban, Keren Or Amar and Shmuel Cohen

The Lautenberg Center for Immunology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; E-mail: davidn@ekmd.huji.ac.il



A human CD44-derived 5-mer peptide displays an efficient anti-inflammatory response in collagen-induced arthritis mouse model, as it can regenerate the normal anatomy and the function of the damaged tissue. Injection of the peptide after the onset of the disease substantially reduced the inflammation as indicated by blind analysis of footpad swelling and histopathology of joint sections. The effect is autoimmune-specific and the peptide injection does not induce neutralizing antibodies. In attempt to understand its mechanism of action we focused efforts to identify the target molecule of this 5-mer peptide. Mass Spectrometry analysis revealed a protein epitope as a potential target for the anti-inflammatory activity of the 5-mer peptide. The protein containing the epitope, which is recognized and neutralized by the peptide, is highly involved not only in the pathology of rheumatoid arthritis, but also in the pathologies of Alzheimer's disease, cancer diseases and cardiovascular diseases. This finding provides additional indications for the therapeutic potential of the 5-mer peptide. The protein containing the target epitope strongly supports cell migration in a rheumatoid arthritis model. This finding can explain why the 5-mer peptide is effective in the inhibition of joint inflammation and regeneration of joint normal tissue, as cell migration is an essential element of the inflammation cascade.

SL-36

Track: Business Development

FINANCIAL VALUATION ALGORITHM FOR THE ASSESSMENT OF THE FUTURES SALES OF A NEW, INNOVATIVE MEDICINAL PRODUCT

Mark Nuijten

Health Care Valuation, Amsterdam, The Netherlands; E-mail: marknuijten@arsaccessusmedica.com



Registration is not the sole success factor anymore for future sales and valuation of the share of company. Reimbursement procedures and the introduction of new business models like value-based pricing and risk sharing agreements, have financial consequences for biotech companies. As the future financial performance of a biotech company is directly related to the revenues of new products, an appropriate assessment of the potential sales forecast of the portfolio of forthcoming new products is an important predictor of the financial value of a pharmaceutical company. Therefore information on positive clinical trials results of a new product should be followed by a reimbursement scan and a sales forecast model including the key global markets.

The objective of this lecture is to present a financial valuation algorithm for the assessment of the futures sales of a new, innovative medicinal product based on the current reimbursement policies and future business models for reimbursement. The algorithm consists of a number pathways leading to an assessment of the market potential of the new drug: no potential, limited potential, moderate potential and expected potential. The algorithm is applied to possible new innovative products for treatment of depression and multiple sclerosis.

Keywords: Valuation, reimbursement, algorithm.

SL-147*Track: Pharmaceutical Biotechnology***HYPOLIPIDEMIC AND HYPOGLYCEMIC ACTIVITIES OF ETHANOL EXTRACT OF CYPERUS ROTUNDUS RHIZOME****Gloria N. Okwu, S.E. Abanobi, U.V. Nnadi, N. Nwachukwu, C.O. Ujowundu and A.C. Ene.***Federal University of Technology, Owerri, Nigeria; E-mail: gnokwu@yahoo.com*

The effect of ethanol extract of cyperus rotundus rhizome on carbimazole - induced hyperlipidemia and on hyperglycemia (using glucose tolerance test) in male wistar rats was investigated. Acute toxicity analysis with the cyperus rotundus extract produced no lethality even at high doses. Hyperlipidemia was induced using 400mg/kg cholesterol and 2mg/kg carbimazole. The lipemic control groups were administered cholesterol and carbimazole but not the normal control group. Cholesterol and carbimazole administration caused a significant ($p < 0.05$) increase in the Total Cholesterol, Triglyceride (TG), Low Density Lipoprotein (LDL), Non-High Density Lipoprotein (non-HDL) Cholesterol and LDL/HDL ratio and a significant ($p < 0.05$) decrease in the levels of HDL in the lipemic control when compared to the normal control. Treatment with cyperus rotundus extract at 250mg/kg, 500mg/kg and the standard hyperlipidemic drug (simvastatin) 5mg/kg significantly ($p < 0.05$) reduced Total Cholesterol, TG, LDL, LDL/HDL ratio, Total non-HDL Cholesterol and significantly ($p < 0.05$) increased the level of HDL when compared to the non-treatment groups (the normal control and the lipemic control). Treatment with cyperus rotundus extract and the hyperglycemic drug (Glucophage) at 7.14mg/kg caused a significant ($p < 0.05$) decrease in the blood glucose level when compared to the non-treatment groups. This study concluded that cyperus rotundus rhizome contains principles that compare effectively and as well as standard clinically used antihyperlipidemic and antihyperglycemic agents.

Keywords: Cyperus rotundus, antihyperlipidemia, antihyperglycemia.**SL-312***Track: Plant and Environment***EFFECT OF TRANSGENE COPY NUMBER ON VECTOR BACKBONE (VBB) INTEGRATION IN TRANSGENIC CASSAVA GENOME****Ihuoma Okwuonu***Ihuoma Okwuonu, Head Tissue culture & genetic transformation unit, Biotechnology programme, NRCRI Umudike, Nigeria; E-mail: ihuomaumezurumba@yahoo.com*

The effect of transgene copy number on vector backbone (VBB) integration in transgenic cassava genome was evaluated using Dot blot hybridization and confirmed by PCR analysis. Transgenic cassava events produced by transforming cassava friable embryogenic callus (FEC) with *Agrobacterium* LBA4404 strains independently carrying three different constructs p8016, p8052 and p900 were randomly selected and DNA samples extracted from these events were probed with specific neomycin phosphotransferase (*nptII*) probe. Copy number was measured based on density of the blot signal using Image J software. The occurrences of low and high copy number events were correlated with the total number of VBB integrated and VBB free events. Correlation coefficient of the data revealed that the number of VBB integrated events and the occurrences of low copy and high copy number events were less significant ($r = 0.396$, $n = 5$, $P < 0.05$) and the correlation coefficient between number of VBB free events and occurrences of low and high copy number shows a high positive significance ($r = 0.7708$, $n = 5$, $p < 0.05$) for low copy number and a high negative significance on ($r = -0.891$, $n = 5$, $p < 0.05$) for high copy number. This study shows that the recovery of low copy number events increases the chances of recovering VBB free events. This means that strategies that favors recovery of low copy number events invariably favors the recovery of VBB free events.

Keywords: Vector backbone, transgenic cassava, copy number, *Agrobacterium* transformation.

SL-23

Track: Other Areas

FOOD SCIENCE AND BIOTECHNOLOGY: INNOVATIVE APPLICATIONS OF NON THERMAL TECHNOLOGIES IN FOODS: HIGH PRESSURE PROCESSING FOR INCREASING THE SHELF LIFE AND SENSORIAL ANALYSIS OF FISH AND SEA FOOD.

Ivana Orlando and Luigi Palmieri

Experimental Station for Food Preserving Industry, Angri Section, Via Nazionale 121/123 84012 Angri (Sa), Italy; E- mail: ivana.orlando@ssica.it



Fish, shellfish and gastropoda are very perishable and the only system of preservation in order to retain their sensorial properties is the application of refrigeration by means of ice that lets a short shelf-life. For their high commercial risks, in order to utilize the surplus it is really important to increase the shelf-life by means this non thermal technology: High Pressure, well settled in many parts of the world. In this paper have been carried out trials on packed fish and not, gastropoda and shell-fish these last inoculated with pathogenic microorganisms. All the samples have been treated at different pressure and different times with a Quintus Food Press 600. The obtained results show an increasing of shelf –life and sensorial properties of the treated products compared to not treated samples.

SL-300

Track: Plant and Environment

INHIBITION OF *CHILO PARTELLUS* (LEPIDOPTERA: PYRALIDAE) GUT PROTEASES BY COMPOUNDS FROM *IPOMOEA BATATA*

Balaji Panchal and Manvendra Kachole

Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra 431004, India; E-mail: panchalbalaji@yahoo.co.in

Chilo partellus is a devastating pest of sorghum bicolor and maize all over the world. *C. partellus* gut proteases were isolated, purified and characterized. We analysed potential trypsin and chymotrypsin inhibitors from tuber plants to identify protease inhibitors (PIs) of *C. partellus* gut proteases. Enzymatic and feeding assays were revealed that non-host PIs inhibited protease activity efficiently. PIs from non-host *Ipomoea batata* inhibited total proeace activity, retarded the growth and led to reduced weight of *C. partellus* larvae. One PI was purified from *I. batata* tubers and identified as a potent PI against *C. partellus* gut proteases. We propose this compound has potential for developing *C. partellus*-resistant transgenic plants.

Keywords: *Chilo partellus* insects; CPGPs; Host and non-host PIs; BApNA; GXCP.

SL-304*Track: Plant and Environment***PLANT STRESS ADAPTATION, SIGNALING AND THE REGULATORY SMALL RNA PATHWAYS: EVOLUTION AND FUNCTIONAL SPECIALIZATION****Shree Prakash Panday**

*Biological Sciences, Indian Institute of Science Education and Research Kolkata, India;
E-mail: shreepandey@gmail.com*



Plants face a plethora of biotic stresses in their agro-ecological environments. Responses of plants tailored to these stresses involve perception, processing and integration of external information into cellular and physiological machinery. This involves elicitation of complex signaling networks. But these signaling networks remain poorly characterized in crop species such as wheat. Moreover, other than the involvement of some transcription factors (TFs), how cellular signaling is modulated during attack of fungal pathogens and herbivores remains poorly understood even in model plants. On the other hand, small regulatory RNAs (smRNAs), such as microRNAs, have appeared as master regulators of cellular signaling events in processes such as development and differentiation. Our functional studies on components small RNA machinery suggest that smRNA biogenesis pathways have evolved in specialized manner during adaptation to specific stresses. Use the Argonaute (AGO) proteins (the central component of the smRNA pathways) as candidates for studying evolution of smRNA pathways; we conclude that the evolution of smRNA pathways has been a dynamic process that could generate signatures of their diversification of function in plants. We have extended our investigations to the wheat genomes to identify, annotate and understand signaling pathways and to determine phylogenetic linkages in other grass genomes. Our results have direct implications for biotechnological applications for crop improvement in cereals in general and wheat in particular.

Keywords: Phytohormone signaling, small RNA, miRNA, transcription factor, stress adaptation.

SL-234*Track: Plant and Environment***A NEW PLANT OIL PRODUCTION PLATFORM: SEED-LIKE OIL YIELD FROM BIOMASS****James Petrie, Thomas Vanhercke, Allan Green and Surinder Singh**

CSIRO Food, Nutrition and Bioproducts Flagship, ACT, Australia; E-mail: James.Petrie@csiro.au



Supply of vegetable oils as a major commodity faces continuous pressure. Global demand is expected to double in the next two decades due to increasing world population and rising petroleum prices. Increasing limitations on arable land and agricultural inputs mean it will be difficult to meet this additional demand with current oilseed-based production systems. The concept of producing oil in the leaves and stems of high biomass species has attracted attention as a way to intensify oil production. The engineering of such a new oil production platform would not only yield greater amount of oil for a given land area but also provide a way to more easily segregate bioeconomy traits such as unusual fatty acids away from food production. We previously reported the accumulation of up to 17% triacylglycerol (TAG, dry weight) in leaf tissue of *Nicotiana* species. This was achieved by combinatorial metabolic engineering in which we increased fatty acid biosynthesis ('Push') by limited overexpression of the WRI1 transcription factor, increased TAG assembly ('Pull') by expressing DGAT1, and encouraged oil body formation ('Packaging') by expressing oleosin in plant leaves^{1,2}.

In this presentation, we will describe some of our second generation construct designs which have more than doubled the previously reported TAG content. Oil content in leaves now matches elite oilseed crop seed levels of +40%. We will describe the implications that this technology has for global plant oil production from a yield and intensification perspective, as well as the challenges that remain for integration into the existing industry. We will also present data demonstrating that the newly produced fatty acids can be modified for industrial or nutritional applications as well as preliminary data of a transcriptome comparison between wild type and high oil leaf tissue, harvested at different stages during plant development.

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SL-268

Track: Other Areas

ATPASE 6/8 GENE ASSISTED GENETIC VARIABILITY STUDIES AMONG THE POPULATION OF LABEO ROHITA

Fayyaz Rasool, Shafat Hussain and Shakeela Parveen

*Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore-Pakistan;
E-mail: fayyazrasool@uvas.edu.pk*

Labeo rohita is a commercially important fish of South Asia. A study on the variation amongst the different populations of on the basis of ATPase 6/8 gene was conducted. The present study evaluates the potential of complete ATPase 6/8 region of mitochondrial DNA as a marker region to determine the phylogeography of Labeo rohita from fish farm of region Punjab, Pakistan. mtATPase6/8 (878 bp) regions was used to investigate genetic variation within Labeo rohita and develop a global genealogy of genus Labeo strains. The mtATPase6/8 region was more variable but the given the wide distribution of Labeo rohita the overall levels of sequence divergence were low. Levels of haplotype diversity varied widely among countries with Chinese and India showing the greatest diversity whereas Japanese Labeo had undetectable nucleotide variation. Chinese and Japanese carp strains were the most divergent, and their relationships do not support the evolution of independent Asian and European lineages and current taxonomic treatments. The results revealed that 878 bp of ATPase 6/8 region could be a promising marker for determining variations at interpopulation as well as intrapopulation levels in Labeo rohita. These results would facilitate conservation and management of this important species

Keywords: ATPase6/8, Variation, Populations, Labeo rohita.

SL-282

Track: Other Areas

METALLOGENIC INFORMATION EXTRACT AND PROGNOSIS RESEARCH OF SEAFLOOR POLYMETALLIC SULFIDE RESOURCES IN SOUTHWEST INDIAN OCEAN

Mengyi Ren, Jianping Chen and Ke Shao

*School of the Earth Sciences and Resources, China University of Geosciences, Beijing, China;
E-mail: renmengyi@163.com*

Seafloor polymetallic sulfide becomes one kind of seabed mineral resources with a huge development prospect after the discovery of the Fe-Mn nodules and Cobalt-rich crusts. Polymetallic sulfides have attracted significant attention as a potential seabed mineral resource due to their high grade of precious metal elements, such as Cu, Zn, Pb, Au, and Ag. Survey for seafloor polymetallic sulfides is still in the general exploration stage at home and abroad, and the prediction and evaluation of seafloor sulfide resource potential is still in the blank. In this paper, we select Southwest Indian Ridge as the prediction area and uses weights-of-evidence method to carry out the comprehensive metallogenic prognosis research with multivariate information. We extensively collect geological, geophysical and other related information, summarize the ore-controlling factors, and establish an ore deposit prediction model of polymetallic sulfides. The result of the prediction shows us the locations of prospecting targets that will provide a basis for the further exploration and development of the seafloor sulfide mineral resources in southwest Indian Ridge.

Keywords: Seafloor polymetallic sulfide, Metallogenic information extraction, Prediction model, Weights-of-evidence.

SL-153*Track: Pharmaceutical Biotechnology***ENDOPHYTIC FUNGUS AS A SOURCE OF BIOACTIVE SECONDARY METABOLITES: RESVERATROL AND VINIFERIN****Chetana Roat and Shailesh R. Dave***Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad, India;
E-mail: chetana.roat@gmail.com*

Resveratrol and Viniferin possess a broad spectrum of pharmacological and therapeutic effect like anti-cancer, antioxidant, anti-atherosclerosis. Endophytes, by residing within the specific chemical environment of host plant tissue, form unique group of microorganisms. Microbially unexplored medicinal plants can have diverse and potential microbial association. The aim of this work is to identify and isolate the endophytic microorganisms that possess resveratrol and viniferin producing capability and optimizes the conditions for their production. Endophytes from *Vitis vinifera* L.cv. Merlot, *Vitis quinquangularis* Rend. and *Cayratia trifolia* L. were studied and the resveratrol and viniferin producing isolates were screened by thin Layer Chromatographic (TLC) with solvents system $\text{CHCl}_3/\text{MeOH}/\text{HCOOH}$, 85/15/3 (v/v) and identification of the compound was done by HPLC. The total of 20 isolates were obtained, 5 were endophytic bacteria and 15 were endophytic fungi and it was found that the one strain *Aspergillus* sp. AB4 isolated from leaf of *Vitis vinifera* using Czapek yeast extract agar medium had stable resveratrol and viniferin producing capability in all subcultures. Cell growth of *Aspergillus* sp. increased during cultivation and reached a stable and high level of biomass after 7 days. The best fermentation conditions for resveratrol and viniferin production in broth cultures of *Aspergillus* sp. AB4 were an inoculum size of 10%, a rotation speed of 100 rpm, and a temperature of 28°C.

Keywords: Resveratrol, Viniferin, Endophytes, *Aspergillus*, HPLC.

SL-313*Track: Pharmaceutical Biotechnology***PAPER-ATTACHED YEAST BIOFILMS. AN ALTERNATIVE FOR LIVING BIOCATALYSTS GROWTH****Edvaldo Antonio Ribeiro Rosa, Rosimeire Takaki Rosa, Alinne Ulbrich Mores Rymovicz, Cristina Rauen Ribas, Marcela Cardoso Selow***Xenobiotics Research Unit, The Pontifical catholic University of Paraná, Curitiba, Brazil;
E-mail: prof.edvaldo.rosa@gmail.com*

Microbial biotransformation (a.k.a. biocatalysis, bioconversion) processes are amongst the smarter and greener ways to obtain new therapeutic molecules. Such processes occur when a microorganism converts a substrate into new molecule(s) by adding chemical functional groups, mainly *via* P450 cytochromes. Consolidated data reveals that immobilized cells tend to carry out biotransformations in a more promptly manner and several processes have been developed for that. In this study, we propose the use of filter paper as a support matrix for microbial attachment and further growth as biofilm.

A dedicated biofilm bioreactor was conceived and built. Among a diversity of microorganisms possible to be employed, *Candida albicans* was elected due to its wide morphological multiplicity. Eight-millimeter paper disks were soaked with ca. 106 cells of *C. albicans* and transferred to the biofilm bioreactor. Sterile culture broth was admitted in a slow flux and consistent biofilms were obtained after 72 h. Scanning electron microscopy revealed the occurrence of pseudo and true hyphae in abundance, even within the paper matrix.

Authors propose that paper-based matrixes may be considered as suitable supports for the growth of biofilms, which may serve as living biocatalysts.

SL-175

Track: Pharmaceutical Biotechnology

PHARMACOKINETICS AND IMMUNOGENICITY OF PLANT-DERIVED BROADLY NEUTRALIZING HIV MONOCLONAL ANTIBODIES IN MACAQUES

Yvonne J. Rosenberg

CEO, PlantVax Inc., 9430 Key West Ave, Suite 120, Rockville, MD 20850, USA;

E-mail: YRosenTher@aol.com



The identification of highly potent broadly neutralizing antibodies (bnAbs) against HIV-1, and success in preventing SHIV infection following their passive administration in macaques, have increased the likelihood that immunotherapeutic strategies can be adopted to prevent and treat HIV-1 infection. However, while broad and potent neutralizing activity is an essential prerequisite, *in vivo* properties such as good circulatory stability and non-immunogenicity are equally critical for developing a human treatment. In the present study, glycoforms of the bnAbs 10-1074, NIH45-46G54W, 10E8, PGT121, PGT128, PGT145, PGT135, PG9, PG16, VRC01 and b12 were produced by Agrobacterium-mediated transient transfection of *Nicotiana benthamiana* and assessed following administration in macaques. The results indicate that (i) N-glycans within the VL domain impair plasma stability of plant-derived bnAbs and (ii) while PGT121 and b12 exhibit no immunogenicity in macaques after multiple injections, VRC01, 10-1074 and NIH45-46G54W elicit high titer anti-idiotypic antibodies following a second injection. These anti-idiotypic antibodies specifically bind the administered bnAb or a close family member, and inhibit the bnAb in neutralization assays. These findings suggest that specific mutations in certain bnAbs contribute to their immunogenicity and call attention to the prospect that these mutated bnAbs will be immunogenic in humans, potentially compromising their value for prophylaxis and therapy of HIV-1.

SL-253

Track: Other Areas

BIOTECHNOLOGICAL APPROACH OF MEMS MICROROBOT SYSTEM WITH ARTIFICIAL NEURAL NETWORKS INTEGRATED CIRCUIT

Ken Saito, Minami Takato, Yoshifumi Sekine and Fumio Uchikoba

Department of Precision Machinery Engineering, College of Science and Technology, Nihon University, Japan; E-mail: kensaito@eme.cst.nihon-u.ac.jp



Microrobot could play an important role in paramedical use such as cleaning inside the blood vessel. However, thin artery of human is less than 5 mm and artery structure of human differs greatly in individuals. Therefore, further miniaturizations and higher functionalization on the microrobot system are required to play an important role in paramedical use. We will talk about less than 5mm width, length, and height in size hexapod locomotive type microrobot system. The microrobot system consisted by micro-mechanical systems which were fabricated by the micro fabrication technology and micro-electro systems which was constructed by the integrated circuit technology. Micro-mechanical systems were equipped with small size rotary type actuators, body frame, link mechanisms, and 6 legs to realize the ant-like switching behavior. Micro-electro system was biologically inspired locomotion rhythm generator of the microrobot using artificial neural networks. Both systems were made from silicon wafer. Therefore, both systems could integrate on same silicon wafer using micro-electro-mechanical systems (MEMS) technology. Artificial neural networks consisted by 4 cell body models and 12 inhibitory synaptic models. Cell body model was analog circuit model which could output oscillatory patterns such as the biological neuron. Cell body models were connected mutually by the inhibitory synaptic models. Thus, artificial neural networks could generate the locomotion rhythms using synchronization phenomena of the cell body models such as biological neural networks. Locomotion rhythm generator using artificial neural networks realized the locomotion of the robot without using any software programs or analog digital converters. As a result, MEMS microrobot performed forward and backward locomotion, and also changes direction by inputting an external single trigger pulse to the artificial neural networks.

Keywords: Biotechnology, MEMS, Microrobot, Artificial Neural Networks

SL-249

Track: Plant and Environment

BIOAUGMENTATION OF CRUDE OIL SPILLS: BIOREMEDIATION WITH A MICROBIAL FORMULA TAILORED WITH SELECTED SOLVENT TOLERANT NATIVE STRAIN

Seema Sambrani, Bharat Shinde and Shailesh Dave

Department of Biotechnology, Fergusson College, Pune, India;

E-mail: seemasambrani@hotmail.com



The activation of natural degradation potentials in environment is currently the challenge in environmental research addressed to remediation methods. Presence of hydrocarbons often inhibits survival and biodegradation capabilities of the responsible organisms. The aim of the work is to check the feasibility of oil degradation in water using bioaugmentation strategy based on the use of the microbial formula tailored with selected native strain. The prepared formula consists of five bacterial strains having multiple solvents resistance. Lipase activity was assayed by Kwon and Rhee method. The maximum lipase production was observed under aerobic conditions, the microaerobic condition also showed comparable results. Lipase enzymes were partially purified and its molecular weight was determined by SDS-PAGE. 16S rDNA sequence analysis by NCBI-BLAST identified four isolates to be *Bacillus* species and one *Pseudomonas* species. Their lipolytic ability in the presence of different temperature, pH, natural oils, synthetic oils, organic carbon, inorganic carbon, nitrogen, surfactants and metal ions was investigated. Indeed this tailored microbial formula efficiently facilitates the breakdown of oils and the solvents in an ecofriendly way. The results of LCMS Analysis are interesting. Till date there are reports of organic solvent tolerant lipases used for bioremediation but very few reports on bioaugmentation.

Keywords: Bioremediation, Bioaugmentation, oil degrading Bacilli, lipases, solvent tolerance

SL-217

Track: Pharmaceutical Biotechnology

NON-INVASIVE DELIVERY OF BIOTECHNOLOGICAL DRUGS

Jun Shao

Department of Pharmaceutical Sciences, College of Pharmacy and Health Professions, St. John's University, Queens, NY, USA; E-mail: shaoj@stjohns.edu



With the advancement of biotechnology, several hundred biotechnological products are developed and approved as drugs. However, most of them are administered by injection which is painful and inconvenient although the absorption through such route is good. Safety is also a concern when dealing with needles. Therefore, for a long time, the demand for non-invasive delivery of these drugs is great. However, low permeability through biological mucosa due to their large size and hydrophilic nature restricts them from being absorbed sufficiently through those non-invasive routes. In addition, most of these drugs will face extensive pre-absorption degradation, especially in the gastro-intestinal tract (GIT), presents another major difficulty for non-invasive delivery. Non-invasive delivery means the drug is administered by routes other than injection, which usually include oral, pulmonary, nasal, vaginal and transdermal routes. Tremendous efforts have been placed on the development of non-invasive delivery of biotechnological drugs in the past several decades around the world. The most successful case is the inhalation of insulin. Otherwise, there has been not much success. Even though, many studies do shed light for future success. In this presentation, the following aspects will be discussed: general physicochemical properties of biotechnological drugs relevant to absorption through non-invasive routes, the physiological features of the various routes related to absorption and their respective advantages and disadvantages, and some

advanced delivery systems showing promising results. The focus of this presentation will be on the various delivery systems such as micro-, nano-particles, nanoemulsions, microneedles, and probiotic bacteria. Several case studies will be presented as well.

SL-259

Track: Plant and Environment

BIOREMEDIATION OF HEAVY METAL POLLUTED AND SALT AFFECTED SOILS EMPLOYING ARBUSCULAR MYCORRHIZAL FUNGI

Satyawati Sharma, Kalpana Arora, Abhishek Sharma and Ashwani Kumar

Centre for Rural Development and Technology, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110016, India; E-mail: satyawatis@hotmail.com



Contaminated soils are hazardous to the environment and human health. Among various soil remedial measures, bioremediation is considered to be ecofriendly and socio-economically a highly suitable method. Microbes are adapted to thrive in adverse conditions of high acidity, alkalinity, toxicity and temperature and can either uptake and/or bio-transform the heavy metals from/in the soil. Arbuscular Mycorrhizal Fungi (AMF) play vital role in soil bioremediation, increasing soil fertility and enhancing plant growth and biomass. The present study deals with the evaluation of potential application of AMF along with *Brassica juncea*, *Sorghum vulgare*, *Panicum maximum* and *P. virgatum* (Switch grass) for reducing the heavy metal toxicity (Pb, Cd) and *Jatropha* for salinity (EC up to 7 mmhos/cm) and alkalinity (pH up to 10) of soils. The results indicated effective role of AMF colonization for biomass production in heavy metal and salt affected soils along with bioremediation. 23-50% heavy metal (Cd and Pb) uptake was observed in different selected plant species. An increase in proline, protein and sugars was also observed. The data pertaining to glomalin, content in the soil (AM-inoculated normal soil) showed a higher amount of glomalin. In comparison to non-saline control, AMF treatment showed 1.59, 1.54 and 1.28 times elevated SOD, 3.46, 3.23 and 3.05 times APX and 2.93, 2.92 and 3.1 times GR enzyme activities at 6, 12, and 18 MAP, respectively. Proteomic analysis of *Jatropha* seedlings, grown under different salt stress regimes, using 1DSDS-PAGE and MALDI-TOF showed up- and down-regulation of some new proteins. Down regulating protein, 49kD was matched with *Neesia* (NADH dehydrogenase subunit) and *Jatropha integrimma* (ribulose 1-5 biphosphate carboxylase/oxygenase large subunit). Up regulated protein 17kD was matched with heat shock class 1 protein and novel protein of 23kD was matched with unnamed protein product *Osterococcustauri*.

The data pertaining to bioremediation of soils (Heavy metal %, EC, pH) and plant biomass yield will be presented at conference.

Keywords: Bioremediation, Efficient microbes, Heavy metals, Salinity and Alkalinity Pollutants.

SL-84

Track: Regenerative Medicine

NOVEL SMOOTH MUSCLE CELL PROGENITORS ARE PRIMED TO MUSCULARIZE IN DISEASE

Abdul Q. Sheikh, Ralf H. Adams and Daniel M. Greif

Yale Cardiovascular Research Center, Yale University, USA; E-mail: abdul.sheikh@yale.edu



Excess and ectopic smooth muscle cells (SMCs) are central to cardiovascular disease pathogenesis, but underlying mechanisms are poorly defined. For instance, pulmonary hypertension (PH) or elevated pulmonary artery blood pressure is a lethal disease with distal extension of smooth muscle to normally unmuscularized pulmonary arterioles. We recently demonstrated that embryonic pulmonary artery wall morphogenesis consists of discrete developmentally regulated steps. In contrast, poor understanding of distal arteriole muscularization in pulmonary artery hypertension severely limits existing therapies that aim to

dilate the pulmonary vasculature but have modest clinical benefit and do not prevent hypermuscularization. Here, we show that most pathological distal arteriole smooth muscle cells derive from pre-existing smooth muscle and the program of distal arteriole muscularization encompasses smooth muscle cell dedifferentiation, distal migration, proliferation, and then redifferentiation. Interestingly, we consistently detect a few SMA+SMMHC+PDGFR- β + cells adjacent to the arteriole muscular-unmuscular transition in normoxia and these cells are primed to dedifferentiate, migrate and colonially expand in hypoxia-induced PH. In sum, novel SMC progenitors are critical for the pathogenesis of PH, and perhaps other vascular disorders, and therapeutic strategies targeting this cell type have profound implications.

SL-273

Track: Other Areas

BOOSTING THE FOOD FUNCTIONALITY (*IN VIVO* AND *IN VITRO*) OF SPIRULINA GROWN IN BANGLADESH BY GAMMA RADIATION: AN INSPIRING APPROACH

Hossain Uddin Shekhar, Emran Kabir Chowdhury and Jahid M M Islam

*Biochemistry and Molecular Biology, Dhaka University, Bangladesh;
E-mail: hossainshekhar@yahoo.com*



Functional food is a natural or processed food contains known biologically active compounds, which provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age. In this experiment Spirulina, a well known functional food worldwide is irradiated by ^{60}Co gamma radiation by different doses 5, 10, 15, 20, 25 and 30 kGy at a dose rate of 5 kGy per hour. Amongst the irradiated samples Spirulina exposed to 15kGy showed most inspiring results both *in vitro* and *in vivo*. 15kGy irradiated Spirulina showed maximum fat binding capacity (FBC,47%) and sugar binding capacity (SBC,119%). Water holding capacity, water retention capacity and swelling capacity of 15kGy irradiated Spirulina was found to be highest and were 33.68%, 40.22% and 86.96% respectively. Mice fed with irradiated Spirulina, exhibited lower weight gain, reduced blood glucose, TG, cholesterol, LDL level and higher HDL level compared to the control group although both of the groups were fed fat rich diet.

Keywords: Functional food, Spirulina, irradiation.

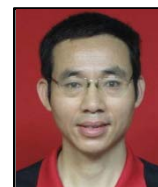
SL-187

Track: Pharmaceutical Biotechnology

MTDLS DESIGN ON ACETYLCHOLINESTERASE (ACHE) AND B-SECRETASE (BACE-1): 3D-QSAR AND MOLECULAR DOCKING STUDIES

Jiancheng Shi, Wentong Tu and Chusheng Huang

*College of Chemistry and Material Sciences, Guangxi Teachers Education University, China;
E-mail: wsf668@sina.cn*



To find promising new multitargeted Alzheimer's disease (AD) inhibitors, the three-dimensional quantitative structure-activity relationships (3D-QSAR) models for 32 AD inhibitors were established by using comparative molecular field analysis (CoMFA) and comparative similarity indices analysis (CoMSIA) methods. Results showed that the CoMFA and CoMSIA models were constructed successfully with a good cross-validated coefficient (q^2) and a non-cross-validated coefficient (R^2), and the binding modes obtained by molecular docking were in agreement with the 3D-QSAR results, which suggests that the present 3D-QSAR model has good predictive capability to guide the design and structural modification of novel multitargeted AD inhibitors. Meanwhile, we found that one side of inhibitory molecule should be small group so that it would be conducive to enter the gorge to interact with the catalytic active sites of AChE, and the other side of inhibitory molecule should be large group so that it would be favorable for

interaction with the peripheral anionic site of AChE. Furthermore, based on the 3D-QSAR models and the binding modes of AChE and BACE-1, the designed multitargeted AD molecules could act as dual binding inhibitors on AChE (act at catalytic and peripheral sites) and dual targets inhibitors (act at AChE and BACE-1). We hope that our results could provide hints for the design of new multitargeted AD derivatives with more potency and selective activity.

SL-264

Track: Plant and Environment

MULTI-OMICS STUDY ON ANTHOCYANIN AND RESVERATROL ACCUMULATIONS IN GRAPE BERRY SKIN AND CULTURE CELLS

Katsuhiko Shiratake, Mami Suzuki, Hiroshi Sakamoto, Moeko Taki, Ryo Nakabayashi, Yoichiro Fukao, Yoshiyuki Ogata, Nozomu Sakurai, Toshiaki Tokimatsu, Susumu Goto and Kazuki Saito

Graduate School of Bioagricultural Sciences, Nagoya University, Japan; E-mail: shira@agr.nagoya-u.ac.jp

Grape is an important crop as a horticultural crop and for wine production. Grape accumulates different polyphenolic compounds, such as anthocyanin, catechin and resveratrol, in the berry skin. These compounds decide color and taste of fruit and wine and are known as beneficial compounds for human health. We performed transcriptomics and metabolomics of grape berry skin after ultraviolet irradiation, and proteomics and metabolomics of grape culture cells after light irradiation or elicitor and jasmonic acid treatment. The metabolic pathway maps projected the obtained omics data clearly show specific induction of anthocyanin or resveratrol synthetic pathway by different stimuli. Proteomics of microsomal fraction revealed known anthocyanin transporters and putative ones. We will discuss the possibility of anthocyanin and resveratrol productions using the grape culture cells with metabolic engineering, too.



Keywords: Grape, anthocyanin, resveratrol, omics, culture cell.

SL-99

Track: Medical Biotechnology

APPLICATION OF NEWER MOLECULAR METHODS FOR DIAGNOSING AND DRUG RESISTANCE DETECTION IN *MYCOBACTERIUM TUBERCULOSIS*: EVALUATION IN INDIAN SETTING

Sarman Singh, Beenish Syed Rufai, Parveen Kumar and Jitender Singh

Division of Clinical Microbiology & Molecular Medicine, All India Institute of Medical Sciences, New Delhi, India; E-mails: Sarman_singh@yahoo.com; sarman.singh@gmail.com

Tuberculosis is global health problem, specially after AIDS epidemic. India is having largest pool of drug resistant tuberculosis. The most important concern is its early diagnosis and drug resistance. The conventional methods of diagnosis and drug resistance detection are cumbersome, takes several weeks and lack reproducibility. Therefore in recent years several molecular methods such as conventional monoplex and multiplex polymerase chain reactions, real time PCR assays, Line probe assay and Xpert MTB/RIF have been made commercially available. The MTBDR_{plus} line probe assay (LPA) and Xpert MTB/RIF have been endorsed by World Health Organization and both these tests can be used for detecting Tuberculosis as well as for drug resistant detection in *Mycobacterium tuberculosis*. However, there is no clarity regarding the superiority of one over the other. Therefore, for the first time from India, we carried out a prospective study, to evaluated the efficacy of Xpert MTB/RIF and LPA on culture confirmed samples. A total of 405 sputa of suspected drug resistant tuberculosis patients were included. Of these, 285 samples were smear positive and all these were subjected to LPA. Seventy-two (25.8%) samples showed multi-drug resistance, 62 (22.2%) showed rifampicin monoresistance, 29 (10.3%) isoniazid monoresistance and 116 (41.5%) were pan-susceptible. All 62 rifampicin monoresistant samples detected by LPA were tested by Xpert MTB/RIF using cartridge version G4. Of these, 38 (61.4%) showed concordance with LPA showing rifampicin resistance while 21 (33.8%) were found discordant susceptible to rifampicin by Xpert MTB/RIF using cartridge version G4. Of the 116 pan-susceptible samples, only 83 were available for Xpert MTB/RIF testing; of which 4 (5.1%)

were found rifampicin resistant, 74 (94.8%) were susceptible. The 25 discrepant samples were further subjected to MGIT960 drug-susceptibility testing. The MGIT960 results showed 100% agreement with LPA results but only 64.4% agreement with Xpert MTB/RIF results. Sequencing analysis of discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with Xpert MTB/RIF assay. These isolates were characterized by spoligotyping and mycobacterial interspersed repetitive-unit-variable-number-tandem-repeat (MIRU-VNTR) analysis. Interestingly, most of these strains were CAS (CAS-1_Delhi [SIT 26] and 2 of SIT 846), followed by Orphan (SIT 27), and only 1 belonged to the MANU2 (SIT 1976) genotype. NO isolate was Beijing type.

This study shows that Xpert MTB/RIF may not be the first choice of molecular test to be used as point of care as primary screening test.

SL-296

Track: Plant and Environment

METABOLIC ENGINEERING OF LONG CHAIN OMEGA-3 FATTY ACIDS FOR THE OILSEED INDUSTRY

Surinder Singh, James Petrie and Malcolm Devine

CSIRO Food, Nutrition and Bioproducts Flagship, ACT, Australia; E-mail: surinder.singh@csiro.au

Omega-3 long-chain ($\geq C20$) polyunsaturated fatty acids ($\omega 3$ LC-PUFA) like EPA and DHA have critical roles in human health and development with numerous studies indicating that deficiencies in these fatty acids can increase the risk or severity of cardiovascular and inflammatory diseases in particular. These fatty acids are predominantly sourced from fish and algal oils. In order to meet the increasing demand for these oils there is an urgent need for an alternative, safe and sustainable source of EPA and DHA. Over the last 10 years, we have focused on the production of $\omega 3$ LC-PUFA in oilseeds. In particular, we have had world leading success in engineering the synthesis of DHA in oilseeds. My talk will describe the transition of DHA production in seed of our model species *Arabidopsis* through to camelina and our target crop canola. DHA levels that exceed the amount typically found in bulk fish oil have now been achieved in all three species and involved transfer of a 7 gene algal pathway into oilseed crops. The talk will describe gene isolation and characterisation, design and assembly of complex multi-gene expression vectors harbouring a high efficiency $\omega 3$ LC-PUFA synthesis pathway and transgenic seed oil fatty acid profiles.



SL-51

Track: Other Areas

MICROBIAL RESPONSE(S) TO THE 2010 DEEPWATER HORIZON GULF OIL SPILL: A 5-YEAR STUDY OF A COASTAL ALABAMA SALT MARSH

P. A. Sobecky

The University of Alabama, Tuscaloosa, AL 35487, USA; E-mail: psobecky@ua.edu

On April 20, 2010, the explosion and sinking of the *Deepwater Horizon* (DWH) oil rig in the Gulf of Mexico resulted in the loss of eleven lives and the release of an estimated 3.1 to 4.2 million barrels of oil along with methane gas into the overlying water column during an 87-day period. The spill has been referred to as one of the worst environmental disasters in U.S. history. We have been conducting a long term study of a coastal Alabama ecosystem that was impacted by the DWH oil spill. Sediments, water samples, and tar balls have been collected at our Alabama study site over the past 5 years beginning in June 2010. Nucleic acids from the microbial communities have been subjected to cultivation-independent genomics-based analyses including high density microarrays, 16S rRNA amplicon and metagenomic (shotgun) sequencing during this period. The goal of our 5-year study is to gain insights into the responses and changes to the salt marsh microbial community structure and function resulting from and/or associated with the DWH spill. Our objectives are to better understand the metabolic properties of microbial populations in marsh sediments from coastal Alabama in response to the oil so as to identify their roles in hydrocarbon (oil) degradation and their contributions to the recovery of ecological functions in this economically vital coastal marsh system. In addition, we are applying the same genomics-based

approaches to highly weathered tar balls washed ashore in coastal Alabama during 2010 and 2011 to identify the roles and contributions of microbial populations capable of promoting aerobic and anaerobic hydrocarbon degradation of weathered oil.

SL-98

Track: Regenerative Medicine

INITIAL RESULTS OF GENE THERAPY FOR β THALASSEMIA MAJOR VIA TRANSPLANTATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSDUCED EX-VIVO WITH A LENTIVIRAL β A T87Q GLOBIN VECTOR

Sandeep Soni, Alexis A. Thompson, Mark C. Walters, Philippe Leboulch and Marina Cavazzana

Department of Hematology/Cellular Therapy, Bluebird Biotech, Inc., USA;

E-mail: ssoni@bluebirdbio.com

Background: Hematopoietic stem cell (HSC) gene therapy has the potential to induce globin production in the red blood cell lineage to diminish the need for blood transfusions in patients with β -thalassemia major. Transplantation with autologous CD34+ cells transduced with a replication-defective, self-inactivating LentiGlobin BB305 lentiviral vector containing an engineered β -globin gene (β A-T87Q) is being tested in a Phase 1/2 studies.

Subjects and Methods: The Phase 1/2 studies are designed to evaluate the feasibility, safety and efficacy of LentiGlobin (BB305) drug product in the treatment of subjects with beta-thalassemia major (clinicaltrials.gov NCT01745120). Subjects undergo HSC collection *via* mobilized peripheral blood apheresis and CD34+ cells are selected. Estimation of the mean *ex-vivo* vector copy number (VCN) is obtained by quantitative PCR performed on pooled colony-forming progenitors. Subjects undergo myeloablation with intravenous busulfan (Bu), followed by infusion of transduced CD34+ cells. Subjects are monitored for hematologic engraftment, β A-T87Q -globin expression (by high performance liquid chromatography) and transfusion requirements. Integration site analysis (ISA, by linear amplification-mediated PCR and high-throughput sequencing on nucleated cells) and replication-competent lentivirus (RCL) assays are performed for safety monitoring.

Results: As of 1 December 2014, seven subjects have undergone gene-therapy *via* infusion of transduced CD34+ cells. All four subjects, with >3 months of follow up, are producing significant β A-T87Q globin and are transfusion independent after an overall median follow up of 94 days (range: 21-372 days). All adverse events to date were related to Bu conditioning, without any serious or gene therapy-related adverse events. Details of patient eligibility, vector design, efficiency of transduction and safety parameters for gene therapy will be provided.

Conclusion: *Ex-vivo* gene transfer of β A T87Q globin to autologous HSCs has resulted in clinically beneficial production of β A-T87Q globin and is a promising approach for the treatment of patients with β -thalassemia major

SL-279

Track: Plant and Environment

ENZYMATIC DE-EPOXIDATION OF VIOLAXANTHIN AND DIADINOXANTHIN IN THE XANTHOPHYLL CYCLE REQUIRES PRESENCE OF NON-BILAYER LIPIDS

Kazimierz Strzalka, Dariusz Latowski, Susann Schaller, Malgorzata Jemioła-Rzeminska, Christian Wilhelm and Reimund Goss

Faculty of Biochemistry, Biophysics and Biotechnology, and Malopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa Str. 7, 30-387 Kraków, Poland;

E-mail: kazimierzstrzalka@gmail.com

In present work we investigated influence of lipids on the activity of two de-epoxidases: violaxanthin de-epoxidase (VDE) engaged in the violaxanthin cycle and diadinoxanthin de-epoxidase (DDE) operating in the diadinoxanthin cycle. These cycles play important photoprotective role in plants and



diatoms, respectively. We studied the effect of various lipids differing in their chemical character and physical properties on VDE and DDE activity. Among the parameters studied were thickness of the hydrophobic fraction of the lipid aggregates, molecular dynamics of the membrane, size of the inverted micelles and lipid solubility of violaxanthin and diadinoxanthin. We found that the main factors determining the VDE and DDE activity are the type of structure formed by lipids and solubility of xanthophylls in different lipids. The presented results indicate that VDE and DDE constitute a new family of enzymes which require presence of non-lamellar lipids for their activity.

SL-86

Track: Plant and Environment

THE FIRST EXTENSIVE CASE-STUDY ON THE EFFECTS OF ARTIFICIAL MYCORRHIZATION OF ACER BUERGERIANUM MIQ. IN HUNGARY

Krisztina Szabó, Vilmos Lovas and Zsolt Erős-Honti

Garden and Open Space Design, Corvinus University of Budapest, Faculty of Landscape Architecture, Hungary; E-mail: krisztina.szabo9@uni-corvinus.hu



Owing to the increasing environmental disturbances, afforestation of our cities became a difficult objective. To elaborate liveable environments, the most important mission of experts is to find plant species and cultivars tolerating urban conditions and to elaborate methods for helping their survival. A possible solution is using artificial mycorrhizae which have manifold beneficial effects for the host tree. In our research we present the first results on a large-scale investigation on artificially inoculated *Acer buergerianum* species in urban domains.

Members of the genus *Acer* are rarely planted in treelines *Acer buergerianum* is one of the most popular trees in Easter Asian cities and it was included also in the Urban Green Project 2021 as well. Although in Hungary it is found only as solitaire tree mainly in collection garden, owing to its good tolerance and high decorative value it may have a potential role in Hungarian urban afforestation.

In our study, endomycorrhizal products available in Hungary were tested on 120 specimens of *Acer buergerianum* seedlings. We evaluated the effects of different products and differing inoculation methods. We examined the colonisation level of the roots and evaluated the vegetative growth intensity of the plants.

Keywords: Urban trees, Afforestation, Endomycorrhiza, Inoculums.

SL-263

Track: Other Areas

HELIO-GERO PROTECTED DRINK-WATER AND FOOD: THE NEW POSSIBILITIES AND PERSPECTIVES OF THE PREVENTIVE MEDICINE

Alexander Trofimov

International Scientific Research Institute of Cosmic Anthropoecology (ISRICA), Novosibirsk, Russia; E-mail: isrica2@rambler.ru



In recent years, in the era of nano-technologies of information and knowledge, in Novosibirsk intensively conducted research biotropic weakly energy avant-garde influences on the physiological condition of the plants, animals and humans heliogeophysical numerous factors. Scientists of ISRICA has developed and patented the technology for creating holograms with physiologically active information (Patent RU 2239860,2004,A.Trofimov,K.Evstratov, 2015) sing of a new type of hologram, presumably, containing "analog information about the quantum states" of antioxidants, vitamins, amino acids and other essential

compounds of the best examples of the cluster structures of clean drinking water, provided the use of the original screening device "TRODR" (authors A. Trofimov, G. Druzhinin, 2011) [1] repeatedly weakening of the magnetic induction field of the Earth, it was possible to create a technology to produce helio-hero tread water, which reduces the functional dependence of biological systems, including humans, are very high, often pathological helio-dependent from extreme impacts of solar and geomagnetic disturbances (Patent RU2342149). At last years we are created else one new technological complex «HELIOSTAR» for the exposition at hypogeomagnetic conditions not only drink water, but and the water contained food (seeds, milk, honey, caviar of fish). We want propose for the realization the new scientific-practical program «STOP-TIME». The main aim of this program is the decreasing of speed of human aging. It is the new scientific reality now! According to the our data, received with the use of only one in the world hypogeomagnetic installation, and about 70% of the population of Siberia can be attributed to helio-magnetically sensitive people (high and medium), responding to sudden changes heliogeophysical medium high blood pressure, and often hypertensive crisis with severe consequences (heart attacks, strokes, etc.) [2]. At many congresses in gerontology, held in St. Petersburg, Amsterdam, Paris, in the first decade of the XXI century, were discussed the new scientific data these units on the dependence of the human life-span of the solar activity in the period of intrauterine development. It is shown that the «threshold of longevity reach, mainly those of intrauterine development which took place with minimal activity of the Sun» [3,4]. These data were obtained from the use of the developed and registered in Russia of the computer program «Helios» (ISRIC, SCCEM RAMS), the world community was first introduced to the world EXPO- 92 in Sevilla (Spain) [5]. How do you change the «Sunny code», which prevents the solar-addicted people «get sick less and slower aging»? Effective non-drug prevention helio-magnetotropic reactions and states rights, as well as possible ways to slow the rate of aging of the body have not existed until quite recently. Now for this role claims helio-geroprotective water, developed and tested in ISRIC. It is a real help in the confrontation, in this sense, a defenseless person, over the raging solar element! This a protective tool can be indispensable to all of us, as the crew spacecraft «Earth» in collapsible currently very dangerous for all living cosmic-physical situation, when the magnetic field of the Earth, this natural buffer against solar corpuscular flows continues to decrease and, accordingly, more and more high-energy particle reaches the upper layers of the atmosphere, causing showers of secondary ionized corpuscles, threatening the biosphere and man [6]. We have shown that the plants watered by helio-tread water, and also Wistar rats, who took this water for drink, unlike the controls, show a high resistance to stress in animals males and we noticed increase testosterone in blood and tissues and relative abundance of stable isotopes of carbon (^{13}C) which usually with age irreversibly lost by the body [4]. In people with hypertension after 2-3 weeks of taking this water manifested distinct helio-tread effect: in periods of solar and geomagnetic storms they have not reacted to the solar-magnetospheric disturbances rise in blood pressure and deterioration of their health, so they went out of the zone of risk of development of crises and many complications [7]. Helio-trade effect was confirmed by rigorous mathematical analysis, when was observed significant INVERSE correlation between the majority of the functional parameters of the man and intensity of cosmophysical factors. Exist the necessity for the creation of a global system of geoeological human life support in the conditions of spreading heliogeophysical changes of our biosphere on the basis of new biotechnologies.

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SL-72*Track: Plant and Environment***BIOTECHNOLOGICAL APPROACH TO INCREASING NUTRITIONAL QUALITY IN HIGH YIELDING RICE****Herry Utomo, Steve Linscombe and Ida Wenefrida***Rice Research Station, Louisiana State University Agricultural Center, Louisiana, USA;**E-mail: HUtomo@agcenter.lsu.edu*

Rice is a crop that feeds billions of people around the world, particularly in Asia and Africa. Adaptable to a wide range of environmental conditions from river deltas at sea level to mountainous regions, rice can be grown in both irrigated and rain fed systems. In addition to increased efficiency in rice culture through pest and disease control, land leveling, and efficient fertilization, water use, and harvesting, some rice breeding programs have successfully developed new cultivars with a steady yield increase in the past 10 years. In the U.S., for example, the average annual increase is at an approximate rate of 1.4% (0.1 ton ha⁻¹). Demands for rice are strong, as an additional 116 million tons will be needed by 2035 due to the growing world population. Without area expansion, an annual yield increase of about 1.4% world-wide will be needed. Although rice serves mainly as an energy food, its nutritional value has been realized and is becoming increasingly important. Incorporation of high protein and other grain nutritional properties in modern high yielding rice will provide some solutions to solve malnutrition problems associated with the world's population growth. A better understanding of mutational techniques, genomics, and an increased efficiency in sequencing and genotyping, have provided essential tools to incorporate high nutritional properties into high yielding rice. A number of potential high yielding rice lines with improved nutritional quality have been successfully developed through biotechnological approaches. Genomic sequencing was applied to determine target genes corresponding to the nutritional changes. The information will improve the understanding of how the traits are regulated and provide the basis for efficient incorporation into future high yielding rice varieties.

SL-238*Track: Medical Biotechnology***THE EXPRESSION OF PLASMA MIR-199A-5P, MIR-200C-3P IN GASTRIC CARCINOMA AND ITS CLINICAL SIGNIFICANCE****Tang Jinli, Xiaoming Wang, Ge Mengyuan, Gui Zhen, Li Jinchang, Zhu Mingchen and Yan Feng***Department of Clinical Laboratory, Nanjing Medical University Cancer Hospital, Jiangsu Key Laboratory of Molecular and Translational Cancer Research, Nanjing 210009, China; E-mail: jsszlyy1346@163.com*

Objective To explore the relative expression of plasma miR-199a-5p and miR-200c-3p in gastric adenocarcinoma cancer(GAC) patients and its clinical value.

Methods: Case-control study was used in this research. The relative expression of plasma miR-199a-5p and miR-200c-3p from 47 GAC patients and 50 healthy controls were determined by RT-PCR(TaqMan Probe method). Meanwhile, the association with age, gender, tumor location, size, degree of differentiation, TNM stage, lymph node metastasis and other clinical pathological parameters were analyzed. The expression of these two miRNAs in plasma of 30 GAC patients during preoperation was compared with their expression 6-8 days after radical surgery. The sensitivity and specificity of plasma miRNAs expression for the diagnosis of GAC were analyzed using the receiver operating characteristic (ROC) curve. SPSS20.0 statistical software was used for statistical analysis. *T*-test, paired *t*-test and one-factor ANOVA were used for normal distribution of quantitative data.

Results: The plasma level of miR-199a-5p in GAC patients was significantly lower (1.05 ± 0.22) ($t=3.058, P=0.003$), while miR-200c-3p was significantly higher (15.15 ± 3.02) ($t=-2.854, P=0.006$), when they were compared with those in controls ($26.80 \pm 8.38, 3.39 \pm 0.87$). Low miR-199a-5p expression in GAC patients were associated with lymph node metastasis ($F=4.725, P=0.029$) and the differentiation degree of gastric cancer ($F=3.854, P=0.032$). The relative

expression of miR-199a-5p in postoperative plasma was significantly increased ($t=-3.814$, $P=0.001$), but the relative expression of miR-200c-3p was significantly reduced when compared to the preoperative samples ($t=2.978$, $P=0.006$). Area under the ROC curve of miR-199a-5p, miR-200c-3p and combined miR-199a-5p and miR-200c-3p were 0.692, 0.792 and 0.798, the sensitivity and specificity were 87%, 97%, 92.5% and 43%, 54%, 65%, respectively.

Conclusion: Combined detection of miR-199a-5p and miR-200c-3p in plasma has a higher sensitivity and specificity than the conventional tumor marker CEA and CA19-9, and may be a useful biomarker for gastric cancer diagnosis.

Key words: Gastric adenocarcinoma cancer, plasma microRNAs, tumor markers, real-time fluorescent quantitative PCR.

SL-277

Track: Regenerative Medicine

NEUROPROTECTIVE DRUG DISCOVERY AND NEURAL STEM CELL RESEARCH IN ALS

Xin Wang

Neuroapoptosis Drug Discovery Laboratory, Department of Neurosurgery, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA; E-mail: xwang@rics.bwh.harvard.edu



Amyotrophic lateral sclerosis (ALS) is a debilitating disease characterized by progressive loss of voluntary motor neurons leading to muscle atrophy. Because riluzole, the only Food and Drug Administration (FDA)-approved treatment, prolongs the ALS patient's life by only 3 months, new therapeutic treatments that may delay disease onset, slow progression, prolong survival, and ultimately reduce the burden of disease are urgently needed. Harnessing the regenerative potential of the central nervous system would be a novel approach for the treatment of motor neuron death resulting from ALS. The impact of neural stem cell therapies and neuroprotective drugs such as melatonin on disease onset and progression of ALS will be summarized. Furthermore, the increased cell proliferation in the adult mSOD1^{G93A} ALS transgenic mice, and the Wnt/ β -catenin signaling pathway promotes the proliferation and differentiation of neural stem cells in the adult spinal cord of mSOD1^{G93A} ALS transgenic mice will be discussed.

SL-205

Track: Pharmaceutical Biotechnology

THE DEVELOPMENT OF A HIGH RESOLUTION LC-MS PEPTIDE MAPPING METHOD FOR AUTOMATED QUANTIFICATION OF N-LINKED GLYCANS IN IMMUNOGLOBULINS

Tian Wang, Lily Chu, Wenzhou Li, Ken Lawson, Izydor Apostol and Tamer Eris

One Amgen Center Dr, MS 30E-1-B, Thousand Oaks, CA 91320, USA; E-mail: tianw@amgen.com

In traditional methods for N-linked oligosaccharide characterization, all the glycans are released enzymatically from proteins prior to labeling and analysis. A significant drawback of these methods is that they do not provide site specific glycosylation information. In this paper, we describe the development, qualification, and application of a LC-MS-based peptide mapping method for site specific quantitation of N-linked glycans present on an IgG1 molecule containing two distinct N-linked glycosylation sites; one on HC Fab region and the other on HC Fc region. LC and MS conditions were optimized to achieve optimal separation and quantitation of glycoforms using a high-resolution, accurate-mass Orbitrap MS instrument, Q ExactiveTM, with automated quantitation software, Pinpoint (Thermo). The LC/MS method was shown to have acceptable accuracy, reproducibility, precision, and linearity for routine glycan profiling. The results from the quantitation of individual glycan species obtained from the LC-MS method were consistent with those obtained from traditional HILIC analysis of enzymatically released and fluorescently labelled glycans. Our work has demonstrated the quantitative LC/MS method using automated software is suitable for providing site-specific glycan information for routine profiling of N-glycans on immunoglobulins. This high resolution analysis is important for determining product comparability and biosimilarity.

SL-79*Track: Plant and Environment***NUTRITIONAL QUALITY ENHANCEMENT IN A NON-GMO HIGH YIELDING RICE****Ida Wenefrida, Herry S. Utomo and Steve D. Linscombe**

*Rice Research Station, Louisiana State University Agricultural Center, Louisiana, USA;
E-mail: iwenefrida@agcenter.lsu.edu*



Advancements in nutrigenomics and nutrigenetics have improved public knowledge of the importance of developing nutritional regimes for optimum fitness and health. Greater knowledge of the human genome and understanding the molecular basis for human health and genetic predisposition to certain diseases enable individuals to personalize their nutritional requirements. These recent advances together with worldwide accessibility to the most current information on nutrition and health have transformed and elevated the need for more nutritious food products. Non-GMO high yielding rice lines possessing improved protein content and better amino acid profiles have been successfully developed. A combination of potent mutagenic agents and sufficient genomic information has allowed the program to streamline product development through specific, oriented targets and direct identification of nucleotide changes associated with the new traits. Utilization of reverse and forward genetics in the target genes provided a framework for large-scale production of rice mutants. Since rice is a model crop, the technique can potentially be applied to other crops. High protein rice can provide the base for developing novel foods and fibers or nutrient-dense food products. It can also be tailored into functional foods to meet the needs for individuals with specific genetic traits. Rice feeds nearly half of the world's population and, therefore, enhancing rice nutritional quality is of a significant value.

Keywords: Rice, high protein, nutritional quality, nutritious food product, amino acids.

SL-146*Track: Regenerative Medicine***HUMAN THYMUS DERIVED PROGENITOR/STEM CELLS IMMUNOTHERAPY IN AUTOIMMUNE DISEASES****Nawrot I, Wozniwicz BM, Maruszewski B, Janas R, Wozniwicz M and Nazarewski s.**

*Department of Pathology, Childrens' Memorial Health Institute, Warszawa, Poland;
E-mail: b.wozniwicz@czd.pl*



Introduction: Thymus is important organ of the immune system. From discovery of thymosine alpha 1 play essential role in maturation of peripheral T lymphocytes. Many factors was discovered that cause precocious thymus atrophy and developing of autoimmune diseases. Many methods are described to stop autoimmune diseases including revolution in oncology (Ipilimumab) , and stem cell therapy. This paper describe a new method to improve natural thymus defence system by ectopic thymus derived stem cell implantation.

Material and Methods: Thymus explants were cultured for 34 days to observe secretion of thymosine alpha 1 *in vitro* and after implantation into volunteers. Cells were implanted into the subcutaneous adipose tissue and Tha1 level was observed during 12 years. Number of implanted cells was adequate to the size of 3,0 thymus remnants of adult man.

Results: Thymus derived progenitor/stem cells secrete thymosin alpha 1 and may be used as natural method improving immune system.

Conclusion: Thymus progenitor/stem cells may be used in future as a new additional tools in case of thymus deficiency and in autoimmune diseases. Instead of very expensive pharmaco-therapy using synthetic thymosine (Zadaxin) and other phatmaceuticals.

SL-243

Track: Medical Biotechnology

THE PHYSICAL PROPERTIES AND BIOCOMPATIBILITY OF BIOMEDICAL POLYURETHANE REINFORCED BY NATIVE SILK FIBROIN POWDER

Weilin Xu, Hongjun Yang, Yuping Pi, Ruiqing Hu, Mei Zhou, Chenxi Ouyang, Dujin Wang and Xia Dong

College of textile science and engineering, Wuhan Textile University, China; E-mail: weilin-xu@hotmail.com

Native silk fibroin powder (NSFP) deprived from silk fibroin fiber by physical method was firstly used to enhance the biocompatibility of biomedical polyurethane (BPU). BPU/NSFP hybrid membranes were prepared by a phase separation technique using distilled water as coagulant at room temperature. The physical properties and biocompatibility of BPU porous membrane reinforced by NSFP was investigated. Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords and seeded on the upper surface of the hybrid membrane to evaluate the *in vitro* biocompatibility of BPU/NSFP hybrid membrane. When NSFP percentage in hybrid membrane was no more than 50 %, the amount of HUVECs proliferated on the upper surface increased with the increase of NSFP content. Strip-shaped cells were observed on pure BPU membrane. However, the morphology of cells distributed on 70/30 BPU/NSFP hybrid membrane exhibited a cobblestone pattern. The change in morphology of HUVECs was attributed to the increase of hydrophilicity, porosity and stiffness of BPU/NSFP hybrid membrane. *In vivo* biocompatibility of BPU/NSFP hybrid membrane was studied using rat model. The characterization on *in vitro* and *vivo* biocompatibility indicated that biocompatibility of BPU membrane was improved by NSFP. The BPU/NSFP hybrid membranes with excellent biocompatibility may be find applications in tissue engineering and small-diameter vascular grafts.

Keywords: Biocompatibility, Polyurethane, Silk fibroin, Hydrophilicity, Vascular graft.

SL-49

Track: Regenerative Medicine

MESENCHYMAL STEM CELL TREATMENT IMPROVES DIABETIC WOUND HEALING THROUGH MODULATION OF MICRORNA EXPRESSION

Junwang Xu, Carlos Zgheib, Junyi Hu and Kenneth W. Liechty

University of Colorado Denver School of Medicine, Department of Surgery, Aurora, CO, USA; E-mail: JUNWANG.XU@UCDENVER.EDU

INTRODUCTION

Impaired wound healing following injury in diabetics represents a major clinical problem, resulting in prolonged hospitalizations and significant healthcare expenditures. Diabetic wounds have been shown to have an abnormal inflammatory response, decreased angiogenesis and collagen deposition. MicroRNAs (miRNAs) are short non-coding RNAs that negatively regulate the translation of target mRNAs at the post-transcriptional level, and have been shown to play pivotal roles in the regulation of inflammation, angiogenesis, and collagen production. We have shown that Mesenchymal stem cell (MSC) treatment can improve healing in diabetic mice. However, the role of microRNA in this correction remain unclear. We hypothesize that MSC treatment improves diabetic wound healing, in part, by correcting abnormal miRNA gene expression.

METHODS

To test our hypothesis, we created 8 mm wounds on the back of diabetic (Db/Db) and non-diabetic (Db/+) mice. A subset of wounds were treated with 10^6 MSC. The wounds were harvested at 0, 1, 3, 7 and 14 days and total cellular RNA isolated. Real-time PCR was performed for miRNAs and their target genes expression.



RESULTS

Treatment of diabetic wounds with MSC resulted in a significant improvement in wound closure. At day 7 after wounding, MSC treated wounds showed significantly reduced wound size, a significant reduction in the number of CD45+ inflammatory cells, a significant induction of CD31+ cells, and increasing production of collagen content. We found miR-146a, an anti-inflammation molecule, was significantly down-regulated in diabetic wounds. Decreased miR-146a levels closely correlated with increased gene expression of its pro-inflammatory target genes in NF- κ B signaling pathway. More interestingly, the correction of the diabetic wound healing impairment with MSC treatment was associated with a significant increase in miR-146a expression level and decreased gene expression of its pro-inflammatory target genes. MiR-15b, an anti-angiogenic molecule, was found significantly increased in diabetic wounds and associated with decreased expression of its target VEGF α through BCL2/HIF1/VEGF signaling pathway. MSC treatment resulted in significantly decreased expression of miR-15b, and increased expression of VEGF α . MiR-29a has been shown to repress type I collagen (Col1a1 and Col1a2) and type III collagen (Col3a1) mRNA and we found that miR-29a expression was significantly increased in murine diabetic wounds. Diabetic wounds treated with MSC demonstrated a significant decrease in miR-29a expression compared to controls and this was associated with a significant increase in collagen protein content.

CONCLUSION

These findings demonstrate that stem cell correction miRNA plays a role in the diabetic wound healing impairment and MSC mediated correction of the diabetic wound healing impairment is due, in part, to modulation of microRNA expression. These data also provides a new layer of regulatory mechanisms that could be targeted for potential therapeutic intervention in diabetic wounds.

SL-106

Track: Medical Biotechnology

PHOTOCROSS LINKABLE MALEIC ANHYDRIDE ACYLATED CHITOSAN/METHACRYL POLY (VINYL ALCOHOL) NANOFIBROUS MEMBRANE AS POTENTIAL WOUND DRESSING FOR SKIN REGENERATION

Hongjun Yang, Xin Liu, Yingshan Zhou, Shaojin Gu, Shuoshuo Yang, Qi Dong and Weilin Xu

College of material science and engineering, Wuhan Textile University, China; E-mail: h_j.yang@yahoo.com

Biocompatible water-insoluble nanofibers based on maleic anhydride acylated chitosan (MCS)/methacryl poly (vinyl alcohol) (MPVA) were successfully prepared by electrospinning combined with photopolymerization. The composite nanofibrous membranes were subjected to detailed analysis by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD). SEM images showed that the morphology and diameter of the nanofibers were mainly affected by the weight ratio of MCS/MPVA. XRD and DSC demonstrated that there was strong intermolecular hydrogen bonding between the molecules of MCS and MPVA. The crystalline microstructure of the electrospun fibers was not well developed.

The composite nanofibrous membranes could keep the morphology without changed when immersed in water for 48h. The potential use of the photocrosslinkable MCS/MPVA electrospun fiber mats as scaffolding materials for skin regeneration was evaluated *in vitro* using mouse fibroblasts (L929) as reference cell lines. Indirect cytotoxicity assessment of the nanofibrous membranes indicated that the photocrosslinkable MCS/MPVA electrospun mat was nontoxic to the L929 cell. Cell culture results showed that fibrous mats were good in promoting the cell attachment and proliferation. This novel photocrosslinkable electrospun matrix would be used as potential wound dressing for skin regeneration.

Keywords: Nanofiber, Chitosan, Photopolymerization.

SL-181

Track: Pharmaceutical Biotechnology

HUMAN CANNABINOID RECEPTOR CB2: EXPRESSION, FUNCTIONAL AND STRUCTURAL STUDIES**Alexei A. Yeliseev**

National Institute on Alcoholism and Alcohol Abuse, NIH, Rockville, MD, USA;

E-mail: yeliseeva@mail.nih.gov



The human cannabinoid receptor CB2 belongs to the class A of heptahelical G protein-coupled receptors (GPCR) and is an attractive target for the development of drugs for management of pain, inflammation, osteoporosis and treatment of immunological disorders. High resolution structural studies are critical to obtain insights into the molecular mechanisms of ligand binding and activation of CB2.

We developed methods for expression in milligram quantities, purification, reconstitution in lipid bilayers and stabilization of the functional recombinant CB2 as well as efficient stable isotope labeling of CB2 by high density fermentation were developed, enabling NMR studies. NMR analysis of labeled receptor reconstituted in proteoliposomes in agonist or inverse-agonist-bound form will be reported.

Monoclonal antibodies were raised against the purified CB2, and the affinity of interaction with CB2 was determined by surface plasmon resonance. Finally, we demonstrate the specific effects of lipids with negatively charged head group in activation of CB2 reconstituted in proteoliposomes as measured by an *in vitro* G protein activation which may have important physiological significance as manifested in natural membranes of various lipid compositions.

Keywords: Cannabinoid Receptor, G Protein-Coupled Receptors and NMR studies.

SL-254

Track: Plant and Environment

THE SIGNIFICANCE OF DOUBLED HAPLOID PRODUCTION VIA MICROSPORE EMBRYOGENESIS IN PLANT BIOTECHNOLOGY AND CROP BREEDING**Ming Y. Zheng**

Professor of Biology, Gordon College, Wenham, MA 01984, USA; Ming.Zheng@gordon.edu



Production of doubled haploids (DH) *via* microspores embryogenesis is a proven method to obtain homozygous individuals in a single step, thus the method is very useful in genetic engineering and crop breeding. The instant homozygosity saves plant breeders multiple years of inbreeding necessary in conventional cross breeding and substantially reduce the population sizes required for effective selection of superior trait combinations. An efficient doubled haploid platform is also highly sought after in generating transgenic plants. Large number of embryogenic microspores are ideal target for genetic transformation. Dominant or recessive target gene(s) to be transferred into gametic cells can be made homozygous in a single generation. In addition, doubled haploids are important tools in plant genome mapping and many areas of basic research, including *in vitro* embryogenesis, signal transduction and developmental biology. Since the initial success in reprogramming microspores for embryogenesis in our laboratory, many factors associated with microspore embryogenesis were investigated in our research, including stress variables, isolation protocol, media components, nuclear divisions, embryogenic abortion and chromosome doubling. An artificial manipulation, in the form of physical stress, and /or chemical treatment, is employed to reprogram microspores from gametophytic to sporophytic development. Induced embryogenic microspores, characterized by unique morphological features, undergo an interlocked cascades of cell divisions and differentiation that lead to the formation of embryoids. These embryoids “germinate” to give rise to haploid or doubled haploid plants. We also investigated “nursing factors”, released by live ovaries co-cultured with microspores, and found their effects in promoting embryogenesis and

reducing the abortion rate among developing microspores. In addition, nuclear fusion inside embryogenic microspores within the first several days of culture is a leading cause for chromosome doubling. These studies have yielded useful information for understanding the developmental process of embryogenesis. As a result, a more efficient doubled haploid system is in place to accelerate crop breeding aiming at rapid responses to climate change, sudden outbreak of pests, diseases and other environmental distresses.

SL-278

Track: Other Areas

DETERMINE THE ROLE AND MECHANISM OF GENE CODON USAGE IN REGULATING PROTEIN EXPRESSION AND FOLDING

Mian Zhou, Jinhu Guo, Joonseok Cha, Michael Chae, She Chen, Jose M. Barral, Matthew S. Sachs and Yi Liu

*School of Biotechnology, East China University of Science and Technology, Shanghai, China;
E-mail: zhoumian333@163.com*



During heterologous protein expression, the aim is to increase expression levels without altering native activities. Gene expression is mostly known to be regulated at the transcriptional level, while protein function can be regulated post-translationally. However, as a co-translational mechanism, the role of codon usage in regulating protein expression and function is less well studied.

In many organisms, there is a bias for preferred codons and it is thought to be a mechanism to enhance expression for highly expressed genes. For example, *Neurospora crassa* prefers to use G/C rather than A/T at the 3rd position of codons in highly expressed genes. FREQUENCY (FRQ) is a key component in the negative feedback loop of *Neurospora crassa* circadian clock. Compared with other genes, frequency (frq) exhibits a very non-optimal codon usage. It's not clear whether this "poor" codon usage has any biological significance.

To test the role of codon usage in frq, here we made a series of codon optimized frq constructs, and introduced them into a frq null strain. We found that strains carrying the N-terminal optimized frq exhibited severe circadian clock phenotypes, with higher FRQ protein levels but similar frq mRNA levels compared with wild-type. Surprisingly, shown by both trypsin sensitivity assay and freeze-thaw assay, FRQ proteins in these strains were less stable. Besides, they were defective in binding their WCC partners, and optimized FRQ had a compromised function to support WC-1 and WC-2 levels in the positive feedback loop, suggesting that codon optimization also influences protein function even though its sequence is unchanged. Taken together, codon usage of frq regulates its protein expression, folding and function. Therefore a scientific analysis is needed during codon optimization design when expressing heterologous proteins.

Keywords: Codon usage, expression, folding.

POSTERS

PO-31

Track: Industrial and Manufacturing: Bio-Fuels

ETHANOL PRODUCTION USING RED BEET JUICE BY *SACCHAROMYCES CEREVISIAE* ATCC 9763

Venegas-Sánchez Josué Addiel and Jiménez Islas Donaji

Huichapan Higher Technological Institute, Renewable Energy, El Saucillo, Huichapan, Hidalgo, Mexico ZIP. 42811; E-mail: djimenez@iteshu.edu.mx

In Mexico, red beet (*Beta vulgaris* L. conditiva) is available all the year, therefore its juice and bagasse can be used for bioethanol production. In this work was investigated the ethanol production by *Saccharomyces cerevisiae* ATCC 9763 using juice red beet at pH = 2.8 and 37 °C. The rates of biomass growth, sugar consumption and ethanol production during batch fermentation of red beet juice were estimated. The logistic, Pirt, and Luedeking-Piret equations were used to model the microbial growth $X(t)$, substrate consumption $S(X)$, and ethanol production $P(X)$, respectively. The volumetric ethanol productivity (Q_p) and ethanol production (P) were 0.86 g L⁻¹ h⁻¹ and 28 g L⁻¹, respectively. The maximum specific growth rate (μ_{max}) and maximum biomass concentration (X_{max}) were 0.23 h⁻¹ and 1.95 g L⁻¹, respectively. These results indicate that a significant portion of the carbon source was used for maintenance of strain, which agrees with the fact that the lowest values of X_{max} obtained under these fermentation condition. The strain of *Saccharomyces cerevisiae* ATCC 9763 utilized in this study was able to produce ethanol with high yield and volumetric productivity under acid and thermal stress condition.

**PO-76**

Track: Industrial and Manufacturing

HETEROGENEOUS NANOBIOCOMPOSITES ON THE BASIS OF CARBONIZED SORBENTS

Nuraly Akimbekov, Abdieva Z. Gulzhamal, Ualyeva S. Perizat, Kaiyrmanova K. Gulzhan, Digel Ilya and Zhubanova A. Azhar

Department of Biotechnology, Al-Farabi Kazakh National University, Kazakhstan;
E-mail: akimbeknur@gmail.com

Microbial cells immobilized on solid surfaces cover nowadays a wide area of applications in biotechnology. A unique combination of valuable physical and chemical characteristics makes nanostructured carbonized sorbents of plant origin very attractive as a constituent of heterogeneous composite systems containing microbial cells. Due to their remarkable properties, nanostructured carbon rice husks (CRH) can be used as sorbents for adsorption of different industrially important microorganisms. Our research is aimed to creation of cost-effective and sustainable bio-composite materials on the basis of microbial cells adsorbed CRH. Electron microscopy studies confirmed that multiple probiotic and protein producing valuable cells can successfully attach, survive and proliferate inside the porous network of the CRH. In our model experiments, the carbon material specifically adsorbed up to 95% microbial cells from various solutions. The resulting biocomposite materials possess outstanding probiotic and nutraceutical properties accompanied by high specificity, depending on the particular microbial strain used. The *in vivo* and *in vitro* studies strongly suggest that the use of the CRH as carrier for the oral administration of probiotic microorganisms has a very big potential for improving functionality and safety of probiotic preparations. This interdisciplinary knowledge could significantly stimulate development of novel immobilized biocatalysts possessing high activity, selectivity and stability.



Keywords: Nanobiocomposites, carbonized sorbents, immobilization.

PO-27*Track: Plant and Environment***PHYSIOLOGICAL AND GROWTH PERFORMANCE OF CYANOBACTERIUM, PHORMIDIUM FRAGILE UNDER EXPOSURE TO DIFFERENT LIGHT CONDITIONS****Jehan Saud AL-Abraham, Mudawi Mukhtar Elobeid and Afrah Eltayeb Mohammed***P.N.U Princess Nourah Bint Abdulrahman University, KSA; E-mail: JSAA242@hotmail.com*

Light is the most important environmental factor that critically affects growth and development of algae through its immediate influence on photosynthesis. In such a process, the initial phase (light reactions) involves light absorption resulting in the formation of intermediate energy compounds (ATP and NADPH), which would later be utilized for driving the second phase of photosynthesis (carbon fixation) in the Calvin cycle. In the current investigation the principal objective was to evaluate the effect of different light conditions on the physiological and growth behaviour of the Cyanobacterium, Phormidium fragile. To realize that goal carbohydrates content as well as dry mass production were analyzed and determined. Phormidium fragile tissues were propagated in sterilized flasks containing an appropriate algal culture and subjected to four different light conditions: sun light, laboratory light, white lamb light (control) and darkness. Analysis of carbohydrates content showed that the algal growth under both laboratory and darkness conditions exhibited significantly higher values (106 mg and 81 mg, respectively) relative to control (38 mg). On the other hand, the dry matter content was significantly lower in darkness and laboratory conditions (30 and 35 mg, respectively) compared to that of the control (75 mg). Based on these findings our present study provided additional supporting evidence for the significance of light energy as a determinant factor in the photosynthetic activity of algae in relation to dry mass accumulation and carbohydrate metabolism. Further studies need to be carried out in order to validate our conclusions.

Keywords: Phormidium fragile, photosynthesis, light, carbohydrate, dry mass.

PO-4*Track: Plant and Environment***MOLECULAR ANALYSIS OF ACETOLACTATE SYNTHASE LOCUS REVEALS POTENTIAL NEW ALS- RESISTANT ALLELES AMONG SAUDI LOLIUM RIGIDUM POPULATIONS****Abdullah A. Al-Doss, Abdelhalim I. Ghazy, Sulieman A. Al-Faifi, Hussein M. Migdadi, Muhammad A. Khan, Megahed H. Ammar and Mohammad I. Althamrah***Collage of Food and Agriculture, Riyadh, KSA; E-mail: aaldoss@live.com*

Annual ryegrass is one of the most common weed species in Saudi wheat fields. Resistance to ALS herbicides has evolved widely in Saudi Arabia, however, no earlier attempt has been dedicated to elucidate or identify mutations conferring resistance in annual ryegrass populations (*Lolium rigidum*) invading wheat fields in this region. Six reported ALS-linked primers were used to identify potential ALS-herbicides resistant alleles of seven *L. rigidum* populations collected from wheat fields across Saudi Arabia in addition to international sensitive accession. Molecular screening revealed no apparent polymorphism among or within populations. Therefore, genomic sequences were determined from cloned amplified PCR fragments. Sequence alignment of ALS cDNA sequence in public domain with our sequences revealed ten base pair substitutions. Five of those mutants had a potential phenotype alteration since they led to amino acid change. The Pro-197-Thr in Tabouk and Pro-197-Gln in Harad populations occurred at same position reported earlier, with a novel amino acid change, threonine (Thr) that was not reported. Other mutant Lys-98-Met represents potential new ALS-allele, with amino acid methionine detected in all Saudi populations except the resistant population (Tabouk), where it was Lysine. One more potential allele was detected in Asp-185-Tyr, where it was Aspartic acid in known resistant allele as well as in all Saudi populations except in Tabouk, the resistant population, where it converted to Tyrosine. These two potential alleles are of a particular interest and needs further confirmatory studies. Other mutant, Glu-105-Gly may represent ecotype-specific mutant; it showed consistent amino acid change among all Saudi

populations regardless their resistance level. These mutants may be utilized as ecotype specific mutants that differentiate Saudi from other annual ryegrass populations. Furthermore, Glu-213-Lys and Ile-462-Thr mutants occurred only once each in international sensitive check, and Wadi El-Dawaser Population, respectively. The obtained sequences were deposited to NCBI gene bank through individual submission (KM000067, KM000068 and KJ953895). Results obtained in this study will help to implement new strategies for annual ryegrass weed management.

Keywords: Herbicide resistance, annual ryegrass, point mutation, base pair substitution.

PO-20

Track: Medical Biotechnology

ANTIMICROBIAL EFFECT OF MELITTIN ISOLATED FROM SYRIAN HONEYBEE (APISMELLIFERA) VENOM AND ITS WOUND HEALING POTENTIAL

Omran Alia, Massouh Laila and Al-Daoud Antonious

Faculty of Pharmacy, Damascus University, Syria; E-mail: aliapharmacy@yahoo.com

In this study, bee venom was collected from 12 Syrian beehives during June and July 2011 using electric shock method. Melittin, the major component of BV, was isolated and identified using RP-HPLC C18 column and MALDI-TOF-MS analysis. The obtained melittin exhibited a potent antibacterial activity particularly against Gram-positive bacteria as its MIC was 12.5 µg/ml for *Listeria monocytogenes* compared with 200 µg/ml for *Yersinia kristensenii* (a Gram-negative bacterium) indicating that melittin has significant antibacterial effects. Additionally, melittin treatment was found to significantly accelerate wound contraction and re-epithelialisation as wound sizes decreased dramatically and healed within 5 days in all melittin treated rats compared with 8 days in the controls in a rat full-thickness excision wound model. These findings suggested that topical melittin treatment for skin defects should be very effective in preventing and reducing the wound and scar sizes. However, further studies are needed to evaluate the precise mechanism of epithelial cell proliferation induced by melittin treatment.

Keywords: Melittin, AMPs, Antimicrobial Effect, Wound Healing.



PO-41

Track: Plant and Environment

STUDIES ON GENETIC FIDELITY OF CRYOPRESERVED CALLI OF DATE PALM CULTIVARS (PHOENIX DACTYLIFERA) FROM SAUDI ARABIA

Fahad Al-Qurainy, Salim Khan, Mohammad Nadeem and Mohamed Tarroum

Department of Botany and Microbiology, College of Science, King Saud University, Riyadh-11451, KSA; E-mail: fahad_alqurainy@yahoo.com

It is important to conserve the genetic diversity of date palm for genetic research and to develop breeding programs. However, conventional methods for conservation of genetic resources, *e.g.*, seed storage, are not suitable for date palm, because its seeds are highly heterozygous, and therefore, they do not reproduce true-to-type. Some *in vitro* culture techniques have been established for date palm, including organogenesis, somatic embryogenesis, and embryo rescue-plant regeneration. The offshoots of date palm cultivars were collected from three locations Riyadh, Al-Qasim and Al-Hasa regions. More than ten cultivars were selected. Among these we are able to cryopreserve 4 cultivars. Callus induction was achieved on higher concentrations of auxins (mainly 2,4-D and NAA) and lower concentrations of cytokinin (2ip and BA). Subsequently somatic embryogenesis and plant regeneration was achieved on low level of auxin and cytokinin. The calli were cryopreserved



by encapsulation and drying method in liquid nitrogen. After cryopreservation, the calli were evaluated for genetic stability by molecular tools.

Keywords: SCoT marker, phytohormone, cryopreservation, regeneration.

PO-43

Track: Medical Biotechnology

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE OXYGENASE DOMAIN (eNOSoxy) ON LIPID NANODISCS

Ghaith Altawallbeh, Mohammad Mahfuzul Haque, Dennis J. Stuehr and Mekki Bayachou

Department of Chemistry, Cleveland State University, USA; E-mail: g.altawallbeh@vikes.csuohio.edu

Even though a lot is yet to be explored about eNOS structure-function relationship, its vital role in cardiovascular activities makes it one of the leading research targets today. eNOS is a complex redox protein, whose function harmonizes a group of partners including the cell membrane which modulates its activity. This enzyme and other related isoforms have been studied exclusively in solution despite evidence that membrane plays a role in at least the eNOS activity and how this affects its physiologic environment.



Therefore, we have incorporated the recombinant oxygenase subunit of the enzyme into miniature lipid membranes called nanodiscs. These lipid bilayers will provide a defined system to elucidate the influence of phospholipid bilayers on the structure and activity of eNOSoxy. The structural homogeneity and stability of nanodisc samples with and without eNOSoxy were determined using size exclusion chromatography, gel electrophoresis, and atomic force microscopy. The calculated stocks hydrodynamic diameter of both empty and eNOSoxy bound nanodiscs were 11.3 nm and 13.7 nm, respectively. The coomassie stained SDS-PAGE gel of selected fractions confirms the co-elution of the complex. The height measured by AFM for empty nanodiscs were ~5 nm consistent with the published data. The average size of nanodisc diameter was confirmed by dynamic light scattering. Griess assay is used to measure activity of free and nanodisc-bound enzymes. As compared to the free enzyme, the specific activity of the enzyme drops by 2 folds when compared to the free form. These data suggest that the membrane environment affects the catalytic properties of eNOS heme domain.

Keywords: Nanodiscs, eNOSoxy, Cardiovascular, enzyme activity.

PO-42

Track: Industrial and Manufacturing

STUDY OF THE EFFECT OF MOBILE PHONE RADIATION ON ANTIBIOTIC SENSITIVITY IN MICRO ORGANISMS

Arunkumar G

Lekshmi Bhavan Maruthoorkonam, Kottukal PO, India; E-mail: akg911247@gmail.com

Mobile phone radiation exposure for long term is harmful to human beings and other living system. Nowadays antibiotic resistance is the common tragedy in our modern allopathic treatment especially in the case of Tuberculosis. This study was based on the effect of mobile phone radiation on the antibiotic sensitivity in *Escherichia Coli*. The difference in sensitivity of *E. Coli* that exposed to mobile phone radiation was studied. The mechanism of resistance of these pathogenic bacteria has to be found out as soon as possible for improved patient care. This study may be repeated with other type of microorganisms, both gram positive and gram negative with other antibiotics for further investigations. This study has



found that, such radio frequency radiation exposed *E. Coli* shows decreased sensitivity than other non-radiated *E. Coli* towards Gentamycin. Anyway this topic helps to take preventive measures to withstand our healthy living system.

This study throws light in to resistance developed by microorganisms to normally used antibiotics. This research indicates that, the organisms achieve resistance not only due to the numerous commonly known reasons, patients' noncompliance, *etc* but also due to invitro exposure of RF waves. Now our world has been surrounded by numerous mobile phone towers & this may cause serious health problems. All of them may know about some hazardous effects of mobile tower and mobile phone radiation but not known about this effects on drugs through microorganisms. Due to the single cell structure, the microorganisms absorb radiation through their entire surface, which were surrounded by mobile tower radiation. When a healthy individual infected with microorganisms which has previously developed resistance or any change in susceptibility from its environment, it may cause failure to response of the individual to the normally used drugs or its dose. On the basis of this study, further research should be necessary about the hazardous effects of the mobile phone radiation to the pathogenic gram positive & gram negative bacteria, virus and fungus. Then only this study will achieve the success in protection of human health.

Keywords: Mobile phone, Radiation, Antibiotic sensitivity, Resistance, *Escherichia Coli*, Gentamycin.

PO-26

Track: Pharmaceutical Biotechnology

BLOCKADE OF NMDA RECEPTOR AFFECTS PROLIFERATION AND DIFFERENTIATION OF HUMAN CORTICAL PROGENITORS

Inseyah Bagasrawala, Nevena Radonjic and Nada Zecevic

Department of Neuroscience, Uconn Health, Farmington, CT, USA; E-mail: bagasrawala@uchc.edu

Viral infections in an expecting mother can lead to devastating neurodevelopmental defects and part of the damage is believed to be due to pro-inflammatory cytokines secreted from reactive astrocytes and microglia. Kynurenic acid (KYNA), an antagonist at the glycine-binding site of the NMDA receptor (NMDAR), activates these glial cells. According to the KYNA hypothesis of schizophrenia (Sch), excessive production of KYNA antagonizes NR1, the obligatory subunit of the excitatory glutamate receptor NMDAR, and possibly leads to an excitation-inhibition (E/I) imbalance of cortical circuits. Animal studies point to a possible role of KYNA in generating behavioral deficits (anhedonia, prepulse inhibition), similar to those observed in Sch patients. The NMDAR-antagonizing effects of KYNA are yet to be established in the developing human cerebral cortex. We hypothesize that KYNA will downregulate NMDAR expression on human cortical progenitors, and thereby alter their proliferation and differentiation into neurons and glial cells. We studied the effects of increasing doses of KYNA (0.01 mM, 0.05 mM and 0.1 mM) on NR1 expression and on the proliferation and differentiation of radial glial cells (RGCs) isolated from human fetal cerebral cortex at 17 gestational weeks (gw). RGCs (GFAP+) are multipotent progenitors capable of generating intermediate progenitors (Tbr2+), interneuron progenitors (Nkx2.1+), glutamatergic neurons (Tbr1+), and interneurons (GABA+, Calretinin+). KYNA treatment for 48 hours resulted in a significant and dose-dependent increase in cell death (21.02%) compared to control cultures (8.1%). In addition, cell proliferation measured after 3 days *in vitro* (DIV) in proliferation medium (using Ki67 as a proliferation marker) was reduced in KYNA treated cultures as compared to controls ($p = 0.001$). More specifically, the number of GFAP+ astroglia cells from total number of cells was reduced in half (76.25% in controls to 35.06%), similar to a reduction in other progenitors (Tbr2: 72.86% to 38.16%; Nkx2.1: 67.76% to 40.80%). KYNA treatment for 7DIV influenced differentiation into neurons, reducing significantly the number of GABAergic (33.17% to 5.52%) or Calretinin (29.71% to 3.63%) cells, but not glutamatergic neurons (Tbr1: 21.18% to 21.02%). Using immunocytochemistry and Western blotting, we find that the 48 hour KYNA treatment results in a 30% reduction of NR1 expression at 3 DIV cultures in proliferation medium, and a 12% reduction after 7 DIV in differentiation medium, as compared to controls. Furthermore, KYNA treatment resulted in a decrease in NR1 expression on all studied cell types: (RGCs, intermediate progenitors, interneurons and glutamatergic neurons). These results indicate that exposure of the human fetal cerebral cortex to KYNA at midgestation can lead to an imbalance between the number of interneurons and glutamatergic neurons, and a reduction of NMDAR subunit NR1 expression, possibly laying the



foundation for an imbalance in cortical circuits during development.

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PO-79

Track: Pharmaceutical Biotechnology

BIOTRANSFORMATION OF EXISTING DRUGS INTO NEW ANALOGUES-AN APPROACH TOWARDS COST-EFFECTIVE DRUGS

Elias Baydoun, Saira Bano, Hina Mahmood and M. Asif Iqbal

Department of Biology; American University of Beirut, Lebanon; E-mail: eliasbay@aub.edu.lb

Biotransformation is an important tool for food additive, pharmaceutical, agrochemical, and other industries. Biotransformations catalyzed by microorganisms have been used in industry for the production of pharmaceuticals. We used biotransformation to create new analogues of existing drugs with improved pharmacological activity and lower toxicity. This approach appears to be a solution to problems in the pharmaceutical industry including production and high cost.

Exemestane was biotransformed to produce various structural analogues that were evaluated for their cytotoxicity against cancerous cell lines. An analogue of exemestane showed significant activity against HeLa and PC3 cancer cell lines.

Similarly, biotransformation of melengestrol acetate resulted in the production of new analogues. They were found to be six-fold more potent in inhibiting T-cell proliferation compared to the standard drug, prednisolone. This discovery of potent anti-inflammatory compounds can lead to the development of new immunosuppressant compounds for clinical applications.

Furthermore, the biotransformation of nandrolone resulted in the synthesis of new analogues with significant activity against the protozoal tropical disease leishmaniasis. Some of these analogues could form the basis for the development of effective therapies against leishmaniasis.

PO-30

Track: Plant and Environment

DENITRIFICATION BARRIERS – AS A BIOREMEDIATION TOOL FOR NITROGEN REMOVAL FROM POINT SOURCE AND DIFFUSED POLLUTION IN RURAL AREAS

Bednarek A., Szklarek S., Gągała I., Dziedziczak K., Serwecińska L., Zaborowski A., Dziadek J., Mankiewicz–Boczek J. and Zalewski M.

European Regional Centre for Ecohydrology of the Polish Academy of Sciences, Poland; E-mail: agnik@biol.uni.lodz.pl

The application of methods based on a denitrification process has created an opportunity to increase the efficiency of groundwater protection in the catchment scale. The aim of the study was to test the efficiency of nitrates removal in denitrifying barriers, composed with different organic carbon sources – harl, mix of: pinus sawdust/straw, lignite/charcoal lime and straw/lignite. Pollution emitted from point and non-point sources ranged respectively from 200 mg/L to 2000 mg/L, and from 50 mg/L to 200 mg/L. Barriers constructed around the point-sources indicated that nitrogen was reduced from 52% to 72% and it was mainly dependent from incoming nitrogen load. The removal of nitrate from diffused sources amounted to 15-56%. In turn, the time of the removal of nitrogen compounds in barriers installed in rural areas was about 6-7 weeks. The results suggest



that denitrification barriers, applied around point sources, compared to barriers for nitrates removal from diffused pollution, have the best cost- effectiveness ratio of nitrates removal in the area of intensive farming.

Study supported by the NCRD DP NR14 0061/2009, "GEOFIBROUS" and NCRD, PBS1/A8/5/2012 "MIKRAZO"

Keywords: Denitrifying barrier, carbon substrates, agricultural pollutions.

PO-64

Track: Other areas

MODULATION OF ENDOPROTEASES BY TRICHOPHYTON RUBRUM GROWTH ON ELASTIN AND KERATIN SUBSTRATES

Tamires Aparecida Bitencourt, Matheus Eloy Franco, Mozart Marins and Ana Lúcia Fachin

Unidade de Biotecnologia, Ribeirão Preto, Brazil; E-mail: tabitencourt@yahoo.com.br

Dermatophytes are keratinolytic fungi, classified as geophilic, anthropophilic and zoophilic species on the basis of their primary habitat association. The anthropophilic specie *Trichophyton rubrum* is the most common in superficial mycoses worldwide. Although dermatophytes do not normally penetrate beyond the epidermis, deeper penetration and systemic infections can occur in immunocompromised hosts. The epidermis of skin is composed by keratin, while derme is constituted by collagen, fibers and elastin. The secretion of proteases is considered a key factor for virulence of dermatophytes, and arthroconidia play a central role in pathogenesis, since is responsible for skin adherence. The aim of this work was to verify the modulation of Subtilisin3 and Subtilisin1 in germinating conidia of *T. rubrum* growth on elastin and keratin substrates. The modulation of two encoding genes of Subtilisin3 and Subtilisin1 was carried out by qPCR using a pool of RNA of 24h, 36h and 72h of *T. rubrum* strain CBS118892 growth on keratin and elastin. The results show that the Subtilisin3 is similar induced by *T. rubrum* in both protein substrates, whereas subtilisin1 is more induced on keratin substrate. The modulation of these genes may be important to elucidate the superficial and deep infection mechanism.



Keywords: *Trichophyton rubrum*, Subtilisin1, Subtilisin3, keratin, elastin.

PO-11

Track: Industrial and Manufacturing

FERMENTATION OF BACILLUS LICHENIFORMIS TO PRODUCE POLY (GLUTAMIC ACID) AT HIGH PRESSURE AND HIGH OXYGEN CONCENTRATION

Alejandra Hernández-Valencia, Jose R. Cascante-Alpízar and Jordi J. Bou

*Department of Chemical Engineering, Universitat Politècnica de Catalunya, Spain;
E-mail: jordi.bou@upc.edu*

One of the main problems observed during the synthesis process of Polyglutamic acid (PGA) *via* aerobic liquid fermentation, is the inability to achieve high yields when working at high culture volumes. This could be due to the reduction of the oxygen transfer rate (OTR) as the culture volume increases.

The aim of this study is to propose two possible methods for dealing with the problem of low dissolve oxygen concentrations: increment the pressure inside the bioreactor and increasing the oxygen partial pressure in the reactor atmosphere.

The fermentations performed with *Bacillus Licheniformis* ATCC9945A, showed that the polymer production slightly increases with increasing the oxygen content in the atmosphere. Thus, as the air pressure increases, the PGA production

increases until the maximum is reached (13.34 g PGA/l), at 2.03 bar absolute. On the other hand, as expected, fermentation under enriched O₂ atmosphere also increases productivity, but not as high pressure experiments.

In addition, it has been found that the pressure also alters the enantiomeric composition of the polymer, since the PGA synthesized under these conditions is mainly formed by L enantiomer while, the one synthesized at atmospheric pressure, contains predominantly D enantiomer.

According to the obtained results, raising the pressure inside the reactor is a more effective way of growing the fermentation yield than increasing the oxygen proportion in the atmosphere, because of the highest PGA concentrations obtained.

Keywords: Polyglutamic acid, *Bacillus Licheniformis*, enantiomeric composition, high pressure fermentation, Oxygen transfer rate.

PO-37

Track: Medical Biotechnology

STUDY OF THE EFFECT OF POLYPHENOLS EXTRACTED FROM GREEN ALGAE *CAULERPA PROLIFERA* IN PREVENTING CELL DAMAGE BY IRI

Bover, E. A., Sanchez, D.A, Belda, M.A, Padrón, C. A, Cejalvo, D.A and Lloris, J.M.B

Facultad de Ciencias Experimentales, Universidad Católica de Valencia "San Vicente Mártir", C/ Guillem de Castro, 94. 46003. Valencia, Spain, E-mail: elena.bover@ucv.es



Seaweeds have developed strong antioxidant systems in response to highly oxidative conditions in which they live. They are considered to be a rich source of antioxidants and have been identified to possess biochemical substances with therapeutic potential [1]. Phenolic antioxidant compounds have gained importance due to their high demand for alternative and safe antioxidants from natural resources. Antioxidant agents have been used to prevent tissue damage in various clinical settings and experimental models and could help in preventing the post-ischemic increases in lipid peroxidation and hydrogen peroxide levels, resulting in reduced IR injury [2, 3].

Therefore, the green seaweed *Caulerpa prolifera*, was evaluated for its Total Phenolic Content (TPC) and for its Radical Scavenging Activity (RSA). The effect of variables such as: solvent, temperature and time, have been studied to optimize the polyphenol extraction.

C.prolifera showed the maximum TPC and RSA in ethanol at 3 h 45°C. Identification and quantification of phenolic compounds was carried out with RP-HPLC/DAD. Biochemical analyses were carried out (MDA, catalase, Gpx, SOD and LDH), protein analyses (cytokines and proinflammatory molecules), Western Blot and the histologies to determine the intestinal morphology and the physiopathological changes during the period of damage due to IRI were also characterized.

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PO-39

Track: Pharmaceutical Biotechnology

ANTINOCICEPTIVE ACTIVITY OF BYRSONIMA DUCKEANA W. R. ANDERSON (MALPIDIACEAE) - AN AMAZONIAN "MURICI"

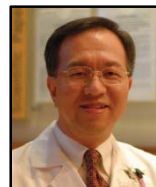
Maria Christina dos Santos Verdam, Fernanda Guilhon-Simplicio, Kleyton Cardoso de Andrade, Karina Lorena Meira Fernandes, Marilis Dallarmi Miguel, Obdúlio Gomes Miguel and Patricia M. Stuelp Campelo

Department of Pharmacy, Pontifícia Universidade Católica do Paraná, Brazil;
E-mail: p.campelo@pucpr.br



Byrsonima duckeana A. W. Anderson is endemic specie in Brazil and is mainly distributed in North Brazil, particularly in the states of Pará and Amazonas and it is popularly know as "sara-tudo" (cureall). This study evaluated anti-inflammatory and antinociceptive activities of the extract and fractions obtained from leaves of this plant. The collection of vegetal samples was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), chaired by the Ministry of Environment of Federative Republic of Brazil. Plant material was collect in Reserva Florestal Adolpho Ducke Manaus - AM, and taxonomically identified by Herbarium of Instituto Nacional de Pesquisas da Amazonia (INPA). The leaves were dried, pulverized and exhaustively extracted with ethanol (EE) in Soxhlet apparatus and was partitioned providing hexane (HF), chloroform (CHLF) and ethyl acetate (EAF) fractions, which were used in the biological tests. Swiss mice were used for the experiments, which were approved by the Ethics Committee for Animal Research of the Universidade Federal do Amazonas and Pontifícia Universidade Católica do Paraná. The acute toxicity was evaluated using five groups of 8 animals received orally the ethanolic extract and the fractions (2000 mg/kg p.o). The animals were observed at the first 4 hours to behavior alterations and diary by 14 days. The anti-inflammatory and antinociceptive activities were tested by intraplantar injection of formalin solution, carrageenan-induced peritonitis, acetic acid-induced abdominal constrictions writhings and hot plate test. In the acute toxicity test, behavioral and physiological alterations were not observed neither animal's death in the dose level of 2000 mg/kg of the crude ethanol extract. EE inhibited the inflammatory phase (15-30 minutes) but not neurogenic phase (0-5 minutes) in the formalin test, decreased the time of licking paw in the inflammatory pain in 57%, demonstrating anti-hyperalgesic capacity. After carrageenan administration, the leukocyte migration decreased 43% and 58% at 500 and 1000 mg/kg, respectively, in comparison to negative control. The results obtained in the writhing test showed that analgesic activity of EE, CHLF and EAF are statistically significant compared to the negative control, reducing ~50% the abdominal writhings. No difference was found in relation to the positive control (indomethacin) and the HF showed no analgesic activity. Considering that the CHLF and EAF showed similar activity, the last one was selected for the hot plate test. EAF showed analgesic activity even with the lowest dose tested (5 mg/kg), which is half the dose of indomethacin used, emphasizing that it is a promising source of substances with analgesic activity. Therefore, considering that one of the objectives of this study was to indicate the most promising way for the isolation of at least one active compound, and the results obtained in this study allowed us to observe that the most polar compounds are more directly related to analgesic activity of the EE, it is suggested that the process isolation and characterization of active compound should be performed with the EAF. Besides, this study is pioneer in investigation of antinociceptive and anti-inflammatory potential of Byrsonima duckeana W.R. Anderson, demonstrating that it is promissory specie of the Amazonia Region, and confirming traditional use.

Keywords: Amazonian rainforest, Byrsonima duckeana, Inflammation, pain, anti-hyperalgesic.

PO-68*Track: Regenerative Medicine***INVESTIGATION OF THE ESSENCE OF PLATELET-RICH FIBRIN (EPRF): CYTOKINE DISTRIBUTION IN RELATION TO THREE-DIMENSIONAL STRUCTURE****Wing P. Chan, Meng-Yi Bai, Ching-Wei Wang, Jyun-Yi Wang and Ming-Fang Lin***Wan Fang Hospital, Taipei Medical University and School of Medicine, College of Medicine, Taipei Medical University, Taiwan; E-mail: wp.chan@msa.hinet.net*

Platelet-rich fibrin (PRF) is a second-generation platelet-containing biomaterial used in regenerative medicine. This study aimed to determine the cytokine content of PRF obtained from centrifugation of whole blood and its relationship with the three-dimensional fibrin network structure. Enzyme-linked immunosorbent assays, histological images, and scanning electron microscopic (SEM) images revealed a cytokine concentration gradient (especially for PDGF-BB and TGF- β 1), which was strongly related to the three-dimensional structure of the fibrin network. Histological images of PRF sections showed the average porosity gradually increasing across PRF segments (6.5% at the red blood cell (RBC) border), reaching a maximum at the serum border (40.3%). SEM images revealed a network structure composed of fibrin fiber with a diameter of 0.11 μ m at the RBC end, gradually increasing to 0.14 μ m at the serum end. Compactness decreased along the longitudinal axis of the PRF gel from the RBC end to the serum end. This fibrin network structure led to trapping of cytokines; the amount of cytokine incorporated was related to fibrin compactness and pore size in various parts of the PRF gel. Knowledge of the cytokine distribution and the three-dimensional structure of PRF are critical to proper use of the PRF gel for various clinical applications.

Keywords: Biomaterials, cytokines, nanomedicine, Platelet-rich fibrin (PRF), regenerative medicine.

PO-8*Track: Other Areas***DIMETHOXYFLAVONE, A FLAVONOID FROM *STEREOSPERMUM KUNTHIANUM* STEM BARK WITH ANALGESIC AND ANTIINFLAMMATORY ACTIVITIES****Ching.F.P and Falodun, A.***Dept. of Pharmacology, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Yenagoa, Nigeria; E-mail: fidelching@yahoo.ca*

Introduction: *Stereospermum kunthianum* is reputed for its ethnomedicinal use in the treatment of various painful and inflammatory conditions in Nigeria and other West African countries [1].

Materials and Methods: The chemical identification and isolation of the stem bark of *Stereospermum kunthianum* led to the isolation and characterization of an alkylated flavonoid (3,7,4/-trihydroxy-3/-(8/-acetoxo-7/-methyloctyl)-5,6-dimethoxyflavone. The structure was confirmed by 2-DNMR [2] and other spectroscopic techniques [3].

The analgesic and anti-inflammatory activities of the isolated compound were studied using the Randall-Selitto test [4], mouse writhing assay [5] and carrageenan-induced paw oedema in rats [6].

Results: Dimethoxyflavone at the dose of 25mg/kg and 50 mg/kg significantly increased ($P < 0.001$) the pain threshold when compared with the rats that similarly had sham treatment with distilled water. The percentage increase in pain threshold was 51.6, 66.7 and 62.3 respectively for dimethoxyflavone at 25 mg/kg, 50 mg/kg and indomethacin (10 mg/kg) respectively. In the mouse writhing assay, the writhes were significantly reduced ($P < 0.001$) in the dimethoxyflavone treated mice with the percentage inhibition of pain of 46.2, 58.3 and 63.1 for the flavonoid at 25 mg/kg, 50 mg/kg and aspirin (100 mg/kg) respectively. Dimethoxyflavone (25mg/kg and 50 mg/kg) caused a significant reduction ($P < 0.001$) in the paw swelling as from the 2nd h and a much more reduction at the 3rd h post carrageenan injection compared with control rats.

Conclusion: The result of the phytochemical study led to the isolation of 3,7,4-trihydroxy-3-(8-acetoxy-7-methyloctyl)-5,6-dimethoxyflavone, a known flavonoid from *Stereospermum kunthianum* stem bark which possesses both analgesic and antiinflammatory activities.

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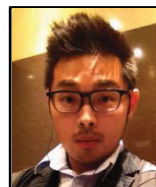
PO-23

Track: Plant and Environment

EFFECTS OF UNIFORM MAGNETIC FIELD REVERSAL ON ARABIDOPSIS THALIANA

Albert M. Chung

Vassar College, 124 Raymond Avenue, Box 1155 Vassar College Poughkeepsie, NY 12604-1155, USA; E-mail: albertchung1238@gmail.com



Uniform magnetic fields stronger than the Earth's have been documented as having various influences on the physiology and morphology of different plant species. Effects resulting from the manipulation of the magnetic field's orientation have not been thoroughly investigated and relevant studies have demonstrated the effects of magnetic field lines directed either parallel or perpendicular to the planet's surface. In this study, I show that no relationship exists between germination potential and the orientation of the magnetic field in either direction along the axis of the planet's geomagnetic field (p-value > 0.05). I further report a positive relationship between wilting and antiparallel field lines, and a negative association between leaf growth and a reversed magnetic field. An initial surge in the germination rate was observed under the presence of an antiparallel magnetic field. Conversely, initial damping in the germination rate was noted for seeds subjected to parallel field lines. These findings indicate a significant reduction in germination success (p-value < 0.05), initial peak in germination rate, excessive plant wilting, and negative leaf growth due to a magnetic field reversal in the model plant organism, thale cress (*Arabidopsis thaliana*).

Keywords: Magnetic field reversal, wilting, germination, leaf growth, *Arabidopsis*.

PO-36

Track: Plant and Environment

EXTRACTING AND TRANSPIRING METALS FROM CONTAMINATED SOILS

Jenna Forte, Megan Corley, Sam Mutiti, and Alyssa Thomson

Georgia College and State University, 145 South Irwin Street Apt. #2315 Milledgeville, GA 31061, USA; E-mail: megan.corley@bobcats.gcsu.edu



Certain plants are known to take up heavy metals directly from the soil through their roots and into their leaves. There are no studies in the literature on whether these plants are able to transpire these contaminants once they get to the leaves. This study, therefore, investigates the ability of common hyperaccumulators of heavy metals, *Hydrangea paniculata* and *Tithonia rotundifolia*, to translocate heavy metals from the soil through the plant to the atmosphere. The metals of choice were copper and lead. At the end of the growth period both translocation and enrichment factors will be calculated. In a preliminary study, 10 pots of *Tithonia diversifolia* were watered with 1000 ppm copper solution, and 10 pots were watered with 1000 ppm lead nitrate

solution for four weeks. A fully grown *Hydrangea* plant was also watered with 1000ppm copper solution for one week. During this time, transpiration was collected from all *T. diversifolia* plants and from three branches on the *Hydrangea* plant. Transpiration from *T. diversifolia* showed a copper concentration presence of 0.04 mg/L and 0.005 mg/L for lead. The transpiration collected from the three *Hydrangea* branches had of 0.026 mg/L copper.

PO-71

Track: Plant and Environment

THE NEUTRALIZATION OF ACID MINE DRAINAGE BY MIXED CULTURE 'ALGAL' BIOMASS

Rosemary Falcon and Evanie Devi Deenanath

Department of Chemical and Metallurgical Engineering, University of the Witwatersrand, South Africa; E-mail: edeenanath@yahoo.com



Background: Contamination by acid mine drainage (AMD) from the mining industry is an on-going environmental concern. The generation of AMD, unfortunately cannot be entirely prevented, however it can be treated in order to minimize and control the harmful effects caused by AMD metals with a growing interest in biological aspects towards AMD treatment. In Middelburg, Mpumalanga-a popular mining area in South Africa, an isolated site known as the NET Energy site was established to investigate the large-scale harvesting of algae biomass to be implemented for carbon sequestration. Other than this carbon technology there is an interest in utilising algae biomass for AMD neutralization. For this research, 'algae' growing in an open-pit, close to the Net Energy site, was collected and harvested on site and the neutralising potential of the 'algal' biomass (as a mixed culture or multiple species culture) will be investigated.

Method: A bioassay was carried out which consisted of acid mine water (collected from Muhanga Mines) inoculated with the harvested culture (open-pit algae). Basic measurements of pH and dry mass were monitored.

Results: During the duration of the bioassay, an increase in pH from 3.3 to 6.8 was recorded and dry weight of biomass ranged from 0.30 to 0.35 g/L.

Conclusion: The biomass, presumably algae, collected from this particular area has a neutralizing ability and can easily sustain its growth within an AMD environment.

Future Work: Experimental work is currently underway to confirm these preliminary results and identify species population of the culture. Additionally, concentration of AMD metals and sulphur will be determined and other analytical methods will be performed to understand the interaction between the AMD metals and the biological cells and the effect of sulphur removal for the utilization of biomass for mine land rehabilitation.

Keywords: Acid mine drainage, Neutralization, Biomass.

PO-82

Track: Plant and Environment

EX-VITRO ADAPTATION AND SCREEN HOUSE EVALUATION OF GENETICALLY MODIFIED CASSAVA GENOTYPES FOR CMD RESISTANCE IN UMUDIKE, NIGERIA

Edoh N.L., Okwuonu I., Onyeka J. and C.N. Egesi

Biotechnology Program National Root Crops Research Institute (NRCRI), Umudike, Nigeria; E-mail: edohlink@yahoo.com

Considerable efforts have been directed to optimize the conditions for *in-vitro* stages of micropropagation of cassava, but the process of acclimatization of micropropagated plants to the soil environment has not fully been achieved. Five hundred and fifty four cultures of genetically modified (GM) cassava were received from University of Zurich (ETH) for *ex-vitro* adaptation and confined field trial in National Root Crops Research Institute, Umudike, Nigeria. The effect of genotype on *ex-vitro* adaptation of

GM cassava was evaluated by considering the survival rate, growth rate and vigor and analysed statistically using ANOVA. There was no significant difference observed between the survival rates of TMS 6044, TME 3, TME 7 and TME 14 in the humidity chamber ($F=0.23$ at $P>0.05$). Their growth rate and vigor equally showed no significant difference ($F=3.71, 4$ at $P>0.05$) in the screen house as they performed same. Effect of transgene showed no adverse effect on survival rate, growth rate and vigor ($F=0.04, 2.59$ and 3.50 at $P>0.05$). TMS 60444 was transformed with different constructs and recovered events were compared with non-transgenic and transformed cassava without transgene (p1300). The individual construct had no adverse effect on the adaptation of the GM cassava as no significant difference was observed in their survival rate ($F=0.21$ at $P>0.05$), growth rate ($F=1.5$ at $P>0.05$) and vigor. The transgenic event performed favourably in comparison with the non-transgenic. In this study, the transgene had no adverse effect on *ex vitro* adaptation of genetically modified cassava genotypes for CMD resistance.

Keywords: *Ex vitro*, acclimatization, transgenic, cassava

PO-61

Track: Marine Biotechnology

WATER QUALITY CONTROL FOR INTENSIVE SHRIMP CULTURE PONDS IN DEVELOPING COUNTRIES USING INORGANIC NITROGENS UPTAKE BY SEAWEED

Ryuichi Egashira, Hiroaki Habaki, Kosuke Sato, Shin Tajiri, Yuta Ueno and Tippabust Eksangsri

*Department of International Development Engineering, Tokyo Institute of Technology, Japan;
E-mail: regashir@ide.titech.ac.jp*



This presentation reviewed our application of inorganic nitrogens uptake by seaweed to the water quality control for intensive cultivation of shrimp in developing countries, where simple technique of water quality control is required. In the first, the performances to take in ammonia- and nitrate-nitrogens by seaweed of sterile *Ulva* spp. collected in a bay of Yokohama, Japan were examined under various conditions. The seaweed could take in and remove inorganic nitrogens in the culture medium effectively. The ambient nitrate-nitrogen did not affect the ammonia-nitrogen uptake. The phenomena of these nitrogens uptake by the algae could be correlated by both of a Michaelis-Menten model with an inhibitory effect and a model based on the permeation through cell membrane. Secondly, the water quality control in the pond of the practical shrimp culture batch was simulated by simple calculation based on the material balance of ammonia-nitrogen with parameters obtained from the above experiments and correlations. The ammonia-nitrogen concentration in the pond could be well controlled by seaweed. Consequently, the water quality control by seaweed was proposed as a simple and convenient method appropriate for the shrimp culture pond in developing countries.

Keywords: Water Quality Control, Intensive Shrimp Culture Ponds, Inorganic Nitrogen Uptake, Seaweed.

PO-77

Track: Medical Biotechnology

LACTIC ACID BACTERIAL EXTRACT AS AN ALTERNATIVE CHOICE IN THE TREATMENT OF *C. ALBICANS* INFECTION: AN APPROACH

Hala El-Adawi, Mahmoud Taha, Abdel Fattah Abdel Fattah and Nagwan Youssef

Medical Biotechnology Dept., Genetic Engineering & Biotechnology Institute, P.O.BOX 21934, Alexandria, Egypt; E-mail: haeladawi@hotmail.com



C. albicans induces infections of different kinds, classified as superficial, locally invasive and systemic infections. The present study aimed at estimating the antifungal activity of Lactic acid bacteria (LAB) extract against 30 resistant clinical isolates of the vegetative form of *candida albicans* (*C. albicans*). LAB was isolated from 100 fermented food samples. *C. albicans* isolates and their biofilm were obtained from private hospitals. The different isolates species of LAB extracts were then

evaluated for its antimicrobial activity on *C. albicans* isolates by using agar well diffusion method. Determination of MIC of LAB extracts were then carried out. Moreover, the effect of LAB extract on the formation of *C. albicans* biofilm was assessed and the inhibition was then expressed as percentage.

All LAB extracts exerted a growth inhibitory effect on the vegetative form as well as the biofilm of *C. albicans* and the most powerful isolate was *L. plantarum*, where, the highest diameter of inhibition zone was 22.80mm and the MIC was 62.67% while the average percentage of biofilm inhibition was 67.33%. The use of probiotic LAB strains may offer an alternative therapeutic choice in the treatment of Candidiasis and effectiveness against drug resistant strains since all LAB extract proved inhibition.

PO-78

Track: Plant and Environment

EFFECT OF GAMMA IRRADIATION ON THE BIOACTIVITY OF *ARTHROSPIRA FUSIFORMIS*

Hala El-Adawi, Mahmoud Taha, Abdel Fattah Abdel Fattah and Nagwan Youssef

Medical Biotechnology Dept., Genetic Engineering & Biotechnology Institute, P.O.BOX 21934, Alexandria, Egypt; Email:halaeladawi@hotmail.com

The edible cyanobacterium *Arthrospira*, is used commercially as feed and food additive and is well known for its bioactive compounds having antiviral, antibacterial or anticancer activity.

In the present work *Arthrospira fusiformis* (*A. fusiformis*) was irradiated with different doses of gamma rays (0.5, 1.0, 1.5, 2.0 and 2.5 kGy) to study its effect on the antibacterial and antiviral activity of *A. fusiformis*.

It was found that Gamma rays changed the lipid profile of *A. fusiformis* and the lipids extracted from irradiated cells showed higher anti-hepatitis C (anti-HCV) effect, but the antibacterial effect decreased. It was also observed that increasing Gamma irradiation dose showed increasing anti HCV effect of the respective hot water extracts. Thus Gamma irradiation of *A. fusiformis* could be manipulated to improve the potentiality of its bioactive compounds.



VP-05

Track: Medical Biotechnology

AN EFFECTIVE BCG VACCINATION SCHEME-CONTAINING PRIME AND ANNUAL PERIODIC SUBUNIT BOOST SELECTS AND ACTIVATES TEM AND TH17 CD4+ SUBSETS IN NATURALLY PREINFECTED DAIRY CATTLE

Miranda-Hernández D.U., Ramírez-Cerda E.L. and Estrada-Chávez C

Medical and Pharmaceutical Biotechnology Unit, CIATEJ, 44270 Guadalajara, Mexico ; Email: eramirez@ciatej.mx

Background: bovine tuberculosis (bTB) is an important animal and zoonotic disease that causes significant financial loss and is a public health hazard. *Mycobacterium bovis* (*M.bovis*) is the causative agent of bovine tuberculosis. In many developed countries, the control of bTB is based on test and slaughter programs. Field and laboratory diagnostic test is used to identify potentially infected herds, which are following by additional diagnostic testing and slaughter of all reactor cattle. However, in developing countries with high prevalence of bTB, this policy is a non-viable option, due the high cost that it represents. Therefore, diagnostic improvements and a reasonable vaccination strategy are essential for bTB control. In this work was evaluated the level of protection and immunologic profile of cattle which were immunized with a primary vaccination with BCG and a protein subunit vaccine as well as annual periodic boosters with the subunit vaccine.

Experimental Design: the field trial was performed in two stages. In the first stage was evaluated the general level of protection induced by the vaccination scheme proposed in this work through the time (2010 at 2014) in a representative samples of cattle from each farm, using cattle that belong at dairy farms located within an endemic area with high prevalence of bTB ($20.14 \pm 2.32\%$). In a second stage, we took a representative sample of unvaccinated ($n=25$) and vaccinated cattle ($n=25$) of these herds and were evaluated the immunological profile in cattle whose were vaccinated at birth.

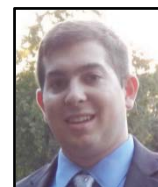
Methods: the general evaluation of the antigen-specific immune response induced by the vaccination was made measured immunological parameters like frequency of reactors and new reactors at Tuberculin Skin Testing (TST), interferon gamma released assay *in vitro* by BOVIGAM® 1G, and the reactivity to MPB83 ELISA; pathological parameters such as pathological score as well as bacteriological isolation. All of these evaluations were made in several points during the development of trial. The evaluation of the immunologic profile was performed measured the frequency of CD4⁺ T cell subsets type 1, CD3⁺CD4⁺CD25⁺CD183⁺ (Th1) cells; type 17 CD4⁺CD25⁺CD161⁺CD183⁺ (Th17) cells; effector memory CD4⁺CD44⁺CD45RO⁺CD197^{low} (T_{EM}) cells, and regulatory T cells CD3⁺CD4⁺CD25⁺FoxP3⁺ were determined by flow cytometry. The activation degree of these subsets was characterized by the level of expression of factors (TF's) in a DNA/Protein array and the capacity to release cytokines, which were measure in culture supernatant of peripheral blood mononuclear cells (PBMC's) by ELISA. ELISpot determined the frequency of antigen specific IFN- γ ⁺ memory T cells.

Results: in the first stage of the research the general evaluation of the antigen-specific immune response shows several findings: the frequency of the reactors to TST in the first phases of the evaluations were lower in vaccinated cattle than unvaccinated cattle, but when the immunization was implemented the frequency of TST was increased. The frequency of the new reactors to TST increased according to the applications of vaccination scheme in the new born cattle inside each herd. Is important to remark that the reactivity to TST in the evaluated cattle is due to the application of the subunit vaccine and not because the cattle were ill. The systemic level of IFN- γ also increased in all vaccinated cattle later to the massive vaccination, but through the time IFN- γ concentration delay gradually without get the same level to initial phases of the evaluation. However when the boosters were administrated the concentrations of IFN- γ increased immediately. At systemic level, vaccinated cattle produced 10 fold more IFN- γ than unvaccinated cattle. The results according to ELISA MPB83 shows that unvaccinated cattle have more positives samples than vaccinated cattle ($p < 0.05$). The evaluation of pathological parameters shows that the reduction of lesions in the vaccinated cattle was relevant a long to time ($p < 0.0402$) ($R^2=0.9161$), with an efficacy of the vaccine scheme was of 34%. Finally the evaluation of bacteriological isolations in vaccinated cattle shows a reduction of number of positive cases ($p = 0.0182$) with a negative correlation a long to time ($R^2=0.8804$). When the efficacy of the vaccination was computed according to this criterion was of 77%. The frequency of Th1 cells was similar between groups ($p > 0.05$), but the expression of STAT-1, STAT-4 and Tbet was higher in vaccinated cattle ($p < 0.05$). The concentration of IFN- γ and TNF- α in culture supernatant of PBMC's was similar between vaccinated and unvaccinated groups. The frequency of Th17 cells ($p < 0.0001$) and the expression of STAT-3 ($p = 0.05$) were higher in vaccinated cattle, while the expression of ROR γ was higher in unvaccinated cattle ($p < 0.05$). However, the IL-17 production was similar in both groups ($p > 0.05$). The frequency of Treg cells and the expression levels of FoxP3 were higher in unvaccinated cattle ($p < 0.0001$), but the IL-10 production was similar between groups ($p > 0.05$). The T_{EM} subset was 50-fold more frequent in vaccinated cattle ($p > 0.0001$) as well as the IFN- γ ⁺ memory T cells ($p < 0.05$).

Conclusions: Protective efficacy, evaluated at slaughter when animals were 64-67 months of age, indicated that in 57% control cows presented visible lesions (44/77) and *M. bovis* was cultured from the tissues of 48% (35/73); in contrast, 44% (32/73) and 11% (8/73) of the vaccinated animals presented visible lesions and were culture positive. These differences were statistically significant ($p < 0.05$). These data indicate that prime-boost vaccination scheme could play a role in the control of bTB in the field. As well the results obtained in this work suggest that BCG vaccination followed by periodic boosters with a subunit vaccine applied in a commercial dairy with high prevalence of bTB, induces a memory specific immune response based in the selection and activation of T_{EM} and Th17 subsets. The vaccination strategy promotes the proliferation of IFN- γ producing memory cells in bovines.

PO-47*Track: Pharmaceutical Biotechnology***LAVANDULA OFFICINALIS ESSENCIAL OIL AGAINST HAEMONCHUS CONTORTUS****Ferreira, L.E., França, S.C., Taleb-Contini, S.H., Chagas, A.C.S. and Beleboni, R.O.***Department of Biotechnology, Ribeirão Preto University (UNARER), Brazil;**E-mail: luis.biomed@hotmail.com*

The sheep farming is traditionally practiced worldwide as a promising economic activity. However, the general unpreparedness of the breeders in the management of animals has contributing to the development of gastrointestinal infections mainly caused by *Haemonchus contortus* species. *H. contortus* sheep infections have been related to serious economic losses on sheep farming and the irregular or erroneous administration of traditional anthelmintic drugs has been associated to the resistance development. Ethnopharmacological information has appointed the *Lavandula officinalis* essential oil as a promising alternative for development of new anthelmintic products. Thus, the main aim of this work was to investigate the popular use of essential oil of *L. officinalis* with emphasis on its potential anthelmintic effects against *H. contortus*. According our best results, *L. officinalis* essential oil showed 92.3% and 40.6% of efficacy in egg hatch and larval motility tests, respectively. In the adult worm motility test, 85% of worms were completely immobilized within the first 6 hours of nematode exposition to different dilutions of oil. Phytochemical analysis revealed the presence of linalool (36.01%), sabinene (31.1%) and 1,8-cineol (5.61%) as the most important compounds, which may be responsible for anthelmintic essential oil activity.

Keywords: Anthelmintic; Ethnopharmacology; Gastrointestinal infections; Sheep.**PO-32***Track: Pharmaceutical Biotechnology***LIPID REMODELING AND LIPOPROTEIN FORMATION BY SERUM AMYLOID A: POTENTIAL FUNCTIONAL IMPLICATIONS****Nicholas Frame, Shobini Jayaraman and Olga Gursky***Department of Physiology & Biophysics, Boston University School of Medicine, 700 Albany St. Boston MA 02118, USA; E-mail: nframe1@bu.edu*

Serum amyloid A (SAA) is an acute-phase protein whose function is subject to debate. In acute injury, plasma levels of SAA can increase 1000-fold. Although functional ramifications of this dramatic increase are unclear, SAA may help remove lipids from damaged cells. SAA circulates mainly on plasma high-density lipoproteins (HDL), which are ~10 nm particles that remove excess cell cholesterol and protect against atherosclerosis. SAA can bind to HDL and replace its major protein, apoA-I. Alternatively, SAA can generate HDL *de novo* at the plasma membrane. To better understand this process, we analyzed interactions of SAA1.1 with physiological phospholipid, POPC. SAA-POPC binding was monitored using circular dichroism spectroscopy, electron microscopy and gel electrophoresis. The results revealed that, in contrast to apoA-I, SAA can spontaneously remodel POPC vesicles to form ~10 nm HDL-like particles. Distinct stages of this remodeling were characterized by varying protein:lipid ratio. The major shift from lipid-free protein to HDL-like lipoprotein was observed between 1:2 and 1:5 SAA:POPC. This novel incremental transition mimics aspects of *de novo* generation of SAA-containing HDL. Similar particles may contribute to lipid removal from injured cells. Moreover, helix folding on the lipid observed in our work may protect SAA from cleavage and amyloid deposition in inflammation-linked amyloidosis.

VP-03

Track: Other Areas

RAPID AND LOW COST PRODUCTION OF hGM-CSF CYTOKINE IN INDUSTRIAL TOBACCO – PROOF OF CONCEPT

Lea Vojšta, Lana Ljuma-Skupnjak, Ankica Budimir, Slobodan Vukičević and Hrvoje Fulgosi

Division of Molecular Biology, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia; E-mail: fulgosi@irb.hr

We report the production of hGM-CSF protein in leaves of two industrial cultivars of tobacco by using *Agrobacterium*-mediated transient expression. For overexpression, two binary destination vectors, p2GW7.0 and pH7WG2.0, were evaluated. The gene hGM-CSF was tagged on the 3' end with the His- and HA-epitopes and cloned into the destination vectors. *Agrobacteria* containing constructs GM-CSF-His-HA/pH7WG2.0 and GM-CSF-His-HA/p2GW7.0 were used for 5- and 10-minute-long vacuum infiltration of tobacco plants. The hGM-CSF accumulation was found to be dependent on the presence of wetting agent supplemented during vacuum-agroinfiltration, duration of vacuum-agroinfiltration, and the age of the leaves. We demonstrate that industrial tobacco cultivars, with very high biomass, are suitable platforms for rapid, low cost production of foreign proteins. Successful transient expression of the GM-CSF in industrial tobacco was achieved in less than three months, opening the possibility for future applications of this approach in rapid response production of various proteins of non-plant origin.

PO-83

Track: Plant and Environment

IMPACT OF WATER PH CHANGES ON FRESH WATER AQUATIC LIFE

Debolina Ghosh

Hathaway Brown High School, Shaker Heights, Ohio, USA; E-mail: lghosh17@hb.edu

Introduction: *Daphnia Magna* (water flea) is a prototype of the freshwater aquatic life. This study noted the effect of pH on its heart rate and life.

Material and Methods: The *Daphnia magna*, kept at a pH of 7, were examined under a microscope to record the heart rate at 0, 2, 5, and 10 minutes. Similar procedures were repeated for the pH 6 (vinegar solution) and 8 (sodium bicarbonate solution). The effect on heart rate was compared by unpaired (different cohorts) and paired (same cohort) t-test. Number of *daphnia* dying in each group was also recorded.

Results: 70 *Daphnia magna* were studied, 10 excluded as heart rate recording was faulty. Finally, there were 20 in each group- pH 7, 6, and 8. Heart rate at pH 7 was unchanged with time; it progressively decreased when exposed to the acid (pH 6) or alkali (pH 8). 10 *Daphnia* died in acid, 5 in alkali, and none in neutral pH after 10 minutes. Heart rates dropped significantly at 5 minutes of acid and 10 minutes of alkali exposure when compared to the neutral PH group. Within the same cohort, heart rates dropped significantly after 2 minutes of acid ($P < 0.01$) and alkali ($P < 0.05$) exposure.

Discussion/Conclusions: The heart rate of *Daphnia magna* decreased significantly on exposure to mild acidic (pH 6) or alkaline (pH 8) environment, often leading to death. This study proves the impact of environmental pollution, greenhouse gas emission and consequent alteration of acid base status of the fresh water on aquatic life.



PO-48*Track: Plant and Environment***RESEARCHES REGARDING THE USE OF COACERVATES ESSENTIAL OILS IN SEED TREATMENT IN ECOLOGICAL CROP PRODUCTION****Mihai Gidea, Emil Stepan, Lenuta Iulia Epure, Mihaela Niculesu, Carmen Gaidau and Doru Gabriel Epure***Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania; E-mail: gideam@yahoo.com*

Following the continuous increase of population, the request for assuring the enough food is a fundamental objective for all farmers. In these conditions is increased as well the request for ecological food production. Answering to this problem, the objective of this research paper was to establish if the microencapsulated essential oils, obtained by us in the experiments at laboratory scale, may be used to protect the wheat seed from attack of pathogens and pests in soil after seeding. The research was performed in laboratory and in experimental field.

The laboratory research was focused on microencapsulation of essential oils and on testing the phytotoxicity effect of microcapsule upon the wheat seeds and as well for test upon the population of *melolontha melolontha* (MM) larvae stage I, II. The microcapsules were obtained by a process of complex coacervation, and they have a central core formed by an essential oil, covered with a shell made of crosslinked hydrolyzed collagen. In the experimental field was evaluated the intensity of pests attack frequency. The results of phytotoxicity tests show that all the products used in seed treatments don't have any phytotoxic influence at the used doses, on the contrary we identified a stimulating effect upon the plant growth.

The researches regarding the influence upon the MM populations show the products with essential oil from *thymus vulgaris*, *satureja hortensis*, *Ocimum basilicum* decreasing the intensity of attack by comparing with control variant without any treatment. By comparing with control treated using imidacloprid, in laboratory, the efficacy of treatment was around 50% from that one.

In the experimental field we found the decreasing significantly of dipteris attack frequency. Analyzing the results obtained in this research we can said: "all the seed treatments based on own microencapsulated essential oils, can be used successfully for wheat pest protect in ecological system"

Keywords: Amino acid extract, microencapsulated essential oil, wheat, ecological production, crop protection.

PO-9*Track: Industrial and Manufacturing- (bio-fuels)***PRODUCTION OF BIOHYDROGEN BY COMBINING A TWO STAGE FERMENTATION PROCESS USING SALT-RICH SUBSTRATES****Dayana Muzziotti Gil, Alessandra Adessi, Andrea Sanchini, Laura Dipasquale, Giuliana d'Ippolito, Angelo Fontana and Roberto De Philippis***Department of Agrifood Production and Environmental Sciences (DISPAA), University of Florence, Florence, Italy; E-mail: dayanamuzziotti1983@gmail.com*

The sequential combination of Dark and Photo-fermentation is a promising process for the production of biohydrogen. It is possible to approach the theoretical maximum yield of 12 mol of H₂ (mol glucose)⁻¹. In this study, the substrate tested for the combined process was artificial sea water supplemented with glucose. The aim of this research was to test the H₂ photo-evolution performance of Purple Non Sulfur Bacteria (PNSB), specifically *Rhodospseudomonas palustris* 42OL, growing on two synthetic spent media deriving from the dark fermentation process: ASW (Artificial Sea Water, 40 g/L of sea salts) and DASW (Diluted Artificial Sea Water, 10 g/L sea salts), both supplemented with glucose (5 g L⁻¹). The DASW spent medium showed to be the best for *Rp. palustris* 42OL because, H₂ was produced and showed a high mean H₂ production rate. Furthermore, a yield of 4.74±0.37 mol H₂ per mol of glucose was obtained. It has to be stressed that, this yield is much higher than typical yields obtained in the single process

operating by *Rp. palustris*. As it seems from the results, it was possible to obtain H2 with a high yield from a salt-rich medium, which usually is not suitable for growing PNSB.

PO-51

Track: Plant and Environment

AGROBACTERIUM MEDIATED TRANSFORMATION OF SOMATIC EMBRYOS OF PERSIAN WALNUT (JUGLANS REGIA L.) USING FLD GENE FOR OSMOTIC STRESS TOLERANCE

Mohammad Ali Sheikh Beig Goharrizi, Atta Dejahang, Koroush Vahdati, Masoud Tohidfar, Ali Izadi Darbandi, Carrillo Nestor and Mohammad-Reza Hajirezaei

Department of Genetic and Biotechnology, Russian Timiryazev State Agrarian University, Moscow, Russia; E-mail: Sheikhbeig@gmail.com



Walnut somatic embryos were transformed with two strains of *Agrobacterium tumefaciens*, LBA4404 and C58 respectively harboring pBI121 with Gus and neomycin phosphotransferase (nptII) genes and p6u-ubi-FVTI plasmids containing the fld gene and hpt gene as reporter and selectable markers. Also the transgenic and non-transgenic somatic embryos of Persian walnut were exposed to four salinity levels (0, 50, 100 and 200 mM NaCl) and four different levels of osmotic stress (0, 1.5, 5 and 10% PEG) treatments. After 20 days, number of survived, secondary and cotyledonary somatic embryos, as well as fresh and dry weights of embryos were evaluated. In addition, the transgenic and non-transgenic plantlets were subjected to 200 mM NaCl. In both experiments, the main effects of fld- transformation and stress treatments on evaluated parameters were significant. Transgenic somatic embryos showed no significant differences in measured parameters at 0 and 200 mM NaCl and 0 and 1.5% PEG as compared to non-transgenic ones. Significant differences in measured parameters of transgenic vs. non-transgenic somatic embryos were observed at 50 and 100 mM NaCl and 5 and 10% PEG. With medium supplemented with 200 mM NaCl non-transgenic plantlets showed complete necrosis and died after 10 days, while transgenic lines continued their growth until 45 days. Our results show clearly that over-expression of fld partially increased stress tolerance in fld transformant lines of walnut and that expression of this specific cyanobacterial protein constitute a powerful tool to improve plant fitness towards environmental adversities.

Keywords: Somatic embryo, transformation flavodoxin gene, walnut gene transformation, Salinity and osmotic stress tolerance in walnut.

PO-21

Track: Pharmaceutical Biotechnology

DEVELOPMENT AND EVALUATION OF A GENETICALLY MODIFIED MICROORGANISM FOR HUMAN EPIDERMAL GROWTH FACTOR EXPRESSION USING GATEWAY® TECHNOLOGY

Isaura Patricia Torres G, Sinar David Granada G, Ana María Aristizábal O, Juan Sebastian Herrera, Yuri Maritza Zapata, Cesar Segura L, Carlos Uribe, Orville Hernández R, Ligia Luz Corrales G, Ana María García, C, Oscar Mauricio Gómez

Department of Cellular and Molecular Biology Laboratory, Corporación para Investigaciones Biológicas (CIB), Colombia; E-mail: oscargomez24@hotmail.com



EGF is a 6.2 kDa peptide with cell proliferation activity, with cosmetic and medical applications at industrial level and recombinant microorganisms are the most cost effective way to produce it. We intend to obtain a recombinant *Escherichia coli* isolate producing hEGF using the Invitrogen™ Gateway® system.

A synthetic hEGF gene was cloned in pDEST17 and transformed into *E. coli*-BL21-AI. Characteristics of the vector and insert were confirmed by restriction analysis, PCR and sequencing. Effectiveness of hEGF induction with L-arabinose was confirmed by SDS-PAGE and Western blot. Quantification of the expression was made by Byuret and the characteristics of pure hEGF were evaluated by SDS-PAGE, Western blotting, HPLC, mass spectrometry and cell proliferation assays.

Clone 100-1 produces a 10 kD protein (hEGF-CIB-TQBL21-A1) coincident with hEGF but with an extra His-Tag sequence on inclusion bodies, corresponding to 70% of total protein and had an expression yield of 450 mg/L. SDS-PAGE and RP-HPLC showed a purity >99% of the protein. The refolded active protein showed similar biological activity compare to standard hEGF (Roche).

A productive clone, *E. coli*-BL21-AI-hEGF-clon-100-1, was obtained. The protein hEGF- CIB-TQBL21-A1 was successfully purified. All its features, including were almost indistinguishable from control hEGF (Roche), confirming the biosimilarity of CIB-TQBL21-A1.

Funding: This project is sponsored and is part of the Biotechnology initiative of Laboratory Tecnoquímicas SA.

Keywords: EGF, cloning, *E. coli*, Gateway

PO-58

Track: Industrial and Manufacturing

IMMUNOLOGICAL & RECEPTOR BINDING CHARACTERIZATION OF BEETAL RECOMBINANT CAPRINE GROWTH HORMONE

Roquyya Gul, Farkhanda Ghafoor, Sumbal Mehmood, Rabail Alam and Muhammad Waheed Akhtar

*Centre for Research in Molecular Medicine, the University of Lahore, Lahore, Pakistan;
E-mail: ruqayya.gul@imbb.uol.edu.pk*



The study aims at establishing the biological assays for recombinant growth hormone (GH) of the local Beetal breed of bovidae specie; caprine. For this purpose, a simple and highly sensitive competitive enzyme immunoassay for recombinant caprine growth hormone (rcGH) was optimized. Antiserum was raised in two local breed rabbits and horseradish peroxidase was labeled with rcGH. 96-well microtitre plates were coated with primary antibody (rabbit anti-rcGH). The rcGH standards ranging from 0-400ng/ml were prepared and standard curve was made. Assay parameters like pH, temperature, reaction time, antibody dilution and conjugate were optimized. The assay was simple to perform and was reliable. To determine the functional activity of rcGH, receptor binding assay was optimized. rcGH was iodinated and its biological activity was detected by using caprine liver receptor membrane and was dependent on receptor protein concentration, tracer counts, temperature, time of incubation and assay pH. In cross-reactive study, human GH competed and displaced the iodinated rcGH by binding effectively to the caprine receptor. Scatchard analysis of the rcGH binding suggested a single class of binding site to the caprine liver membrane with an affinity of $337.1 \pm 82.94 \times 10^9 \text{ M}^{-1}$ and capacity of $57.61 \pm 4.23 \text{ fmol mg}^{-1}$.

Keywords: Caprine, Beetal, Recombinant growth hormone, EIA, Receptor binding assay.

PO-13

Track: Pharmaceutical Biotechnology

ENGINEERING OF CYTOKINE GENE FOR CONDITIONAL ACTIVATION

Iram Gull, Muhammad Shahbaz Aslam and Muhammad Amin Athar

Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan;

E-mail: iramgull86@yahoo.com

Use of recombinant cytokines for therapeutic purposes has side effects due to the pleiotropic effect. It diminishes the efficacy of recombinant cytokines. Use of therapeutics in the form of pro-drug can eliminate the chances of non-specific binding and subsequent adverse effects. This approach ultimately improves the drug efficacy and increases the trust of consumer on therapeutic cytokines for safe use. In the present study, the gene of interferon alpha 2b has been engineered by fusion with gene of latency associated protein (LAP) of human TGF β using HCV NS3 protease cleavage site as linker for conditional activation of resultant chimeric protein only in HCV infected cells by positive use of hepatitis C virus NS3 protease activity.

Keywords: Engineering, Cytokine, Gene, Conditional activation.

PO-66

Track: Pharmaceutical Biotechnology

ROLE OF WOMEN STUDIES IN INHIBITORY EFFECTS OF GLORIOSA SUPERBA EXTRACT ON RHEUMATOID ARTERITIS

Veena Megaravalli Krishnappa Hegde and H. N. Girish

Department of Studies and Research in English, Sahyadri Arts and Commerce College, Shimoga, 577201, Karnataka, India; E-mail: veenamegaravallihegde@gmail.com



Women in many developing countries are often the protectors of biodiversity and have a developed understanding of the medicinal and nutritional uses of a plethora of rare wild and cultivated plants. Biodiversity is fundamental for the continued growth, sustainability and vitality of individuals and communities across the globe. Women, especially in developing nations, are most vulnerable to change in biodiversity and at the same time most capable of protecting and retaining biodiversity. Here is a case study on inhibitory effects of gloriosa superba extract on Rheumatoid Arthritis.

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting about 1 % of the population in developed countries. *Gloriosa superba* Linn. (family-Liliaceae) is one of the herbaceous climbers distributed throughout Western Ghats and well documented traditionally in Ayurveda system of medicine for various ailments like inflammation, gout, gonorrhea, leprosy, rheumatoid arthritis, jaundice, etc. The plant is highly valued in modern medicine owing to the presence of alkaloids. Despite its wide spread use in traditional medicine for treatment of gout and rheumatism, the present investigation was to evaluate the effect of chloroform extract of tubers of *Gloriosa superba* in Freund's complete Adjuvant (FCA) induced arthritis using albino rats. The chloroform extract of tubers of *Gloriosa superba* has shown a dose dependent and significantly decreased paw edema and ankle diameter in treated groups as compared with arthritic group. Synovial membrane damage and neutrophil infiltration in histopathological examination was restored significantly by the extract as compared to arthritic group. Eco-feminism is a movement that applies feminist principles and ideas to ecological issues such as women's rights, peace, labour, ecological and environmental justice. Moreover, the slogan "glocal", that is from global to local, which emphasizes localized cultures, economies, community based sustainable practices that need to be rejuvenated for a harmonious relation between nature, man and woman.

Keywords: Women, *Gloriosa Superba*, Rheumatoid Arthritis.

PO-16

Track: Others – Genetics

HYBRID DETECTION, DNA BARCODING AND IDENTIFICATION OF RETROTRANSPOSONS IN GENOME OF THAILAND'S NOTABLE WATERLILIES, *NYMPHAEA* SPP.

V. Hongtrakul, C. Khensri, B. Sangsri, R. Tuntipaiboonatana and P. Putanyawiwat

Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand;
E-mail: fscivph@ku.ac.th



Waterlily, known as “Queen of Aquatic Plants”, is in the Nymphaeaceae family and is well distributed all over the world in the tropics, semi-tropics and temperate areas. Its beauty arises not only from its forms but also from the colors and combination of colors. Every year, new varieties have been introduced from hybridizers all over the world. Some of the new varieties come from intersubgeneric and interspecific hybridizations. The International Waterlily and Water Gardening Society (IWGS) is a non-profit organization in USA that supports and promotes education and research in all aspects of water gardens and their associated plants. The IWGS conducts an annual competition where hybridizers display their new spectacular waterlilies. Thailand's waterlilies have won many titles from the competition, for example, *Nymphaea* ‘Mayaranee’ from *N. gigantea* (blue) x *N. ‘A-trans’* won 2013 IWGS 1st place Anecphyra waterlily and *N. ‘Siam Purple 1’* from *N. ‘Supranee Pink’* x *N. ‘Nangkwag Fah’* won 2011 IWGS best new waterlily and 1st place intersubgeneric waterlily. Genetic information of waterlily is very limited. No information on the hybrid confirmation at molecular level has been reported. The objectives of this study were to do the hybridity test and generate DNA barcodes for specific identification of Thailand's native and notable waterlilies. The DNA markers used for hybrid detection included the developed microsatellite markers, SSCP (Single Strand Conformational Polymorphism) gene specific markers developed to span intron of specific genes and parental specific markers developed specific to parental DNA sequence individually. DNA barcodes were generated by PCR amplification and sequencing at the DNA regions of *rbcl*, *matK*, *trnH-psbA* intergenic spacer (plastid-encoded) and ITS of *rDNA* (nuclear-encoded). Retrotransposons were identified using *RT* (Reverse transcriptase) specific primers in order to understand waterlily genome. The information obtained will be useful for waterlily improvement project in the future.

Keywords: Waterlily, *Nymphaea*, Hybrid detection, DNA markers, DNA barcodes, Retrotransposons.

PO-6

Track: Plant and Environment

EMULSIFICATION AND DIESEL OIL DEGRADATION ABILITY OF ACINETOBACTER HAEMOLYTICUS ZN01 ISOLATED FROM PETROLEUM HYDROCARBON CONTAMINATED RIVER WATERS

Bulent Içgen, Gozde Onur and Fadime Yilmaz

Department of Environmental Engineering, Middle East Technical University, Turkey;
E-mail: bicgen@metu.edu.tr



Degradation of poorly water soluble hydrocarbons, like n-alkanes and polycyclic aromatic hydrocarbons are challenged by some bacteria through emulsification of hydrocarbons by bacterial bio surfactants. In diesel oil bioremediation, diesel oil degrading and surfactant producing bacteria are used to eliminate these pollutants from contaminated waters. Therefore, identifying and characterizing bacteria capable of producing surfactant and degrading diesel oil are pivotal. In this study, bacteria isolated from hydrocarbon contaminated river water were screened for their potential to degrade diesel oil. Primary selection was carried out by using conventional enrichment culture technique, emulsification index measurement, gravimetric and gas chromatographic (GC) analyses of diesel oil degradation. A bacterium with 60% emulsification index and 92% diesel oil degradation ability in 14 days was identified as *Acinetobacter haemolyticus* Zn01 by 16S rRNA sequencing. *A. haemolyticus* Zn01 was shown to harbor both catabolic genes *alkB* and *C23O* effective in diesel oil degradation. The bio surfactant of the bacterium was also characterized in terms of surface tension, zeta potential,

fourier transform infrared spectroscopy and scanning electron microscopy. Being able to degrade diesel oil with a high emulsifying index *A. haemolyticus* Zn01 seems to have high potential for the elimination of diesel oil from polluted waters.

Keywords: *Acinetobacter*, petroleum bioremediation, hydrocarbons, E24, alkb, C23O.

PO-19

Track: Plant and Environment

TRANSCRIPTOME ANALYSIS OF WHEAT GLUME UNDER DROUGHT STRESS

Changxin Liu and Jinyin Lyu

College of Life Sciences, Northwest A&F University, China; E-mail: Jinyinlv@nwsuaf.edu.cn

The non-leaf organs of wheat performed higher tolerance than leaf in the adverse environment. To investigate the drought tolerance in wheat spike, we investigated variations of gene expression in the early filing period at 6d post anthesis (DPA) by using Affymetrix Wheat Genome Array. 30,768 probe sets of total 61,703 ones were identified of wheat glumes at the mRNA level. 256 differentially expressed probe sets were identified. A total of 229 probe sets were up-regulated ($FC \geq 2.0$) and 27 were down-regulated ($FC \leq 0.5$) under drought stresses. Most up-regulated genes were involved in functions such as signal transduction, metabolism and transcription. Among the up-regulated genes, we identified signaling proteins, transcription factors and abiotic stress-related genes. The signal pathway networks constructed with KEGG showed three important genes involved in the phenylalanine metabolism, in which phenylalanine ammonia-lyase, cinnamic acid hydroxylase, chalcone synthase and chalcone isomerase located at the center of the pathway, which indicated their pivotal roles in the phenylalanine metabolism and flavonoid biosynthesis derived from carbohydrate metabolism. Real-time PCR analysis confirmed that 10 genes were up-regulated and down-regulated at 0, 6, 12, 18, 24 DPA. Most up-regulated genes were involved significantly regulated by drought stresses were of unknown function; our results provide new insights into the molecular mechanism of the wheat spike responses against drought stress.

Keywords: Wheat glume, drought stress, microarray, qRT-PCR.



PO-17

Track: Medical Biotechnology

NEW THERAPEUTIC POTENTIAL OF LOW-DOSE FARNESYLTRANSFERASE INHIBITORS IN ATHEROSCLEROSIS, SEVERE INFECTION, MAJOR TRAUMA, FULMINANT HEPATITIS AND OBESITY

Masao Kaneki

Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, USA; E-mail: mkaneki@helix.mgh.harvard.edu

Protein farnesylation is a lipid modification of the cysteine residues in the CAAX motif located in the carboxyl terminus of proteins ("C" is cysteine, "A" is aliphatic amino acid, and "X" is any amino acid at the carboxyl terminus, but usually serine, methionine, glutamine, or alanine). Farnesyltransferase (FTase) inhibitors (FTIs) have been developed as anti-cancer/leukemia drugs and clinical trials have been conducted in patients with cancer or hematologic malignancies and in progeria syndrome. It has been proposed that inhibition of protein farnesylation is a mediator of the cholesterol-lowering-independent beneficial effects of statins, inhibitors of HMG-CoA reductase, albeit direct evidence is lacking. We have shown that low-dose FTIs effectively prevents atherosclerosis development in apoE-deficient mice on high-fat diet without decreasing circulating cholesterol levels, improves survival in mouse models of sepsis and fulminant hepatitis, and reverses insulin resistance and metabolic aberration after burn injury in mice. Of note, FTIs exerts anti-inflammatory effects while concomitantly increasing bactericidal activities of immune cells, indicative that FTIs are not a simple anti-inflammatory agent. Moreover, our recent study showed that low-dose

FTI inhibited high-fat high-sucrose diet-induced obesity and diabetes in mice, whereas FTI did not decrease body weight in mice on normal chow. The classical targets of FTIs are Ras and Rheb in cancer and leukemia and lamin A in progeria syndrome. These proteins are constitutively farnesylated and farnesylation is the first step of protein maturation that is followed by further processing (such as methylation and cleavage at the site of farnesylated cysteines). In contrast, we have found that there exists inducible farnesylation where proteins get farnesylated under certain conditions such as inflammation, whereas those proteins are not farnesylated or farnesylated to a small extent under normal conditions. Collectively, our data identify inducible farnesylation as a novel molecular target to prevent and/or treat a broad array of human diseases where inflammatory response plays a critical role.

Keywords: Therapeutic agent, inflammation, farnesylation inhibitor

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PO-57

Track: Plant and Environment

POPLAR *GIGANTEA* REGULATE CROSS-TALK BETWEEN FLOWERING TIME AND SALT RESPONSE

Qingbo Ke, Zhi Wang, Ho Soo Kim, Seyeon Park, Jae Cheol Jeong, Haeng-Soon Lee and Sang-Soo Kwak

Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology, 125 Gwahak-ro, Daejeon 305-806, Korea; E-mail: keqingbo@kribb.re.kr



The timing of floral transition determines successful reproduction in higher plants and is precisely monitored by multiple environmental cues. The *GIGANTEA* (*GI*) gene participates in the network connecting environmental stress and developmental stage transition in *Arabidopsis*. We first identified *GI* gene orthologous from the perennial growth poplar (*Populus alba* × *P. glandulosa*) as *PagGla*, *PagGlb* and *PagGlc*. *PagGIs* are predominantly localized at nuclear and its transcripts are diurnally regulated with a peak around zeitgeber time 12 under long-day conditions. Ectopic over-expression of the *PagGIs* in wild-type *Arabidopsis* plants resulted in early flowering and enhanced salt sensitivity; while *gi-2* mutant *Arabidopsis* plants expressing *PagGIs* are rescued from delayed flowering and enhanced salt tolerance. Furthermore, *PagGIs* negatively regulate the salinity stress tolerance by interacting with *PagSOS2* in poplar. In addition, transgenic poplars with down-regulation of *PagGIs* by RNAi display enhanced salt tolerance. Taken together, our results reveal new insights on the link between flowering time regulator and adaptation to salt stress in poplar.

Keywords: Poplar, *GIGANTEA*, flowering, salt stress, RNAi.

VP-01

Track: Other areas

ENCAPSULATION-DEHYDRATION TECHNIQUE EXPLORATION FOR CHRYSANTHEMUM 'LADY SALMON' CRYOPRESERVATION

Dariusz Kulus, Monika Rewers, Agnieszka Abratowska and Anna Mikula

University of Technology and Life Sciences in Bydgoszcz, Faculty of Agriculture and Biotechnology, Department of Ornamental Plants and Vegetable Crops, Laboratory of Biotechnology, Bydgoszcz, Poland; E-mail: dkulus@gmail.com



Chrysanthemum is among the most popular species on the ornamental plants market. The number of available cultivars is constantly growing and they constitute a great breeding material source. Furthermore, the species constitute a valuable source of numerous secondary metabolites useful in medicine (*e.g.* in cancer or AIDS treatment). A fast and easy access to high quality gene banks of a great material variety is the key for ornamental plant producers, as well as for pharmaceutical laboratories and so an efficient method for long-time conservation of the plant material may be extremely valuable for both horticultural production and medicine development. Over time several medium- and long-term plants storage methods have been developed. Traditional storage (cultivation in the ground or in the glasshouse), however, is expensive, work-intensive and threatened with loss due to pests and diseases or bad climate conditions. *In vitro* storage, on the other hand, may lead to the occurrence of somaclonal variation, decrease in metabolic/embryogenic activities and material loss due to human errors and development of bacterial/fungal contaminations. Today, cryopreservation (*i.e.* maintenance of biological material at the temperature of liquid nitrogen; -196°C in Dewar flasks), developing rapidly for the last 25 years, is believed to be the most effective long-term storage method. Usually cryopreservation does not influence on the characteristics of the plant material. Still, there are some reports about (epi)genetic disturbance after storage of chrysanthemum in liquid nitrogen, especially with chimeras, which are very popular among the species (about 50% of all available cultivars). These reports emphasize the need to monitor the stability of samples stored in liquid nitrogen by using different markers (molecular, phenotypical and/or biochemical).

The aim of this study was to determine the effect of sucrose concentration during preculture and the time of osmotic-dehydration on the efficiency of chrysanthemum 'Lady Salmon' shoot tips cryopreservation by encapsulation-dehydration. In addition, the regenerated plants were verified at the phenotypic, biochemical and molecular levels. Shoot tips were precultured on MS medium supplemented with different sucrose concentrations of 0.09, 0.25 or 0.5 M for 14 days, encapsulated in sodium alginate and then osmotically dehydrated in sucrose gradient for 4 or 7 days. The best explant survival after cryopreservation reaching about 50% was obtained with the lowest (0.09M) sucrose concentration, and 4-day-long osmotic dehydration. It was found that higher sucrose concentrations slow down shoot growth, stimulate their vitrification and conduce to the regeneration *via* callus, while encapsulation inhibits rooting. Longer dehydration also led to increased formation of multiple shoots. Microscopic analyzes confirmed the protection of not only all of the initial meristem layers, but also of the leaf primordia and even larger leaves. Transmission electron microscopy (TEM) revealed accumulation of starch as a sole alternation observed in the cryopreserved cells. The analysis of the phenotype (inflorescences and leaf colour, diameter and weight, flowering time and plant habit) and biochemical activity (pigment content in ligulate flowers and leaves), as well as, cytogenetic analysis (DNA content, the number of chromosomes) and genetic markers (RAPD and ISSR) confirmed the stability of the plants obtained after liquid nitrogen treatment. However, it was noted, that the leaves of shoot tips cryopreserved-derived plants were smaller and had a reduced amount of chlorophyll, and their internodes were shorter when compared to the control. Furthermore, their inflorescences often opened slower. Finally, these phenotypic changes are positive from the horticultural production point of view. This confirms the validity of utilizing cryopreservation in the protection of valuable plant material.

PO-73*Track: Plant and Environment***LENTINULA EDODES LACCASES: RECOMBINANT ENZYME PRODUCTION AND APPLICATION IN SOFTWOOD HYDROLYSIS****Hoi-Shan Kwan, Kin-Sing Wong, Valdeir Arantes and Jack N. Saddler***School of Life Sciences, the Chinese University of Hong Kong, Shatin, Hong Kong;
E-mail: hoishankwan@cuhk.edu.hk*

Laccases from basidiomycetes, and their laccase-mediator systems (LMSs), are versatile biocatalysts for “green” applications. We determined the genome sequence of Shiitake mushroom *Lentinula edodes* and clone and express recombinant laccases in *Pichia pastoris*. We purified and characterised two allelic recombinant laccases, multi-copper oxidases that catalyse the oxidation of phenolic compounds using molecular oxygen as oxidant. With mediators, substrate specificity of laccases relaxes can oxidize substrates with high redox potential. The laccase Lcc1A and its LMSs were the most efficient in biodegradation of synthetic dyes and polyaromatic hydrocarbons, and could improve the enzymatic saccharification of steam-pretreated softwoods by 37% to 46%. The laccases can be used as “accessory” enzymes, when added to a typical cellulase enzyme mixture, enhanced the hydrolysis of cellulose in pretreated softwoods. We tested the supplementation of the recombinant laccase and mediators to a cellulase cocktail to hydrolyze steam pretreated lignocellulosic substrates. The optimised LMS enhanced the hydrolysis of pretreated softwoods but not agricultural residues or hardwoods. The enzyme-mediator mixtures were further tested to hydrolyze different steam pretreated softwoods (to obtain $\geq 70\%$ hydrolysis). We demonstrated the “green” applications of recombinant *L. edodes* laccases and provided new enzyme recipes for efficient bioconversion of softwoods.

PO-45*Track: Pharmaceutical Biotechnology***IN VIVO ANTI-HYPERGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF THE LEAF EXTRACTS OF HOLARRHENA ANTIDYSENTERICA ON ALLOXAN-INDUCED DIABETIC RATS****Latha K.P and Shwetha C***Department of P.G. Studies and Research in Chemistry, Sahyadri Science College, Nandeshwara Nilaya, 2 Main, 2 Cross, Chanappa Layout, Shimoga, Karnataka, India; E-mail: latha119@gmail.com*

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. The present study was undertaken to evaluate the anti-hyperglycemic activity of the crude extracts of leaves of *Holarrhena antidysenterica*. The pet ether, chloroform and ethanolic extracts have been subjected to estimate the anti-hyperglycemic activity in alloxan-induced diabetic rats. Blood glucose levels were measured using the commercially available glucometer. Glibenclamide was used as a reference drug at a dose of 0.6 mg/kg. The antioxidant activity of the extracts has been studied in the liver tissue of diabetic rats by measuring catalase and lipid peroxidation levels. The results showed that, ethanolic extract possessed a significant anti-hyperglycemic and antioxidant activity, which is equipotent with the reference drug.

Keywords: *Holarrhena antidysenterica*, anti-hyperglycemic, antioxidant, lipid peroxidation.

PO-55

Track: Industrial and Manufacturing

MORPHOLOGY ENGINEERING OF BASIDIOMYCETES FOR IMPROVED LACCASE BIOSYNTHESIS

Anna Antecka, Michał Błatkiewicz, Marcin Bizukojć and Stanisław Ledakowicz

Department of Bioprocess Engineering, Faculty of Process and Environmental Engineering, Lodz University of Technology, 213 Wolczanska Street, 90-924 Lodz, Poland; E-mail: stanleda@p.lodz.pl



It is well known that productivity in biotechnological processes is often correlated with morphological form of fungi in particular in submerged cultivation. Filamentous organisms such as *Aspergillus niger* can grow either in dense, spherical pellets or as disperse and viscous mycelia. Freely dispersed mycelium seems favorable and regarded as prerequisite to ensure high productivity of enzymes like amylase phytase *etc.*, or production of penicillin. The desired morphological characteristics can be achieved by variation of operating parameters. In pioneering study the use of inorganic microparticles (such as aluminium oxide or magnesium silicate) added to the culture was recently introduced to influence morphology of filamentous fungi. It was observed that intentional supplementation to the culture might generally stimulate growth of these organisms as well as increase biosynthesis of selected enzymes. Following this suggestion we attempted to apply this morphology engineering method to Basidiomycetous white rot fungi such as *Cerrena unicolor* and *Pleurotus ostreatus*, which are well known producers of very robust enzyme laccase. Laccases (EC 1.10.3.2) are copper containing oxidases that catalyze reduction of O_2 to H_2O using variety of phenolic substrates as hydrogen donors. Laccases are used in paper, textile bioremediation and other industries.

The influence of aluminium oxide particles (diameter 10 μm) on laccase biosynthesis and fungal morphology was studied in the shaken culture medium. The results show the positive effect of the microparticles at all tested concentrations of aluminium oxide in the range 0-30 g/L. Concentration of 15 g/l was selected as the optimal one because of highest laccase activity and complete incorporation of aluminium oxide into biomass. The higher concentration of aluminium oxide caused the decrease in pellets size and their surfaces got more hairy up to transformation into dispersed morphology at concentration of 30 g/L. The laccase activity increased up to 3.5 times at the Al_2O_3 concentration of 15g/L, compared to non-supplemented culture of modified Lindeberg & Holm's medium. Further increasing the concentration of aluminium oxide up to 30 g/L did influenced the morphology of fungi decreasing the size of fungi pellets, however the laccase activity was not influenced much more. Therefore the concentration 15 g Al_2O_3 /L was chosen as the optimal one for the laccase production in the submerged cultivation.

Keywords: Laccase biosynthesis, fungal morphology, microparticle

PO-56

Track: Industrial and Manufacturing

BIOCHEMICAL METHANE POTENTIAL OF MAIZE HYBRIDS

Ledakowicz S., Pazera A. and Krzystek L.

Department of Bioprocess Engineering, Faculty of Process and Environmental Engineering, Lodz University of Technology, 213 Wolczanska Street, 90-924 Lodz, Poland; E-mail: stanleda@p.lodz.pl



When the world in XXI century faces problems with growing energy consumption and reducing fossil fuels it is essential to develop suitable cultivars of crops being renewable sources in order to obtain maximum yield of methane production per hectare, all the more so because in recent years more and more attention has been paid to the usefulness of maize for industrial purposes.

The aim of this paper was to perform Biochemical Methane potential (BMP) analysis of 33 hybrids of maize in the form of silage in order to select the best cultivars for the biogas production and to determine the efficiency of biogas

production per hectare. The results showed that it is important to evaluate the usefulness of energy crops for biogas production in order to obtain the best yields. Significant differences both in the efficiency of biogas production of various hybrids of maize and in methane content were observed, which ranged from 7087 to 15021 m³h⁻¹ of biogas and 41-55% of methane. The most efficient for the production of biogas proved to be hybrids of maturity class FAO 240-250 and 260, all trilinear ones, which showed an average level of green mass yield and dry matter content, approx. 836-860 dt/ha and 270 dt/ha, respectively.

PO-81

Track: Plant and Environment

IMPROVEMENT OF THE ANAEROBIC DIGESTION EFFICIENCY OF WASTE ACTIVATED SLUDGE THROUGH CHEMICALLY-ASSISTED MICROWAVE IRRADIATION

Jaeho Lee, Jeongmin Lee, Sanghoon Lee, Hyanggi Kim, Youngwoo Kim, Taejoo Park and Imgyu Byun

*Department of Civil and Environmental Engineering, Pusan National University, South Korea;
E-mail: jhlee6119@hanmail.net*

Microwave (MW) is a promising method for the solubilization of waste activated sludge (WAS) due to the accelerated reaction rates, environmental friendliness and low overall cost compared to conventional heating. In this study, several chemical agents, including H₂SO₄, NaCl and food wastewater, were applied to enhance the MW effect on the solubilization of WAS. NaCl had a positive effect on WAS solubilization compared to that of the MW only-assisted pretreatment by 30.1%. Therefore, food wastewater was used to enhance the MW efficiency in WAS solubilization owing to its high NaCl content. Food wastewater also improved the WAS solubilization efficiency by 60.3%. In the experiment of H₂SO₄-assisted MW pretreatment, the WAS solubilization efficiency was improved by 50.6% compared to that by MW-only assisted pretreatment. After the WAS pretreatment by microwaves with the chemicals, a biochemical methane production (BMP) test was conducted. In these experiments, the amount of biogas produced from WAS pretreated with NaCl, food wastewater and H₂SO₄ assisted microwaves was 11.3, 31.6 and 29.7% higher, respectively, than that of the non-pretreated WAS. Therefore, the most appropriate chemical is food wastewater because of solubilization efficiency as well as waste reuse, cost and environmental friendliness.

Keywords: Anaerobic digestion, Solubilization, Microwave, Pretreatment, Waste activated sludge

PO-25

Track: Medical Biotechnology

THE C-TERMINUS OF CORE B-LADDER DOMAIN IN FLAVIVIRUS NONSTRUCTURAL PROTEIN 1 IS FLEXIBLE FOR ACCOMMODATION OF A HETEROLOGOUS EPITOPE FUSION

Li-Chen Yen, Yi-Ling Lin, Hwei-Jen Lee, Jia-Teh Liao and Ching-Len Liao

National Institute of Infectious Diseases and Vaccinology, Taiwan; E-mail: chinglen@gmail.com

The NS1 is the only nonstructural protein that enters the lumen of ER, where NS1 is glycosylated, forms dimer, and subsequently is secreted during flavivirus replication as hexamers, which appear to be highly antigenic to the infected host as protective immunity can be elicited against homologous flavivirus infections. Here, by using trans-complementation assay, we identified the C-terminal of NS1 derived from Japanese encephalitis virus (JEV) that was more plastic than other regions for housing foreign epitopes without significant impact on virus replication. Such mapped flexible region is located in the conserved tip of core β-ladder domain of multimeric NS1 crystal structure also known to reside certain linear epitopes readily triggering specific antibody responses from



the host. Despite becoming attenuated, recombinant JEV with insertion of neutralizing epitope derived from Enterovirus-71 (EV71) into the C-terminal of NS1 could not only be normally released from the infected cells but also induce dual protective immunity for the host to counteract the lethal challenge of either JEV or EV71 in neonatal mice. These results indicate that the secreted multimeric NS1 of flaviviruses may serve as a nature protein carrier to make epitopes of interest more immunogenic in its C-terminus of core β -ladder domain.

Keywords: Flaviviral NS1 fusion proteins, β -ladder domain, dimer, hexamer, JEV/EV-71 recombinants.

PO-14

Track: Plant and Environment

LONG TERM EVALUATION OF ALGAE OCCURRENCE IN DOWNSTREAM THE NAKDONG RIVER, SOUTH KOREA: 1998~2014

Ji- Sung Lim, Jae-Ho Lee, Young-Woo Kim, Sang-Hoon Lee, Hyang-Gi Kim, Tae-Joo Park and Im-Gyu Byun

*Civil and Environmental Engineering, Pusan National University, South Korea;
E-mail: js1330@hanmail.net*

Nakdong River is one of the major rivers in South Korea. Mulgeum site, which is downstream of Nakdong River, is the intake station of Busan metropolitan city. This study examined the chlorophyll-a(chl-a) concentration as a representative parameter of the occurrence of algae at the Mulgeum site from 1998 to 2014. The water quality, hydraulics and climate conditions were used to determine the parameters significantly affecting the chl-a concentration. The correlations between chl-a and the other parameters were analyzed by the Pearson coefficient. As a result, the chl-a concentration showed clear patterns according to the seasonal changes. High chl-a concentrations by *Stephanodiscus* spp., which is the dominant species in low water temperature at Mulgeum site, were observed in winter. On the other hand, low chl-a concentrations were observed in summer. The Monsoon climate characteristics have a significant effect in summer. Correlation analysis also showed different significant parameters to seasonal changes. In winter, pH, DO, BOD, COD, and PO₄-P showed a significant correlation with the chl-a concentration.

Keywords: Nakdong River, Algae, Correlation, Water quality, Hydraulics, Climate, Dominant species, Seasonal change.



PO-50

Track: Medical Biotechnology

NORMAL TISSUE COMPLICATION PROBABILITY MODELING FOR COCHLEA CONSTRAINTS TO AVOID CAUSING TINNITUS AFTER HEAD-AND-NECK INTENSITY-MODULATED RADIATION THERAPY

Ming-Hsiang Liou, Tsair-Fwu Lee, Shyh-An Yeh, Pei-Ju Chao, Hui-Min Ting, Yu-Jie Huang, Hsiao-Yi Lee

*Division of Radiation Oncology, Yuan's General Hospital, Taiwan;
E-mail: minghsiang.liou@gmail.com*

Purpose: To determine the best-fit parameters for the logistic and the Lyman-Kutcher-Burman (LKB) normal tissue complication probability (NTCP) models for optimizing cochlea constraints to avoid causing tinnitus after head-and-neck cancer (HNC) intensity-modulated radiation therapy (IMRT).



Methods and Materials: A total of 422 ears from 211 patients with HNC were included in the study. The analytical endpoint was defined as grade 2+ tinnitus that occurred within 1 year of IMRT. The logistic and LKB NTCP models were used to assess the incidence of grade 2+ tinnitus. The best-fit values for TD_{50} were determined using maximum-likelihood estimates. A guideline of TD_{25} is recommended for the tolerance dose to produce a 25% complication rate within a specific period of time.

Results: Forty-five of the four hundred and twenty-two samples (10.7%) developed grade 2+ tinnitus symptoms after IMRT, as diagnosed by a clinician. The NTCP-fitted parameters were $TD_{50} = 37.35$ Gy (CI, 36.50-38.20), $\gamma_{50} = 1.60$ (CI, 1.58-1.62), and $TD_{50} = 35.92$ Gy (CI, 35.07-36.77), $m = 0.20$ (CI, 0.18-0.22) for the logistic and LKB models, respectively.

Conclusion: To maintain the incidence of grade 2+ tinnitus toxicity <20%, we suggest that the mean dose to the cochlea should be <29 Gy.

Keywords: Tinnitus, NTCP, cochlea, head and neck, constraints.

PO-49

Track: Medical Biotechnology

NORMAL TISSUE COMPLICATION PROBABILITY MODELING FOR CONTROLLING THE PERCENTAGE OF IPSILATERAL LUNG IRRADIATED-VOLUME TO AVOID CAUSING SYMPTOMATIC RADIATION PNEUMONITIS FOR BREAST CANCER PATIENTS

Ming-Hsiang Liou, Tsair-Fwu Lee, Pei-Ju Chao, Hsiao-Yi Lee and Yu-Jie Huang

Division of Radiation Oncology, Yuan's General Hospital, Taiwan;

E-mail: minghsiang.liou@gmail.com



Purpose: To investigate the incidence of symptomatic radiation pneumonitis (SRP) in breast cancer patients after intensity modulated radiotherapy (IMRT)/hybrid IMRT.

Methods: In total, 120 patients with breast cancer were analyzed. All participants were treated with IMRT or hybrid IMRT. The final endpoint was defined as those who with symptomatic pneumonitis combined with computerized tomography (CT) measured density changes grade ≥ 1 . The risk factors for a multivariate logistic regression normal tissue complication probability (NTCP) model of SRP were determined using the least absolute shrinkage and selection operator (LASSO) technique.

Results: Three risk factors were selected using LASSO: the percentage of the ipsilateral lung volume that received more than 20-Gy (IV20), age, and body mass index (BMI). Our analyses indicate that the risk of SPR following IMRT/hybrid IMRT in elderly or low-BMI breast cancer patients increases when the percentage of the ipsilateral lung volume receiving more than 20-Gy is controlled below 30%.

Conclusions: We suggest to define a dose-volume percentage constraint of IV20Gy < 30% for the irradiated ipsilateral lung in radiation therapy treatment planning to maintain the incidence of SPR below 10%, and pay attention to the sequelae especially in elderly or low-BMI breast cancer patients.

Key words: Symptomatic radiation pneumonitis, LASSO, NTCP, multivariable logistic regression.

PO-15

Track: Industrial and Manufacturing

RHODOCOCCUS ERYTHROPOLIS NTU-1: FROM BIO-DEGRADATION TO BIO-FLOCCULATION AND BIO-DEMULSIFICATION

Hwai-Shen Liu

*Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan 106;
E-mail: hsliau@ntu.edu.tw*



An alkane-biodegrading bacterium identified as *Rhodococcus erythropolis* (NTU-1 strain) was isolated from petroleum-contaminated soil. When long-chain alkanes are supplied as the carbon source, NTU-1 tends to form pellets, ranging from 0.1 to 2 cm in diameter, and thus remove significant amount of alkanes by biodegradation and physical trapping in a short period. Quantitatively, more than 95% of each alkane (~2000ppmv) could be efficiently removed within 40–68 h. Further, *Rhodococcus erythropolis* strain NTU-1 was also confirmed to be able to degrade C10–C32 n-alkanes in diesel oil or crude oil. While degrading these n-alkanes, NTU-1 also trapped most other non-degradable oil constituents in pellets. In batch cultures with 10,000ppmv diesel or crude oil, approximately 90% oil removal was achieved within 4 days. In bioreactors with aeration and pH adjustment, an intermittent feed of 42,000ppmv n-hexadecane resulted in approximately 87% removal within 4 weeks and an intermittent feed of 35,000ppmv diesel or crude oil resulted in more than 90% removal within 2 weeks.

It was also found that, if NTU-1 was properly cultured and heat-dried, the cells could work as bio-flocculating agent that could shorten the clean up time to 12h, as compared to 40-68h. Meanwhile, the cells further showed good capability to de-emulsify oil/water/surfactant emulsion system and may play a role in separate oil from tertiary recovery in petroleum industry.

These interesting and versatile phenomena of *Rhodococcus erythropolis* NTU-1 involving bio-degradation, bio-flocculation and bio-demulsification will be presented and discussed.

PO-1

Track: Plant & Environment

REMOVAL OF NITROGEN AND PHOSPHORUS FROM WASTEWATER IN AN OSMOTIC MEMBRANE PHOTOBIOREACTOR USING MICROALGAE

Prashant Praveen and Kai-Chee Loh

*Department of Chemical & Biomolecular Engineering, National University of Singapore, Singapore;
E-mail: chelohkc@nus.edu.sg*



Microalgae are one of the most promising and sustainable feedstock for the production of biofuels because of their high growth rate, efficient CO₂ fixation and high lipid content. However, despite making significant progress, production of biodiesel from microalgae remains an expensive process. One approach to reduce the cost of microalgae cultivation is the use of urban wastewater for microalgae growth. Wastewater can not only be a cheap and readily available source of water, it can also provide fertilizers for microalgae growth.

Another challenge in microalgae cultivation is the low biomass concentration, which increases the harvesting costs. This can be alleviated by cultivating the microalgae in a osmotic membrane photobioreactor (OMPBR). While the OMPBR will allow continuous supply of wastewater to the microalgae without any constraint regarding the hydraulic retention time, the microalgal biomass will be completely retained in the bioreactor, resulting in high biomass concentration.

In this research, an OMPBR was designed and operated for continuous cultivation of microalgae in tertiary wastewater containing NH₄⁺, PO₃-4 and NO₃⁻. Sparging the bioreactor with 5% CO₂-enriched air, and in the presence of light at an intensity of 200 μmol photo/m²-s, the microalgae exhibited exponential growth rate and the biomass concentration increased from 21 mg/L to 190 mg/L in 22 days. The removal efficiency of NH₄⁺-N, PO₃-4-P and NO₃⁻-N were 85.9%, 99.5% and

96.6%, respectively. The salt concentration profiles in the bioreactor and in the effluent were different, suggesting that membrane rejection of the pollutants played a key role in improving the effluent quality. The permeate flux through the membranes remained stable, indicating that membrane biofouling was negligible during the operating period. These results suggest that the use of OMPBR can be quite promising in tertiary wastewater treatment and in microalgae cultivation.

PO-12

Track: Plant and Environment

DESTABILIZING INFLUENCE OF MICROORGANISMS ON EFFICIENCY OF IMMOBILIZATION OF ARSENIC BY BOG IRON ORES

Lukasz Drewniak, Katarzyna Kucharek and Pola Lomza

Department of Biology, University of Warsaw, Poland; E-mail: pola.lomza@gmail.com

Arsenic is a natural, toxic and carcinogenic metalloid. Occurrence of arsenic contamination in drinking water is the cause of many serious diseases around the world. Various physico-chemical methods were developed and used to treat contaminated waters. The materials dedicated for arsenic removal are dominated by low cost sorbents, such as bog iron ores, dead biomass or alginate. Among them bog iron ores seem to be the most effective adsorbent since have high potential in bonding both arsenite and arsenate. The main limitation of their use may be susceptibility to microbial dissolution, since organic matter from the deposit can be used as a source of energy by the bacteria.

The main aim of this study was assessment of stability of bog iron ores saturated with arsenic under influence of activity of microorganisms occur in contaminated waters. As source of microorganisms we used surface water from the Trujaca Stream in Zloty Stok in Poland. The following physiological group of bacteria were present in the waters: nitrifying, denitrifying, arsenate-reducing, arsenite-oxidizing, sulfate-reducing, sulfite-oxidizing and aerobic heterotrophic bacteria. Growth analysis showed that indigenous bacteria can directly and indirectly promote the mobilization of arsenic adsorbed on bog iron ores.

Keywords: Bog iron ores, sorbents, arsenic, bacteria.



PO-22

Track: Other areas

MAQUI (ARISTOTELIA CHILENSIS) AMELIORATES ENDOTHELIAL DYSFUNCTION IN RATS FED WITH HIGH FAT/CHOLESTEROL DIET.

Oscar Fuentes Mardones, Susana Badilla Wagner and Carlos Céspedes Acuña

University of Biobio, Chile; E-mail: ofuentes@ubiobio.cl

Maqui (*Aristotelia chilensis*) is a natural berry from Patagonia region of South America. Due to its huge content of antioxidants it is called as the super-berry. Mapuche ethnica consume maqui to treat health complaints such as skin inflammation, intestinal disorders, sore throats, wound healing and ulcers. The present study evaluated the beneficial effect of hydroalcoholic extract of maqui against vascular reactivity impairment, hyperglycemia and dyslipidemia on high fat fed rats (HFFR). Forty female outbred Wistar rats were fed with a high-fat diet for 16 weeks to induce obesity. Half of them received maqui supplementation (60 mg•Kg⁻¹/day) during the final 8 weeks. Aorta rings of HFFR displayed attenuated vasorelaxation responses to the endothelium-dependent vasodilator acetylcholine (ACh), and reduced nitric oxide (NO) bioavailability. Removal of the endothelium and addition of nitric oxide synthase (NOS) inhibitor NG-methyl-L-arginine (L-NMA) increased the phenylephrine response in obese rats. Sensitivity to sodium nitroprusside (SNP) did not differ between tested groups. Maqui extract markedly ameliorated hyperreactivity to phenylephrine, reduced impairments of acetylcholine (ACh)-induced relaxation of aorta and decreased plasma levels of glucose, cholesterol, LDL, triglycerides



and accumulation of visceral and subcutaneous fat. The results suggest that maqui extract has remarkable potential to prevent obesity and associated metabolic disorders

Keywords: *Aristotelia chilensis*, obesity, endothelial dysfunction.

PO-53

Track: *Plant and Environment*

REAL TIME PCR TO ELUCIDATE ENDOPHYTIC STAGE OF RAMULARIA COLLO-CYGNI IN BARLEY SEED

Pavel Matusinsky, Leona Svobodova-Leisova, Pavel Marik and Ludvik Tvaruzek

Agrotest fyto, Ltd., Havlickova 2787, Kromeriz, Czech Republic; E-mail: matusinsky@vukrom.cz

Ramularia collo-cygni (Sutton & Waller) (RCC) is a fungal pathogen infesting spring and winter barley (*Hordeum vulgare* L.) and causes the disease known as Ramularia leaf spot (RLS) manifested by necrotic spots on leaves. The symptoms usually appear during late growth phases of the host plants. When severe, the disease progresses by very rapid development of spottiness. In addition to reducing yields, it also decreases grain quality. A real-time PCR assay was designed to quantify RCC in barley tissues. PCR primers and a TaqMan probe were designed to target an RCC-specific DNA sequence. The method was optimized for pure fungal DNA and plasmid standard dilutions. Kernels were dissected into lemma, pericarp, testa, endosperm and embryo, and a suspension was acquired after washing the kernels. All these parts were tested for amounts of RCC, and the presence of other potential seed-borne pathogens was analyzed by standard PCR diagnostics. The highest level of Ramularia DNA was in the lemma, and it occurred in lower amounts in the pericarp and embryo. Ramularia DNA was also evidenced in the suspension after washing the kernels. Our findings indicate that DNA of RCC is present on the emerging leaves of plants sown from infected seed. This confirms that RCC can be seed-borne. DNA of RCC is detectable by molecular methods also in cases when visible symptoms are absent. DNA content in leaves need not necessarily correspond to the intensity of RLS symptoms. The work was supported by the Ministry of Agriculture of the Czech Republic, Projects QJ1310091, QJ1310226, QJ1210008 and RO0211.

Keywords: Ramularia leaf spot, molecular diagnostics, cereal diseases.



PO-10

Track: *Regenerative Medicine*

SYNAPSE FORMATION IN EMBRYONIC NEURAL STEM CELLS TRANSPLANTED INTO ISCHEMICALLY INJURED MOUSE BRAIN

Nina Kosi, Ivan Alić, Srećko Gajović and Dinko Mitrečić

Laboratory for Stem Cells, Croatian Institute for Brain Research, University of Zagreb, Croatia; E-mail: dinko.mitrečić@mef.hr

To reach a satisfactory level of survival and healing properties, it is hypothesized that transplanted neural stem cells (NSCs) need to enter the process of formation of synaptic contacts with both host neurons and in-between themselves. Thus we induced ischemic brain injury by middle cerebral artery occlusion in C57/Bl6 mice, labeled cells with a fluorescent dye and stereotactically injected them 5 days after injury at the dorsal cortico-callosal boundary. Animals were sacrificed at 2, 4, 8 and 14 weeks following transplantation and we analyzed expression of Cell adhesion molecule 1 (CADM1), Neuroligin 1 (NLGN1) and Synapsin I (SYN1). Migration of transplanted NSCs from the implantation site to the site of the lesion was observed and cells aggregated robustly in the ischemic core. They were positive for Nestin, and majority of them for CADM1 and Neuroligin 1. No SYN1 staining was present, even after 14 weeks. This *in vivo* observed pattern was similar to our *in*



vitro results. NSCs are exhibiting a marked and early visible potential for interaction with other cells and extracellular matrix which suggests that formation of synapses contributes to survival, integration and healing properties of NSCs.

Keywords: Neural stem cells, stroke, MCAO, synapse.

PO-72

Track: Other areas

PHYSIOLOGICAL EFFECT OF SODIUM ALGINATE DECOMPOSED PARTIALLY BY *VIBRIO ALGINOLYTICUS* SUN53 ON THE INHIBITION TO DISACCHARIDASE AND GLUCOSYLTRANSFERASE

Sadako Nakamura, Michiru Hashiguchi, Kenichi Tanabe and Tsuneyuki Oku

Department of Food and Nutritional Sciences, Institute of Food, Nutrition and Health, Jumonji University, Japan; E-mail: nacsacj@jumonji-u.ac.jp

Alginate is a co-polymer of alpha-L-guluronate and beta-D-mannuronate, and sodium alginate is a dietary fiber which is gelling polysaccharides found as a part of cell wall and intracellular material in the brown seaweeds. First, we prepared partially decomposed sodium alginate (Alg53) by the co-culture of sodium alginate and *Vibrio alginolyticus* SUN53, and partial purification. The average molecular weight of Alg53 was approximately 1,800. We hypothesized that Alg53 inhibits small intestinal disaccharidases and glucosyltransferase (GTase) based on the facts that several mono- and disaccharides inhibit disaccharidase competitively.

GTase was purified from *Streptococcus sobrinus*. Rat and human small intestinal disaccharidase was prepared by modified Kessler methods. Disaccharidase activity was measured by Oku method using glucose oxidase and GTase activity was evaluated based on the production of glucan using sucrose as a substrate. The study protocol using human intestine was approved by the ethical committee of University of Nagasaki.

The activities of rat and human disaccharidases were inhibited strongly by Alg53 and sucrase, maltase and lactase were competitively inhibited. The production of glucan by GTase was inhibited Alg53 and the acid production by *S. sobrinus* was clearly inhibited. We believe that these results may contribute to the prevention of diabetes and dental caries.

Keywords: Sodium Alginate, disaccharidase, glucosyltransferase.

PO-52

Track: Medical Biotechnology

A NEW ASSAY FOR MEASURING TRACE ELEMENTS IN SERUM FOR THE ESTIMATION OF BREAST CANCER RISK

Naoyuki Okamoto, Haruo Mikami, Youko Nakamura, Hiroki Nagase, Katsura Katou, Seiichi Inagaki, Hideo Tanaka and Yohei Miyagi

Kanagawa Cancer Center, Research Inst., Yokohama, Japan; E-mail: okamoto@renatech.net

An assay to determine the concentrations of trace elements in serum can reflect the metabolic status of the whole body, as trace elements are linked to all of the body's metabolic systems as catalysts. Such an assay could also be measured simply, without imposing physical burdens on subjects. Our group investigated the balance of trace elements in serum to elucidate the correlations between trace elements and cancers. We then used our data on the correlation to develop a new method of measuring trace elements in serum. Venous blood samples were collected from Japanese breast cancer patients before medical treatment (n=30), and from age-matched controls undergoing medical examinations (n=30). After serum separation, 16 elements (Na, Mg, Al, P, K, Ca, Ti, Mn, Fe, Cu, Zn, Se, Rb, Ag, Sn, S) were measured by ICP-MS. The



"metallo-balance" determined by a multivariate analysis of the trace element profiles of the two groups was effective marker for breast cancer risk. The ROC curve calculated to evaluate performance had an AUC of 0.999. Multivariate discriminating classifiers based on trace element profiles measured by ICP-MS were successful in discriminating breast cancer. Our results suggest that serum trace element profiling has great potential for the diagnosis of breast cancer.

PO-67

Track: Other areas

BIOAVAILABILITY AND AVAILABLE ENERGY OF NEWLY DEVELOPED DIETARY FIBRE MATERIALS, RESISTANT GLUCAN AND HYDROGENATED RESISTANT GLUCAN, IN RATS AND HUMANS

Tsuneyuki Oku, Kenichi Tanabe, Norihisa Hamaguchi, Fumio Shimura and Sadako Nakamura

Institute of Food, Nutrition & Health, Jumonji University, Japan; E-mail: okutsune@sun.ac.jp

Resistant glucan (RG) and hydrogenated resistant glucan (HRG) are new dietary fibre materials developed to decrease the risk of metabolic syndrome and lifestyle-related diseases. This study investigated the bioavailability and available energy of purified RG and HRG in rats and humans. The human study used a within-subject, repeated measures design. Effects of RG and HRG on blood glucose and insulin levels and hydrogen excretion were compared with those of resistant maltodextrin (RMD) and fructooligosaccharide (FOS). Available energy was evaluated from breath hydrogen excretion. When rats ingested RG or HRG (400 mg), blood glucose and insulin levels increased slightly. Hydrogen started to excrete about 120 min after administration of RG with a small peak at 180 min, thereafter scarcely excreted until 1440 min. Hydrogen excretion after HRG administration showed a larger peak than RG at 180 min, but much smaller than FOS. RG and HRG were excreted in faeces, but not urine. When RG or HRG (30 g) was ingested by humans, glucose and insulin levels increased scarcely. Breath hydrogen excretion increased slightly, but much less than FOS. After RG or HRG (5 g) ingestion to evaluate available energy, glucose and insulin levels and breath hydrogen excretion increased scarcely. Available energy was 0 kcal/g for RG and HRG. Although oligosaccharides (minor component of RG and HRG) were only slightly digested and fermented slightly, high molecular weight carbohydrates (main component) were digested scarcely and fermented barely in humans. RG and HRG could be used as new dietary fibre materials with low energy.



Keywords: Bioavailability, available energy, dietary fibre materials, resistant glucan, hydrogenated resistant glucan.

PO-74

Track: Other Areas

EFFECTS OF OLEUROPEIN AND PERACETIC ACID AS SANITIZING AGENTS FOR INACTIVATION OF LISTERIA MONOCYTOGENES BIOFILMS

Laura C. C. Dominciano, Sarah H. I. Lee, Roice E. Rosim, Carlos H. Corassin and Carlos A. F. Oliveira

University of Sao Paulo, Department of Food Engineering, Av. Duque de Caxias Norte 225, 13635-900 Pirassununga, SP, Brazil; E-mail: carlosaf@usp.br

The development of more efficient sanitizers for reducing the biofilm formation by pathogenic bacteria is of great importance, especially in the food industry. The aim of this study was to evaluate the efficiency of oleuropein (OLE), a phenolic compound extracted from olive leaves, and peracetic acid (PAA), alone or in combination, to inactivate biofilms formed by *Listeria monocytogenes* (ATCC 7644). Duplicate biofilm assays were prepared in stainless steel coupons during 48 h without stirring. After, the biofilms were washed (NaCl 0.85%) and rinsed with solutions of OLE (5.0 mg/mL) and/or PAA (2.0%) for 1 min. The



reaction was stopped with sodium thiosulfate 0.1 M for 5 min. The inactivation of biofilms was assessed by confocal microscopy. OLE alone in contact with biofilms had low bactericidal activity on the biofilms, when compared with PAA. However, the treatment of OLE in combination with PAA resulted in greater inactivation of biofilms. Results indicate a potential application of OLE for enhancing the bactericidal effect of PAA against *L. monocytogenes* biofilms, although further studies are necessary to understand the mechanisms of action of OLE in combination with commercial chemical sanitizers.

Funding: National Council for Scientific and Technological Development (CNPq, Grant # 309348/2013-7).

Keywords: *L. monocytogenes*, microbial biofilms, organic sanitizers.

PO-75

Track: Other Areas

COMPARISON OF THE INACTIVATION EFFECT OF PERACETIC ACID AND ATMOSPHERIC PLASMA TREATMENTS ON STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES STRAINS

Sarah. H. I. Lee, Julia Jacob, Antje Fröhling, Oliver Schlüter and Carlos A. F. Oliveira

University of Sao Paulo, Department of Food Engineering, Av. Duque de Caxias Norte 225, 13635-900 Pirassununga, SP, Brazil; E-mail: carlosaf@usp.br



In this study, the inactivation effect of peracetic acid (PAA, 0.5%) and atmospheric plasma jet (APJ, power: 20 W) on three strains of *Staphylococcus aureus* and one strain of *Listeria monocytogenes* were evaluated at different treatment times. PAA treatment was performed directly on liquid phase, with the inoculum suspended in brain-heart-infusion (BHI) broth. APJ assays were conducted on platelets made of polysaccharide gel with 1% calcium chloride. Each platelet was inoculated with 10 µl of each bacterial BHI suspension. For all experiments, the initial concentration was set at 10⁹ CFU/mL, and treatment times were 5, 10, 15, 30, 60 and 120 sec. Results were obtained using flow cytometry and plate count methods. For PAA treatment, inactivation was observed at 120 s for all strains tested, with a decrease of >7 log cycles. However, no inactivation was observed after 120 s of APJ treatment, although the colony counts decreased up to 2 log cycles. Results indicate that APJ had lower efficiency than PAA for inactivation of *S. aureus* and *L. monocytogenes* in all treatment times. Further studies with APJ using longer treatment times are necessary to fully describe the kinetics of this technology for inactivation of important food pathogens.

Funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, PDSE Grant # 99999.004419/2014-06)

Keywords: *S. aureus*, *L. monocytogenes*, cold plasma, sanitizers, inactivation.

PO-33

Track: Industrial and Manufacturing

FORMIC ACID REINFORCED AUTOHYDROLYSIS OF WHEAT STRAW FOR HIGH YIELD ENZYMATIC SUGAR PRODUCTION AND MINIMAL LIGNIN PRECIPITATION DURING PRETREATMENT

Omer Ozyurek and Adriaan van Heiningen

University of Maine, Chemical & Biological Engineering 5737, Jenness Hall, USA;

E-mail: ozyurekomer@gmail.com



Wheat straw is the second largest agricultural residue in the world. Presently much of the wheat straw with low-economic value is burnt or left in the field after harvest. Similar to other lignocellulosic biomass, wheat straw consists of cellulose (33-40% w/w), hemicelluloses (20-25% w/w) and lignin (15-20% w/w) and a small amount of extractives and mostly silica-containing ash (3-7% w/w). With a significant carbohydrate content of about 65% w/w, wheat straw is a potential cheap and abundant feedstock for production of fermentable sugars for bioethanol production. However a cost efficient pretreatment technology and low enzymatic charge is required for competitive sugar production at industrial scale. Autohydrolysis would be a good pretreatment process because only water/steam is needed. Unfortunately the presence of "sticky lignin" precipitates in the hydrolysate leads to severe plugging in the hydrolysis reactor and downstream equipment in a continuous process. Earlier we found that the presence of formic acid (FA) at a low concentration (5-20 g/L) during hot water (160 °C) treatment of hardwood chips significantly reduced the amount of lignin precipitates in the pre-hydrolysate. In addition, the FA reinforced hydrolysate contained much more monomeric hemicellulose sugars than that without FA supplementation. In this study we will quantify the effect of low FA concentrations during pretreatment of wheat straw on hemicellulose dissolution and lignin precipitate formation, followed by the effect of FA-reinforced pretreatment and refining of the pretreated straw on enzymatic hydrolysis to produce fermentable sugars.

Keywords: Autohydrolysis, bioethanol, enzymatic hydrolysis, formic acid, lignin precipitates, pretreatment, refining, wheat straw.

PO-63

Track: Plant and Environment

PHYLOGENETIC ANALYSIS AND MARKER DEVELOPMENT USING COMPLETE CHLOROPLAST GENOME SEQUENCES OF SOLANACEOUS SPECIES

Tae-Ho Park and Kwang-Soo Cho

Department of Horticulture and Institute of Life and Environment, Daegu University, Gyeongsan 712-714, Republic of Korea; E-mail: thzoo@daegu.ac.kr



Somatic hybridization has been widely applied to introgress several disease resistance from wild *Solanum* species into the cultivated potatoes (*Solanum tuberosum*) and plastome genotyping is one of important processes to select proper breeding lines. In this study, we completed chloroplast genome sequences of two wild *Solanum* species, *S. commersonii* and *S. nigrum*, which have been known and used as one of useful resources for improving resistance to several diseases. The sequences were compared with those of *Capsicum annum*, *Nicotiana tabacum*, *S. lycopersicum*, *S. bulbocastanum* and *S. tuberosum* to develop InDel markers for the application in cytoplasm genotyping. Although gene contents and their relative positions were almost same, detailed comparison of their sequences identified several indels between *S. commersonii* / *S. nigrum* and *S. tuberosum* in the intergenic and intragenic regions. Based on the sequence information of the indels, one and seven allele specific RCR-based markers were developed for discrimination of *S. commersonii* / *S. nigrum* from *S. tuberosum* and the markers were further confirmed with filial generations and other wild *Solanum* species, respectively. Additionally, the

phylogenetic tree revealed *S. commersonii* is located in a same node with *S. tuberosum*, but *S. nigrum* was slightly further from the node.

Keywords: Chloroplast genome sequence, molecular marker, potato, *Solanum commersonii*, *Solanum nigrum*, *Solanum tuberosum*.

PO-38

Track: Medical Biotechnology

PHARMACOLOGY METABOLIC CHANGES ASSOCIATED WITH ISCHEMIA AND REPERFUSION

Beatriz Prieto-Moure, Dolores Cejalvo-Lapeña, Lajara-Romance J.M., Elena Bover-Arbona, Daniel López-Malao Carolina Padrón-Sanz, Javier Torres Gavilà and José Miguel Lloris-Carsii



Facultad de Medicina, Universidad Católica de Valencia "San Vicente Mártir", C/ Quevedo, 2. 46003. Valencia, Spain; E-mail: beatriz.prieto@ucv.es

Ischemia-reperfusion (IRI) is a complex physiopathological mechanism involving a large number of metabolic processes that can eventually lead to cell apoptosis and ultimately tissue necrosis.



Treatment approaches intended to reduce or palliate the effects of IRI are varied, and are aimed basically at: inhibiting cell apoptosis and the complement system in the inflammatory process deriving from IRI; modulating calcium levels; maintaining mitochondrial membrane integrity; reducing the oxidative effects of IRI and levels of inflammatory cytokines; or minimizing the action of macrophages, neutrophils, and other cell types.

This study involved an extensive, up-to-date review of the bibliography on the currently most widely used active ingredients in the treatment and prevention of IRI, and their mechanisms of action, in an aim to obtain an overview of current and potential future treatments for this pathological process.

Active ingredients are classified by mechanism of action into three groups:

- 1) Substances and mechanisms of action that modify the membrane response of IRI-affected cells:
 - Antioxidants
 - Reduction of inflammatory cytokines IL, TNF, and NK- κ B
 - Na⁺/H⁺ (NHE) inhibitors
 - Calcium modifiers
 - Toll-like receptors
- 2) Substances and mechanisms of action that act on cells that lead to IRI:
 - Prevention of leukocyte adhesion
 - Inhibition of the complement system
 - Depletion of macrophages/neutrophils
 - Stem cell treatment
- 3) Special treatments in IRI
 - Preconditioning
 - Apoptosis inhibition
 - Gene therapy
 - miRNAs

- Natural therapies
- Mitochondrial membrane pore inhibitors
- Other treatments

PO-29

Track: Other areas

USE OF MEDIUM DENSITY SNP PANELS FOR DEVELOPING A MULTIVARIATE BREED ASSIGNMENT APPROACH IN CATTLE AND SHEEP

Pulina G., Dimauro C., Stella A., Ciani E., Moiola B.5, Pilla F., Crepaldi P. and Maciotta N.P.P.

*Dipartimento di Agraria, University of Sassari, via E. De Nicola, 07100, Sassari, Italy;
E-mail gpulina@uniss.it*



In livestock species, DNA-based assignment tests are generally used to trace and certify the origin of animal products. A certified traceability process is of great importance for products with Protected Designation of Origin (PDO) or Geographical Indication (PGI) labels. Moreover, it could be useful to valorize niche products obtained from breeds grazing in mountain areas. At present, several animals of different livestock species and breeds have been genotyped in many countries for genomic selection purposes. They could be therefore conveniently used to develop new assignments methods. In the present research, genotypes from the Illumina's BovineSNP50 and OvineSNP50 BeadChips were used to develop an assignment method based on three multivariate statistical techniques: the stepwise discriminant analysis (SDA), the canonical discriminant analysis (CDA) and the discriminant analysis (DA). The SDA was used to select the most informative markers; the CDA tested if the discrimination of involved groups was significant; the DA assigned individuals to the true group of origin. Around 2,300 genotyped bulls belonging to 3 different breeds and 460 genotyped sheep belonging to 21 Italian sheep breeds were involved in the study. In both cases, 10% of randomly selected animals was used in the validation procedure. The SDA selected 48 and 108 top discriminant SNPs for bulls and sheep, respectively that were able to correctly assign animals to the true breed of origin. Those markers could be further used to develop two low cost customized essays to trace foodstuffs derived from the involved breed.

PO-44

Track: Pharmaceutical Biotechnology

INCREASED EXPRESSION OF HUMAN INTERFERON GAMMA THROUGH SELECTIVE PRESSURE ON THE ADJACENT HIS4 GENE IN PICHIA PASTORIS

Ali Razaghi, Roger Huerlimann, Leigh Owens, Kirsten Heimann

*College of Marin and Environmental Sciences, James Cook University, Australia;
E-mail: ali.razaghi@my.jcu.edu.au*

Transcriptional co-regulation of adjacent genes has been observed for prokaryotic and eukaryotic organisms, alike. High levels of gene adjacency were also found in a wide variety of yeast species with a high frequency of co-regulated gene sets. The aim of this research was to study how selective pressure on the Histidinol dehydrogenase gene (HIS4) affects the level of expression and secretion of the adjacent human interferon gamma gene (hIFN γ) in the recombinant *Pichia pastoris* GS115 strain, a histidine-deficient mutant. hIFN γ was cloned into the pPIC9 vector adjacent to the HIS4 gene, a gene essential for histidine biosynthesis, which was then transformed into *P. pastoris*. The transformed *P. pastoris* was cultured under continuous selective pressure in amino acid-free minimal medium for ten days, with five inoculations into unspent medium every second day. Under these conditions, only successfully transformed cells (hIFN γ -HIS4+) are able to synthesise histidine and therefore thrive. As shown by ELISA, exposure to selective pressure improved hIFN γ

expression and secretion by 55% from unchallenged cells to 10-day continuous applied selective pressure. RT-qPCR showed that there was also a positive correlation between duration of selective pressure and increased levels of the hIFN γ RNA transcript. According to these results, it is suggested that these adjacent genes (hIFN γ and HIS4) in *P. pastoris* are transcriptionally co-regulated and their expression is synchronized. To the best knowledge of the authors; this is the first study demonstrating that selective pressure can alter the regulation pattern of adjacent genes in yeast.

Keywords: Transformation, Gene copy number, Selective pressure, Transcriptional co-regulation

PO-18

Track: Industrial and Manufacturing

REDUCING THE TOXIC EFFECT OF WINE INTAKE (NEUTRALISATION OF THE MOST TOXIC ETANOL DERIVATE ACETALDEHYD WITH ESSENTIAL AMINOACIDS L-LYSINE AND L-ARGININ)

Gy. Kóródi, J. Rikk and T. Szarvas

BGF Methodology Department, Budapest, Hungary; E-mail: rikk@devlart.hu

INTRODUCTION: The moderate wine intake causes more healthy benefits than other types of alcohol because of it's high antioxidant content. On the other hand we have to investigate all the methods, which can be useful in reducing the toxic effects of the ethanol metabolites. It has been supposed for a long time that methylation of amines by acetaldehyde occurs only extreme conditions. Recently hungarian authors have reported on the spontaneous reaction between acetaldehyde and L-lysine yielding N-methylated products at room temperature. This reaction extended to L- arginine as well, so a tentative theoretical explanation of the experimental findings is outlined.

METHODS: We did potentiometric titration using combined glass electrodes, UV, H-NMR and mass were recorded by mass spectrometer, respectively. strong cationic exchange plates were used in a citrate bufer for thin layer chromatography. The content of the methylol-arginine derivate was examined by a radiochromatographic method thinlayer chromatographic system. Electrostatic isopotential maps were calculated by the ELPO programme. Geometries of lysine and arginine were taken from neutron diffraction studies.

RESULTS: Changes in pH L-lysine and L-arginine correspond to the Sørensen formole titration While L-lysine reacts slowly, it's methylated products appear only 5 hours after neutralization, N-hydroxymethyl derivatives of arginine can be detected instantaneously on the plate. Methylation reacton products for L-lysine could be identified as N-mono-, di- and trimethyl-L-lysine. No hydroximethyl derivatives could be detected at all. On the other hand, reaction between L-arginine and acetaldehyde is slow; the products could be identified as N-hydroxymethyl derivatives with thin layer chromatography.

DISCUSSION: Two problems arise when studying the reaction between acetaldehyde and L-arginine: 1., what are the products formed; 2., how the essential difference between reactivities of L-lysine and L-arginine can be explained.

It is evident that if the imino nitrogen of the arginin guanidino miety is blocked by methyl groups the reaction gets practically impossible. On the other hand N-monomethyl and N-N-dimethyl derivatives of arginine reacts with acetaldehyde at about a same rate. Consequently it is the imino nitrogen atom which reacts readily with acetaldehyde to yield the monosubstituted hydromethyl derivate. Most probably the second acetaldehyd molecule enters at the terminal amino nitrogen atom. This is understood by inspection of the lectrostatic isopotential map of the zwitterionic, N-protonated form of L-arginine. The N-proteonated form of lysine reacts with acetaldehyde to yield an unstable adduct, afterwards a proton is transformed to the alkoxide group and hydroxymethyl-L-lysine is formed. This reacts at once with a second molecule of acetaldehyde and the intermediate product is reduced *via* an azomethin structure to N-methyl-L-lysine. The difference in the reaction rate between L-lysine and L-arginine with acetaldehyde is explained with the different nucleophilicity of the amino group in lysine and that of the imino group in argininine. The minima of the electrostatic potential around the corresponding atoms are -481 kJ/mol and -749 kJ/mol, respectively. These values explain the higher affinity of arginine, as related to lysine towards the acetaldehyde molecule.

Keywords: L-Lysine, L-Arginin, wine intake.

VP-2*Track: Industrial and Manufacturing***INCLUSION OF RICE BRAN EXTRACT AND ITS EFFECT ON NUTRITIONAL QUALITY OF SUGARCANE BAGASSE HEMICELULOSIC HYDROLYSATE TO XYLITOL PRODUCTION BY YEAST****Rita C.L.B. Rodrigues, Maria das Graças A. Felipe, Arnaldo M. R. Prata and Adalberto Pessoa Jr.***Departamento de Biotecnologia (DEBIQ), Escola de Engenharia de Lorena (EEL), Universidade de São Paulo (USP), 12600-970, Lorena-SP, Brazil; E-mail: rita@debiq.eel.usp.br*

To produce cost-effectively xylitol on an industrial scale by fermentation, more economic nitrogen sources needed to replace peptone, and yeast extract, which are one of the most expensive ingredients of the semi-defined medium. In our work, we used the statistical approach to get the best medium nutritional need and initial cell concentration to improve xylitol production by the yeast *Candida guilliermondii* FTI 20037 in sugarcane bagasse hemicelulosic hydrolysate (SBHH) containing high xylose concentration (~77g/L). We described the effects of rice bran extract (RBE) as an alternative organic complex nitrogen source and the ammonium sulfate as inorganic nitrogen, using a 2³ full factorial design followed by response surface methodology (RSM). The RSM suggests the maximum values for xylitol volumetric productivity (Q_p) and xylitol yield ($Y_{p/s}$) by using ammonium sulfate (0.5g/L), initial cell concentration (1.0g/L) and RBE (5.0g/L) in their lowest levels. The experimental value for Q_p and $Y_{p/s}$ were 0.73g_{xylitol}/L.h and 0.66g_{xilose}/g_{cel} corresponding to a theoretical conversion efficiency (η) of 73.06% and maximum xylitol production of 40.07g/L. The rice bran is a low cost feedstock. Inclusion of RBE improved the nutritional quality of SBHH and could be a viable alternative as an organic nitrogen source for xylitol production by yeasts.

Keywords: Sugarcane bagasse hemicellulosic hydrolysate, xylitol, yeast fermentation, nitrogen source, rice bran extract.

PO-3*Track: Regenerative Medicine***IN VITRO RESTORATION OF SMN PROTEIN BY HOMOLOGOUS RECOMBINATION METHOD****Afroz Rashnonejad, Cumhur Gündüz, Sunde Süslüer, Hüseyin Onay, Burak Durmaz, Mojgan Bandehpour and Ferda Özkinay***Department of Biotechnology, Ege University, İzmir, Turkey; E-mail: afrash99@yahoo.com*

The reduced level of Survival motor neuron (SMN) protein causes a neurodegenerative disorder named as Spinal muscular atrophy (SMA) that characterized by progressive paralysis and symmetrical muscle weakness. Majority of SMA patients have homozygous deletions in SMN gene. Despite extensive efforts to find a cure for SMA, there is still no effective treatment for this devastating disease. In the present study, 'gene targeting' method based on homologous recombination was used to restoration of SMN protein expression in SMA type I fibroblasts. Gene targeting fragment including SMN1 cDNA and homolog upstream and downstream sequences was designed and subcloned into a pGH vector. SMA type I fibroblast cell line (Coriel institute, GM03813) was transfected with DNA fragments using Lipofectamine LTX Plus Reagent (invitrogen, 15338). Single cell colony was achieved by serial dilution and PCR analysis was performed to confirm the occurrence of homologous recombination in transfected cells. Restoration of SMN protein expression was confirmed by RT-PCR and Western Blot analysis. The immunofluorescent results demonstrated localization of SMN protein in the nucleus of transfected cells. Our result show that homologous recombination could present as an alternative method for *in vitro* restoration of SMN protein. *In vitro* correction of patients stem cell by homologous recombination and transplantation of these cells to patients could be investigated to find alternative treatment for SMA disease.

Keywords: Survival motor neuron protein, spinal muscular atrophy, Homologous recombination, Gene targeting.

PO-2

Track: Regenerative Medicine

EXPERIMENTAL BURN WOUND HEALING USING MOUSE EMBRYONIC STEM CELLS DERIVED KERATINOCYTES

Gulinnaz Ercan, Yiğit Tiftikcioglu, Seda Vatansever, Elgin Turkoz, Afrooz Rashnonejad and Cafer Seyid

Department of Biotechnology, Ege University, İzmir, Turkey; E-mail: afrash99@yahoo.com

Stem cell therapy is a promising new approach in burn wound healing. In this study mouse embryonic stem cells (mESCs) were differentiated to keratinocytes by adding BMP-4 into the medium and characterized by cytokeratin-8 and cytokeratin-14 determination using immunoperoxidase technique. After cultured on vaseline gauze keratinocytes were transferred to 2nd degree thermal burn wounds of mice created bilaterally and symmetrically by a copper rod on the back of the mice in order to evaluate its effect in wound healing. Burn wounds on the right side was covered with keratinocytes on vaseline gauze (study group) while the cell-free vaseline gauze was applied to burn wounds on the left side (control group). Biopsy samples were taken from each group on 1st, 3rd and 5th days after the cell transfer. Immunohistochemical analysis showed that IL-8, MCP-1, Collagen-1, EGF expression which act as a regulator of wound healing started at an earlier stage and their densities were higher in the biopsy samples of the study group in comparison to controls. Wound healing began at an earlier stage in samples treated with keratinocytes. Although, keratinocytes migration from the edge of the wounds was observed in the treated samples, it was not observed in the control group. Since, mESCs derived keratinocytes are able to facilitate earlier recovery of thermal burn wound healing in mice their clinical application seems promising in burn treatment by preventing scar formation.

Keywords: Stem cell therapy, wound healing, keratinocyte, vaseline gauze, mouse embryonic stem cells.

PO-34

Track: Plant and Environment

LIPID-BASED SIGNALING IN ARABIDOPSIS LOCAL DEFENSE RESPONSES

Stephen B. Ryu, Jihye Jung and Sunghye Jung

KRIBB, 125 Gwahak-ro, Yuseong-gu, South Korea; E-mail: sbryu@kribb.re.kr

Pathogen effector-triggered immune responses are mediated by host Resistance (R) proteins. The molecular mechanisms that connect these gene-for-gene interactions to the R gene-induced immune responses remain largely unknown. Here, we report that phospholipase A2 (PLA2) and its lipid products mediate R gene-induced immune responses in Arabidopsis. PLA2 is rapidly expressed in an R gene-mediated manner. PLA2 is secreted into the apoplast, where it generates its lipid products, including lysophosphatidylethanolamine (LPE), from invading bacteria and host cells. The Arabidopsis pla2 mutant lines fail to mount an adequate local immune response. The pla2 mutant defects are complemented by supplementation with LPE. Our results demonstrate PLA2-derived lipid signaling that mediates and/or potentiates R gene-induced immune responses following a gene-for-gene interaction.

Keywords: Lipid signaling, defense, Arabidopsis.



PO-35

Track: Medical Biotechnology

APPLICATION OF VARIOUS COLLOIDAL SERS APPROACHES FOR DETECTION OF TRACES OF URIC ACID

Martynas Velicka, Milda Pucetaite, Justinas Ceponkus and Valdas Sablinskas

Vilnius University, Giedros str. 33, Vilnius, Lithuania; E-mail: valdas.sablinskas@ff.vu.lt

Surface-enhanced Raman scattering (SERS) spectroscopy has been increasingly proposed as a method of choice for disease diagnosis and prevention. Advantages of SERS over conventional Raman spectroscopy includes significantly increased signal which allows detection of trace amounts of substances in the sample and fluorescence quenching. On the other hand, if SERS spectroscopy is going to be used in routine analysis several problems, such as reproducibility of the enhancement factor, should be considered. In addition, suitable SERS substrates should be selected according to the sample and available experimental conditions. The aim of this work is to test the suitability of various SERS substrates for detection of uric acid traces in biological fluids.

Several colloidal solutions of Ag spheres, Ag prisms, Au rods and Au branched nanoparticles in different sizes and concentrations were prepared. In order to form a substrate, a drop of the colloid was placed on a silver plated microscope glass slide and dried. In addition, substrates of Au nanodendrites on graphite plates were produced.

It was found that concentrations of uric acid in biological liquids as low as 10^{-5} - 10^{-7} M can be detected only using substrates produced from concentrated Ag prism colloid, while the other substrates give lower enhancement factor of Raman signal.

Keywords: SERS; Uric acid; silver colloids; biological fluids.

VP-06

Track: Medical Biotechnology

INDUCTION OF DNA DAMAGE IN BLOOD CANCER CELLS BY LOW INTENSITY ULTRASOUND

Timur Saliev, Dinara Begimbetova, Aibobek Seitak and Dinara Baiskhanova

Centre for Life Sciences, Nazarbayev University, Unit 9, 53 Kabanbay batyr Ave., Astana 010000, Kazakhstan; Email: tim.saliev@gmail.com

The low intensity ultrasound has been proven to be effective for cancer treatment, particularly for elimination of malignant cells through direct destruction or induction of apoptosis. It has been thought that the underlying mechanism of ultrasound-mediated bio-effects is acoustic cavitation. The cavitation triggers the chain of sono-chemical reactions resulting in generation of reactive oxygen species (ROS). The data of recent *in vivo* studies conducted on commercially available cancer cell lines have showed that the ultrasound with low intensity is capable of inducing DNA breaks [1, 2]. Our study for the first time demonstrated the capability of ultra-low intensity ultrasound (0.18 W/cm^2) to induce single DNA breaks on blood cancer cells derived from the patients diagnosed with acute myeloid leukemia. For detection of DNA damage we employed DNA comet assay (alkaline gel electrophoresis). The acquired results indicate that ultra-low intensity ultrasound caused single DNA breaks in blood cancer cells ($66.05 \pm 13\%$), while DNA damage was minimal ($4.4 \pm 0.99\%$) in normal white blood cells treated with ultrasound. Our findings suggest that ultra-low intensity ultrasound is not mutagenic for normal white blood cells, but it is capable of inducing DNA damage in cancer blood cells.

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PO-5*Track: Medical Biotechnology***STUDY OF GLYCOSYLATION CHANGES WITH LECTIN-BASED PROTEIN MICROARRAY****Alena Šedivá, Martina Zámorová, Dragana Robajac, Goran Miljuš, Miloš Šunderićb Olgica Nedic, Jaroslav Katrlík***Institute of Chemistry, Slovak Academy of Sciences, Department of Glycobiotechnology, Dubravská cesta 9, 845 38 Bratislava, Slovakia; E-mail: a.sediva@savba.sk*

Glycosylation is a form of co-translational and post-translational modification of proteins. Glycans have a variety of structural and functional roles in membrane and secreted proteins. Changes in glycosylation are accompanied with changes in physiological state, which are also associated with various diseases such as cancer, AIDS, rheumatoid arthritis, multiple sclerosis and many others. Glycoprofiling thus could serve as a diagnostics tool for even early stage of diseases. A highly challenging is the fact that analytical detection platform for glycan analysis has to be able to detect very low levels of analytes. The aim of this work was to develop lectin-based protein microarray method for glycoprofiling of proteins and to apply this method for analyses of glycoproteins isolated from the samples by immunoprecipitation. We measured proteins and receptors of insulin-like growth factor (IGF) system isolated from serum, cytosol or membrane protein fraction of two groups of the samples, (1) serum of healthy persons and patients with colorectal cancer and healthy and tumor part of the tissue of the same patient, and (2) placental membranes from healthy mothers, mothers with pre-eclampsia, and mothers with diabetes. The microarray slides with spotted samples were incubated with set of biotinylated lectins with various glyco-specificity, labeled with streptavidin-fluorescent dye conjugate and scanned. The appropriateness of developed lectin-based protein microarray method for biosensing of changes in protein glycosylation of IGF system was evaluated, and the differences in protein glycosylation within each sample group (for colorectal cancer healthy vs. tumor tissue, for placental samples healthy vs. pre-eclampsia vs. diabetes) were quantified.

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PO-28*Track: Other areas***BIOGENIC SYNTHESIS, OPTIMIZATION AND ANTIBACTERIAL EFFICACY OF EXTRACELLULAR SILVER NANOPARTICLES USING NOVEL FUNGAL ISOLATE ASPERGILLUS NIGER MA****Vikas Sarsar, Manjit K. Selwal and Krishan K. Selwal***Department of Biotechnology, DCR University of Science and Technology, Murthal, (Haryana), India; E-mail: kselwal@rediffmail.com*

Silver nanoparticles have various applications in the field of biotechnology and medicine like water purification, biological sensors, plasmonics, optoelectronics and more recently in detection of various colon cancers *etc.* So the present investigation is focused on the optimization of process conditions for AgNPs synthesis by using *Aspergillus niger* MA extract as reducing agent and evaluation of their antibacterial efficacy. Different parameters such as concentration of the silver precursors, reducing agent, time, pH, and temperature of synthesis were optimized. The optimum values of these process parameters were determined using Surface Plasmon Methodology. X-ray diffraction (XRD) pattern peaks at 2θ confirmed crystalline nature of nanoparticles whereas Fourier transform infrared spectroscopy (FTIR) analysis showed that carbonyl and primary amines could be responsible for stabilization of the AgNPs in solution. Transmission electron microscopy (TEM) techniques exhibited morphological structure of AgNPs of size 3-20 nm. The synthesized AgNPs displayed considerable

antimicrobial activity against various pathogenic bacterial strains like *B. cereus* and *A. hydrophila*. In conclusion, optimization process played a pivotal role in the AgNPs synthesis and future work should be carried on detection of various pathological conditions by exploiting AgNPs.

Keywords: AgNPs, Antimicrobial Activity, FTIR, Silver Precursor, TEM, XRD.

PO-70

Track: Plant and Environment

BIOTECHNOLOGICAL POTENTIAL OF INDUSTRIAL RESIDUES AS SUBSTRATES FOR BIONEMATICIDE DEVELOPMENT

Satyawati Sharma and Abhishek Sharma

Center for Rural Development and Technology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi- 110016, India: E-mail: satyawatis@hotmail.com



The sustainable growth of biopesticide industry depends on the cheap and readily available nutrient inputs into fermentation media as substrates. The utilization of by-products of bio-fuel industries such as non-edible oil cakes and biogas slurry (BGS) is nowadays very much preferred to enhance the commercial feasibility of bioprocess technology. In present study, a novel process was designed to produce the dust formulation of biocontrol fungal agent *Paecilomyces lilacinus* 6029 using nitrogen rich Karanja deoiled cake as major substrate against root-knot nematodes- *Meloidogyne incognita* through solid-state fermentation. In order to achieve optimal fungal growth and pathogenicity, the cake was further combined with carbon rich BGS (sundried) in a certain proportion (Patent application No. 3590/DEL/2013). The results indicated that among the four combinations of Karanja cake/ BGS tested, 40/60 ratio gave maximum spores (9.3×10^8 spores/g) and pathogenicity (94 % egg mass hatching inhibition). The remarkable increment in nematocidal efficacy of *P. lilacinus* cultured on Karanja deoiled cake and BGS might have been due to enhanced production of various nematocidal metabolites such as serine protease, leucinostatin and low molecular weight fatty acids, as seen in our studies. We believe that the present study would provide an impetus to future research in this area to enhance the utilization of industrial residues as sources of nitrogen and carbon for economic viability of the bioprocesses industry.

Keywords: Biopesticide, Karanja deoiled cake, Biogas slurry, Fermentation, *Paecilomyces lilacinus*, Enzymes, Metabolites.

PO-7

Track: Medical Biotechnology

APOPTOTIC EVENTS INDUCED BY PROTOTYPE FOAMY VIRUS INFECTION

Md. A. Hossain, Jinsun Kim, Faysal Bin Hamid and Cha-Gyun Shin

*Department of Systems Biotechnology, Chung-Ang University, South Korea;
E-mail: cgshin@cau.ac.kr*

Foamy virus infection induces cytopathology in several cell types from different species. The exact mechanism of cell killing by foamy viruses is still unknown. In this study, we have investigated the mechanism of cell death induced by prototype foamy virus (PFV) infection in baby hamster kidney (BHK 21) cells lines. PFV induces apoptosis by exhibiting morphological alterations such as chromatin condensation, blebbing, and nuclear fragmentation. In addition, PFV infection causes the fragmentation of chromosomal DNA, upregulation of Bax, and activation of caspases-3. Upregulation of Bax initiates the translocation of cytochrome-c from mitochondria to the cytoplasm, suggesting that PFV-induced apoptosis is triggered predominantly *via* the mitochondrial mediated pathway. Blocking apoptosis using caspases inhibitors increased PFV-infected BHK 21 cell viability. Although blocking apoptosis resulted in reduced progeny release, maximal accumulation of PFV was found in apoptosis-blocked cells. This report provides the

first systematic experimental account of the mechanism of apoptosis induced by PFV infection, which will provide valuable insights for understanding why pathology is not associated in animals infected with cytopathic foamy viruses.

Keywords: Apoptosis, prpotype foamy virus.

VP-04

Track: Other Areas

ETHICAL AND LEGAL FACETS OF BIOFEEDBACK IN ICT-BASED ASSISTIVE TECHNOLOGIES FOR SENIORS

Luiza Spiru and Stefan Alexandru Sterea

Ana Aslan International Foundation, Bucharest, Romania; E-mail: lsaslan@brainaging.ro

Smart, ICT-based assistive technologies represent a new, effort and cost-saving solution for the long term assistance of seniors. The use of ICT-based smart technologies involves the gathering of information about vital processes for monitoring their health state. The ethical and legal issues are critical aspects in obtaining this biofeedback. We are presenting an overview of these aspects, paralleled by several lessons learned as medical partner and pilot site in projects dealing with the user-centered design of biosensors prototypes.

Several important ethical aspects must be assured when working with biosensors: the informed consent form; personal data protection, networks security; communication confidentiality; management of user's complaints; withdrawal request and exit strategy issues and ethical auditing. An important ethical issue are the methods for managing the exit strategy (the discomfort experienced by the senior when he decides to withdraw sensors use). Other challenges are the scarcity of standardized ethical guides, as well as their adaptation to the local socio-cultural particularities. The ethical aspects related to the awareness of the end-user about the usefulness of biosensors are also discussed.

The trustiness, accessibility and acceptance of various batteries of biosensors depend, in a great measure, on their compliance with the requested ethical and legal provisions.

PO-60

Track: Plant and Environment

EFFECT OF DIFFERENT SOUND GENRES ON IN VITRO SEED GERMINATION OF GRAMMATOPHYLLUM HYBRID AND GRAMMATOPHYLLUM STAPELIIFLORUM ORCHIDS

Noor Anilizawatima Sulong, Noor Izzah Mohd Khalil, Mohd Ikhwan Dahari and Anis Athirah Zakaria

*Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Malaysia;
E-mail: watima@puncakalam.uitm.edu.my*

Mechanical stress can greatly affect the development of plants. Stress produced by music has been found to induce a positive effect on the plants growth. The aim of this study was to examine the *in vitro* seed germination of two Grammatophyllum species when exposed to five different music genres. The Grammatophyllum species used were *G. hybrid* and *G. stapeliiflorum* while the music genres were Instrumental, Rock, Hip hop, Yassin and Ballad. Each group of seeds culture were exposed to different music genre for 8 hours starting from 9 am to 6 pm every day in a six months period and kept in the dark for the first three months. All cultures were maintained on half-strength Murashige and Skoog media supplemented with 30 g/L sucrose, 0.5 mg/L 6-benzylaminopurine (BAP), 2 g/L peptone, 1 g/L activated charcoal and 2.5 g/L gelrite. At the end of the experiment, it was found that music exposure had a positive effect on the seeds germination as compared to the untreated control group. For *G. hybrid* species, the highest shoots number counted 19.33 ± 3.79 was observed on seeds exposed to Yassin. The highest shoots length measured was 2.01 ± 1.10 cm when it was exposed to Rocks. In contrast, *G. stapeliiflorum* species showed the highest number of shoots of 12.00 ± 2.64 when exposed to Ballad and the highest shoot length was 1.00 ± 0.38 cm when it was exposed to Instrumental. The findings showed that different species of orchids needs a different type of music to influence its germination and growth rate.



Keywords: Grammatophyllum, music, *in vitro* seed germination, plant tissue culture, orchid.

PO-62

Track: Regenerative Medicine

A GENOME-WIDE ASSOCIATION STUDY ON BODY COMPOSITION OF JINGHAI YELLOW CHICKEN

ZHANG Tao, FAN Qing-can, ZHANG Xiang-qian, ZHANG Gen-xi, WANG Jin-yu and GU Yu-ping

*College of Animal Science and Technology, Yangzhou University, Jiangsu Yangzhou 225009, China;
E-mail: zhangtaorenfuyun@126.com*



Body composition traits are important economic traits of chicken. To identify SNPs and candidate genes affecting body composition traits and provide new methods for genetic improvement, a genome-wide association study was carried out using the Illumina chicken 60K SNP Beadchip in Jinghai Yellow Chicken. Thirteen Body composition traits were measured. A total of 13 SNPs reaching 5% Bonferroni genome-wide significance ($P < 1.8E-6$) and 130 SNPs reaching “suggestive” genome-wide significance ($P < 3.59E-5$) with body composition traits were identified. Those SNPs were located nearby or in twelve candidate genes including GRIK1, NCAPG, KCNIP4, CACNA2D2 and so on. A region approximately 1.6 Mb in length on chicken chromosome 4 (74.3-75.9 Mb) was found to be associated with body composition traits of Jinghai Yellow Chicken. Among 130 SNPs reaching “suggestive” significance, 25 SNPs were located in a region 7.4 Mb in length on chicken chromosome 4 (71.5-78.9). 5,650 haplotypes were established and fourteen of them were found to be associated with six body composition traits. Nine out of fourteen haplotypes were located in the region of 74.3-75.9 Mb on GGA4. Five candidate genes of LCORL, QDPR, KCNIP4, LDB2 and FAM184B were located in this region. The present study indicated that the region of 71.5-78.9 on GGA4 and GRIK1, NCAPG, KCNIP4, CACNA2D2, LCORL, QDPR, KCNIP4, LDB2, FAM184B gene might play important role in regulation of body composition of Jinghai yellow chicken.

PO-24

Track: Regenerative Medicine – Tissue Engineering

A TECHNIQUE FOR CONTINUOUSLY IMAGING PANCREATIC ISLETS WITHIN A ROTATING WALL VESSEL BIOREACTOR

Lauren E. Getz, S. Dan Dimitrijevič, Jeffrey L. Coffey and Tristan J. Tayag

*Engineering Department, Texas Christian University, TCU Box 298640, Fort Worth, TX 76129, USA;
E-mail: t.tayag@tcu.edu*



Type I diabetes is a disease that has reached epidemic proportions worldwide and which costs U.S. healthcare over \$100 billion annually. The tissue transplantation of islets (from donors' pancreata) has been shown to make diabetic patients insulin injection independent; however, such treatment is hampered by the scarcity and fragility of the harvested islets and the need for immunosuppression of the patient. Rotating wall vessel bioreactors offer a means of harvested islet maintenance as well as islet tissue engineering. In this paper, we present a technique for continuous imaging of cell aggregates or organoids (such as pancreatic islets) within the dynamic environment of a rotating bioreactor. To achieve this objective, a trade-off exists between the mass density of the aggregates and viscosity of the culture medium in the bioreactor. The balance of these material parameters permits the tissues within the bioreactor to achieve a stable spatial location for continuous imaging. The work presented in this paper will help make possible the engineering of pancreatic islets from constituent cells.

PO-40*Track: Industrial and Manufacturing***APPLICATION OF PRACTICAL MINIATURIZED PROTOCOL TO FACILITATE ENUMERATION OF *ESCHERICHIA COLI* AND COLIFORMS FOR FOOD INDUSTRY****Wipavadee Sangadkit, Anat Deepatana and Aluck Thipayarat**

Department of Food Engineering, King Mongkut's University of Technology Thonburi, Thailand;
E-mail: wipavadeefood@gmail.com; athipaya@yahoo.com



Unlike clinical samples, food product and environmental samples were often characterized by low initial cell count and large volume of samples that call for different strategies to handle, especially in low-resource settings and less advanced food industrial laboratory. This paper was to compare three popular industrial routines (*i.e.*, MPN method, Petrifilm™ by 3M, and standard pour plate technique) against a modified surface spread technique using 96-well microtiter plate format. Chromocult Coliform Agar (CCA) was utilized as the chromogenic agar medium enabling the formation of purple colonies from *Escherichia coli* and pink colonies from coliforms and possible *E. coli* O157:H7 - cells. The obvious drawback of the proposed miniaturized protocol was the limitation of using small inoculum volume (approximately 10-20 µl) generating the minimum detection limit at 102 CFU/ml. However, the common practice of using a glass pipette in most industrial laboratories slightly produced better minimum detection limit, approximately 101 CFU/ml. The cell enumeration results obtained from each technique showed good agreements. The miniaturized rapid protocol was able to effectively manage large number of samples with the use of multichannel autopipettes and high-throughput design of 96-well microtiter plates and able to conclude the colony count within 12-16 hr. The analytical efficiency of miniaturized protocol surpassed those of the three conventional routines. The colony counts from the ready-to-eat product samples showed similar and comparable results to the pour plate technique displaying good agreement to the universally-accepted standard techniques. The feedbacks by QA staffs from a local Thai food factory revealed good overall method acceptance (*e.g.*, usability, protocol design and method efficiency). The proposed miniaturized technique gave highly consistent results of colony count numbers and good colony separation. This cell enumeration consistency supported that the miniaturized rapid protocol can replace the complex standard protocols as an in-house protocol for less restricted samples, like environmental swabs, to enhance its sampling frequency due to the ease and cost effectiveness of the technique.

Keywords: Practical miniaturized technique, rapid colony enumeration, *Escherichia coli*, coliforms, environmental sample.

PO-65*Track: Pharmaceutical Biotechnology***IN VITRO ANTIOXIDANT AND ANTITUBERCULAR ACTIVITY OF LEUCAS MARRUBIODES DESF. ROOT EXTRACTS****Vagdevi H.M., Gowrish and Rajashekar**

Sahyadri Science College (Autonomous), Shimoga-577203, Karnataka, India;
E-mail: vagdevihm@gmail.com



Modern civilization is facing hundreds of disorders associated with free radicals. The natural antioxidants from non-edible plants are gaining importance to fight against these disorders. The purpose of this study is to evaluate the antioxidant and antitubercular activities of *Leucas marruboides* Desf. (Lamiaceae) root extracts of petroleum ether, chloroform and methanol. The antioxidant activity of the extracts has been evaluated using DPPH radical scavenging, reducing power and nitric oxide scavenging methods. The results of the study indicated that, methanol extract possess promising DPPH radical scavenging, reducing power and nitric oxide scavenging activity. The antitubercular activity of all the extracts of *L. marruboides* have been evaluated against *Mycobacterium tuberculosis* H73Rv strain using

Microplate Alamar Blue Assay (MABA). The activity was documented within MIC range of 0.2 to 100 µg/ml. The results of MABA showed that petroleum ether extract exhibited excellent antitubercular activity. The present investigation suggests that *L. marruboides* possess remarkable antioxidant and antitubercular activity.

Keywords: *Leucas marruboides* Desf., antioxidant activity, antitubercular activity, MABA.

PO-80

Track: Medical Biotechnology

TUMOR TARGETING DUAL IMAGING PROBE AND DRUG DELIVERY

J. Y. Wang, Y. Z. Wang, Y. X. Li and Y. P. Li

Jilin University, Japan; E-mail: jingyuan@jlu.edu.cn

Chinese medicine paclitaxel (PTX) is an antitumor drug and can be effective against various types of solid tumors. The clinical applications and the bioavailability of PTX are limited, primarily due to its narrow therapeutic index and very poor solubility in water. So a series of conjugates has been synthesized with the goal of increasing the specificity of PTX toward cancer cells and multifunctional PTX-loaded drug delivery systems are being evaluated. Poly (amidoamine) (PAMAM) is one class of ideal dendrimers because of their unique characteristics such as uniform size distribution, relatively higher transfection efficiency, and lower cytotoxicity (esp. G3-G5) compared to other traditional cationic polymers, when used as drug delivery and probe system. In here, bifunctional therapeutic dendrimer conjugates have been synthesized containing chemotherapeutic PTX and folic acid. We have also demonstrated the applicability of dendrimers as a platform for conjugating Cy5.5 and IONPs forming novel nanoparticles as molecular imaging probes. Therefore, a novel therapeutic delivery system integrating imaging and targeting modalities (FA-PEG-G3.5-PTX-Cy5.5@IONPs) has been constructed. Collectively, animal studies suggest that these novel PTX-loaded conjugates have the potential to enhance the effect of fluorescence imaging, MRI contrast and cancer therapy in the course of delivering drugs to target sites.

Keywords: PTX, dual imaging probe, drug delivery.

PO-54

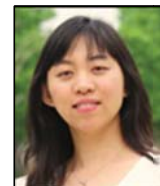
Track: Others – Genetics

BROAD EXISTENCE OF PLURIPOTENCY FACTOR REGULATED TRANSCRIPT ISOFORMS WITH STAGE-SPECIFIC ALTERNATIVE FIRST EXONS (SAFE) IN MOUSE EMBRYONIC STEM CELL

Xiu-Jie Wang

Center for Molecular Systems Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; E-mail: xjwang@genetics.ac.cn

Transcripts with stage-specific alternative first exon (SAFE) usage involve in the regulation of many biological processes, yet their presence and functions in embryonic stem cells (ESCs) are still largely unknown. In this work, we identify 137 mESC SAFE isoforms of 128 genes expressed in both ESCs and somatic cells. Functional analysis revealed that most genes participated in the regulation of stem cell regulated functions. The promoter regions of SAFE isoforms exhibit enriched H3K4me3 and Pol II binding as well as higher DNase I sensitivity in mESCs but not in somatic cells. We found an enrichment of Oct4, Sox2 or Nanog binding sites at the promoter regions of SAFE isoforms, and proved the transcription regulation of SAFE isoforms by these pluripotency factors experimentally. The expression of SAFE isoforms is activated during the reprogramming process of induced pluripotent stem (iPS) cells, and dynamically regulated in early stage embryos or during cell differentiation, indicating their functional importance in regulating pluripotency related cell features.



PO-46*Track: Regenerative Medicine***ESTABLISHMENT OF A NOVEL TUMOR ENDOTHELIAL CELL LINE DERIVED FROM HUMAN HCC****Liping Yang, Yixin Zhang, Wenjing Zhao, Chen Xudong, Qian Hongyan and Shao Jingjing**

Key Laboratory, Cancer Research Center Nantong, Nantong University, Jiangsu 226361, China;
E-mail: Liping.yang@ntu.edu.cn



Tumor angiogenesis plays a crucial role in cancer growth, recurrence and metastasis. However, the studies on the blood vessel formation and regulation in tumor have been hampered by the lack of a steady source of tumor endothelial cell (TEC). In our pilot study, we freshly isolated CD31+ endothelial cells from the tissues of human hepatocellular carcinoma (HCC) and cultured them in ECM medium with several supplements, and found that TECs in primary culture proliferated to confluency in 15 days to 20 days and stopped growing when subcultured to passage 7. To establish a novel TEC line, the cultured TECs were transfected with SV40-LT. The cloned TECs containing SV40-LT have been subcultured to passage 53 to date. The novel cell line of tumor endothelial cell derived from human HCC, with a steady growing rate in ECM medium has been established. These transfected TECs exhibited similar endothelial characteristics as the primary TECs, *i.e.* that appeared the cobblestone and spindle shape, Weibel-Palade bodies in the cells, both CD31 and CD105 expression on the cell surface, chromosome number with 44. Furthermore, functional assays showed that the tube formation and migration also occurred in the transfected cells. These results suggest that a novel TEC cell line established here maintains endothelial characteristics and potencies. Therefore, our TEC cell line should be useful for studying of tumor angiogenesis and vascular targeting therapy.

Acknowledgment: This study is granted by the Natural Science Foundation of China (NSFC), Grant No. 81272378.

Keywords: Tumor angiogenesis, tumor endothelial cell, cell line, human hepatocellular carcinoma.

PO-59*Track: Plant and Environment***ISOLATED AND FUNCTION ANALYSIS OF GAPC GENES AND THEIR PROMOTERS IN DIFFERENT WHEATS****Fenling-Liu¹, Min-Zhang, Shushen-Yang, Xiping-Deng and Lun-Shan**

College of Life Sciences, Northwest A&F University, China; E-mail: yangshushen2014@163.com



In recent years, the cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC) have been emerged as a multifunctional gene with defined functions in numerous subcellular processes, especially in abiotic stress tolerance. But several of them also had been considered as a constitutive housekeeping gene. We have previously identified six GAPC genes from wheat (*Triticum aestivum* L.) and the result of qRT-PCR showed that four of the TaGAPC genes could be a reference gene. In this study, to elucidate the regulatory mechanisms of similar reference gene in different wheats, two corresponding promoters of TaGAPC genes were isolated, which were 87.54% identical. Different cis-acting elements were harbored in two promoters, such as series dehydration and hormone responsive elements (MYBCORE, WREY and ASF1MOTIFCAMV) in P1683 and cold induced response elements (MYC and LTRCORE) in P1693. To further verification, the promoters were fused to GUS reporter gene and Agrobacterium-mediated transient expression assay were conducted. The GUS staining showed that both the two promoters could drive GUS gene and tissues transiently transformed by TaGAPC promoter- β -glucuronidase (GUS) gene fusion were differentially activated by NaCl, abscisic acid and low temperature. The results suggested that promoters from different reference gene contributed different to abiotic stresses.

Keywords: Triticum aestivum L.; GAPDH ; Promoter; Stress response; Abiotic stress.

PO-69

Track: Plant and Environment

BIOREMEDIATION OF WASTEWATER BASED ON MICROALGAE STRAIN - PRODUCER OF FATTY ACIDS, PROMISING FOR BIODIESEL

Bolatkhon K. Zayadan, Asem K. Sadvakasova, Fariza K. Sarsekeyeva, Aizhan A. Userbayeva and Dmitry A. Los

*Department of Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan;
E-mail: zbolatkhan@mail.ru*



Biodiesel production based on microalgae is an environmentally friendly alternative to conventional hydrocarbons. The use of domestic wastewater for the cultivation of microalgae can not only significantly reduce the cost of biodiesel production technology, but also provide the bioremediation of polluted wastewater, which is an actual problem of modern biotechnology. Axenic strains of microalgae were isolated from wastewater treatment systems of Almaty city: *Chlorella vulgaris*-1, *Chlorella* sp.-3, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*. In the results of received cultures screening the maximum increase of biomass was observed in *Chlorella vulgaris*-1 microalgae strain, consequently this strain was selected for next researches.

To study the bioremediation ability of *Chlorella vulgaris*-1 strain, it grown in laboratory photobioreactor with wastewater and Tamia nutrient medium in ratio 1:1. Cultivation carried out in 14 days, at the temperature 27-28°C, at continuous lighting at 120 μ moles of photons $m^{-2} \cdot s^{-1}$. Original number of strain cells was $0,1 \times 10^6$ cell/ml. During the experiment, the number of strain's cells were increased and the maximum level was 150×10^6 cell/ml on 12 day of cultivation. Results of physical and chemical analysis had shown that the overall rate of wastewater treatment in cultivation of *Chlorella vulgaris*-1 strain is equal to 96%.

After the cultivation, we have determined total number of lipids, which constitute 37% of dry weight. The results of fatty acids composition analysis have shown that *Chlorella vulgaris*-1 strain contains more than 26% of polyunsaturated fatty acids in the cell.

Therefore, as a result of this research the ability for bioremediation of *Chlorella vulgaris*-1 strain and its application as a prospective object for biodiesel production were determined.

Keywords: Microalgae, bioremediation, wastewater, lipids, fatty acids, biodiesel.

CUMULATIVE AUTHOR INDEX

A

Abanobi, S.E.	253
Abbade, L.P.F.	231
Abbas, T.	130
Abdellatif, L.	236
Abdin, A.A.	40
Abdo, M.	71
Abdullah, J.M.	195
Abdullah, S.	224
Abdulmalik, O.	108
Abedi, A.	40, 135
Abell, C.	139
Abid, M.	72
Abolfazl, Z.	160
Abrams, J.	106
Aburatowska, A.	298
Acuña, C.C.	305
Acuña, J.F.	155
Adams, R.H.	260
Addiel, V.-S.J.	274
Adegoke, O.A.	136
Aderogba, A.A.	99
Adessi, A.	291
Adi, S.	162
Aeressens, J.	103
Afosah, D.	136, 191
Agboh, K.M.	152
Aghoja, O.C.	137
Agrawal, N.	41
Aharoni, R.	42
Ahlquist, D.A.	49
Ahmad, K.	180
Ahmed, N.	180
Ajala, T.O.	99
Akhtar, M.W.	293
Akimbekov, N.	274
Akin-Ajani, O.D.	99
Akintola, J.A.	176
Akonoghrere, R.O.	138
AL-Abraham, J.S.	275
Alam, M.	171
Alam, R.	293
Alañón, M.E.	210
Al-Daoude, A.	276
Al-Doss, A.A.	275
Al-Faifi, S.A.	275
Al-Hendy, A.A.	113
Al-Horani, R.A.	46, 190
Ali, M.	148
Ali, R.	148
Ali, S.	43
Ali, Z.	216
Alia, O.	276
Alić, I.	306
Aliper, A.	209
Almansoori, A.A.M.J.	216
Alpaslan, F.N.	51
Al-Qurainy, F.	276
Altawallbeh, G.	277
Althamrah, M.I.	275
Althoun, D.	96
Amabeoku, G.J.	138
Amadi, C.N.	139
Amador, A.	140
Amani, V.	40, 135
Amaning, K.	102

Amar, K.O.	252
Amin, H.A.	217
Amin, K.	210
Amir, R.	217
Ammar, M.H.	275
Anaga, A.O.	143
Anastasio, N.C.	134
Anderson, A.	143
Anderson, G.	170
Andrade, K.C.de	282
André-Barrès, C.	48
Andrews, D.	123
Anflous-Pharayra, K.	43
Anteck, A.	300
Anwar, M.M.	131
Aoki, S.	200
Apostol, I.	268
Appella, D.	44
Arab-Tehrany, E.	77, 86, 140
Arafa, F.	247
Arai, K.	90
Arantes, V.	299
Aremu, O.O.	175, 177
Aresh, W.	98
Ari, Ş.	45
Arif, I.	236
Aristizábal, A.M.O.	292
Aristizabal, F.A.	155
Arnold, A.	218
Arora, K.	260
Artemov, A.	209
Arun Kumar, A.G.	277
Aslam, M.	130
Aslam, M.S.	294
Aslam, S.	171
Asori, M.	160
Assiri, A.M.A.	239
Astner, S.T.	115
Asuzu, I.U.	143
Ata, A.	17
Atac, A.O.	51
Athar, M.A.	294
Atkinson, R.L.	1, 201
Atta-ur-Rahman,	9, 208
Azhar, A.	241
Azhar, Z.A.	274
Azimzadeh, A.M.	46
Azizzeddin, A.	240
Azzouni, M.Z.E.	247

B

Bachrach, N.	218
Bacqué, C.	48
Badley, A.D.	47
Baffigo, G.	173
Bagasrawala, I.	278
Bagde, U.S.	219
Bahron, H.	200
Baht, K.	190
Bai, M.-Y.	283
Bai, X.	244
Baig, I.-u.-R.	171
Baishkhanova, D.	316
Bajorath, J.	182
Baklaushev, V.P.	219
Balaji, S.	220
Balcioğlu, H. A.	221, 222
Balcioğlu, N.	221

Ball, J.A.	125
Baltas, M.	48
Bandara, M.N.S.	29
Bandehpour, M.	314
Bano, S.	279
Barbosa, A.N.	231
Barbosa, J.B.	180
Barral, J.M.	273
Barraviera, B.	231
Barraviera, S.R.S.	231
Bartz, U.	182
Barykina, D.V.	60
Baser, K.H.C.	168
Bashir, R.	49
Bat-Erdene, U.	119
Batrakova, E.	107
Bauer, C.	17
Bayachou, M.	277
Baydoun, E.	279
Bayer, C.	115
Bednarek, A.	248, 279
Bedos-Belval, F.	48
Begimbetova, D.	316
Behroo, L.	141
Behroo, L.	141
Behroo, L.	141
Behrouzi, B.	240
Belda, M.A.	281
Beleboni, R.O.	289
Benderitter, M.	209
Benipal, P.K.	46
Ben-Nun, A.	79
Bernacchi, S.	229
Bernkop-Schnürch, A.	150
Bhagawat, S.	190
Bhardwaj, A.	114
Bharthur, A.	56
Bhat, K.	142
Bhat, N.R.	32
Bilal, M.	195
Biljon, R.A.van.	184
Birkholtz, L.-M.	184
Bischoff, F.R.	96
Bitencourt, T.A.	280
Bizukojć, M.	300
Bjorndahl, T.C.	177
Blatkiewicz, M.	300
Bojničić-Kninski, C.v.	95
Bond, M.J.	143
Borger, D.R.	50
Borisov, N.	209
Borshcheva, A.A.	60
Bosha, J.A.	143
Bou, J.J.	280
Bover, E. A.	281
Bover-Arbona, E.	311
Braddock, D.T.	50
Bradford, A.	210
Bravin, A.	224
Breitbart, E.	67
Breitling, F.	95
Breitrück, A.	144
Breitrück, A.	160
Breitsprecher, D.	102
Budimir, A.	290
Bukhari, N.I.	148
Bukreeva, I.	224
Buonamici, S.via	90
Burdorf, L.	46

Burdorf, L.	46
Burris, T.P.	140
Burton, R.A.	222
Busath, D.D.	192
Buyukbingol, M.E.	51
Buyukbingol, Z.	144
Buzdin, A.	209
Bykovskaya, V.	95
Byun, I.	243, 301
Byun, I.-G.	223, 302

C

Cabello-Verrugio, C.A.	171
Cabral, F.A.	150
Çağlar, M.	111
Cahill, C.M.	106
Cai, M.	223
Cai, X.	52
Çakır, Ö.	45
Campelo, P.M.S.	282
Campi, G.	224
Cancedda, R.	224
Cantor, C.	123
Cao, H.	54
Cao, S.	212
Carayon, C.	48
Carvalho, J.E.	150
Cascante-Alpizar, J.R.	280
Cashman, N.	177
Castellana, M.C.	145
Castellar, A.	145
Castro, J.P.	155
Castro, J.P.	181
Cattoz, B.	150
Cavazzana, M.	264
Cedola, A.	224
Cejalvo, D.A.	281
Cejalvo-Lapeña, D.	311
Ceponkus, J.	316
Čeřovský, V.	174
Cha, J.	273
Chae, M.	273
Chagas, A.C.S.	289
Chaguturu, R.	8
Chalivendra, V.B.	123
Chan, B.	90
Chan, W.P.	283
Chandran, P.	103
Chang, C.-C.	146
Chang, C.-C.	146
Chang, T.Y.	52
Chang, X.-R.	53
Chao, P.-J.	302, 303
Chao, W.	53
Chapel, A.	209
Chaudhuri, G.	8
Checler, F.	17
Chekhonin, V.P.	219
Chekler, E.L.P.	146
Chekler, E.L.P.	18
Chen, B.	147
Chen, C.	244
Chen, C.-D.	147
Chen, J.	197
Chen, J.	256
Chen, P.-H.	147
Chen, S.	273
Chen, S.-R.	148

Chen, X.	54, 66, 197, 132
Chen, Y.	61, 88, 110, 120
Chen, Y.-P.	147
Chen, Z.	194
Cheng, K.	54
Cheng, X.	46
Cheng, X.F.	46
Chhikara, A.	55
Ching, F.P.	283
Chinnakannu, P.	32
Chiou, T.-W.	148
Chmielinska, J.J.	16
Cho, K.-S.	310
Choi, M.	119
Chong, K.P.	224
Chopra, A.	143
Chopra, M.	55
Chopra, M.	56
Chopra, N.	13
Chopra, N.	56
Choudhary, M.I.	208
Choudhary, M.I.	9
Chowdhury, E.K.	261
Chu, L.	268
Chuan, J.-I.	127
Chuang, H.-M.	148
Chuang, S.-E.	148
Chung, A.M.	284
Chung, T.D.Y.	19
Ciani, E.	312
Clasen, B.	225
Cleary, J.	57
Coffer, J.L.	320
Cohen, L.	79
Cohen, S.	252
Cohen, Y.	67
Colquhoun, A.	184
Comet, M.	34
Cooper, D.K.C.	46
Cooper, M.	69
Corassin, C.H.	308
Corley, M.	284
Corrales, L.L.G.	292
Corson, L.	90
Corti, O.	17
Corvese, F.	173
Costa, C.A.da.	17
Coulthard, R.W.	165
Coyne, A.G.	139
Coyne, C.P.	58
Crepaldi, P.	312
Crivat, G.	58
Cross, T.A.	192
Cui, B.	132
Cunha, L.E.R.da.	231
Cunningham, K.A.	134
Curiel, D.	113

D

d'Ippolito, G.	291
D'Souza, G.G.M.	59
Dahari, M.I.	319
Dahi, S.	46
D'Andria, D.	173
Danish, M.Z.	148

Danish, Z.	180
Danish, Z.	181
Darbandi, A.I.	292
Darling, T.N.	52
Darvesh, S.	19
Datta, D.	119
Dauda, M.	60
Dave, S.	259
Dave, S.R.	257
Dedeoglu, A.	72
Deenanath, E.D.	285
Deepatana, A.	321
Defant, A.	79
DeFronzo, R.A.	226
Dejahang, A.	292
Delicato, G.	173
Demerzhan, S.	132
Demirci, B.	161, 167
Demorest, Z.	225
DeOliveira, C.	234
Desai, U.R.	20, 46, 108, 136, 191
Deshpande, T.	108
Desobry, S.	77, 140, 163
Devine, M.	263
Dewprashad, B.	210
Dhapare, S.	108
Dhar, G.	8
Diab, R.G.	247
Diamond, M.	113
Diaz, F.	155
Díaz-Maroto, C.	210
Díaz-Maroto, I.	210
Dickey, I.D.	225
Dikmen, G.	151
Dikmen, G.	156
Dimauro, C.	312
Dimitrijevič, S.D.	320
Dimitrov, D.S.	11
Ding, C.	134
Ding, H.	107
Ding, J.	196, 197
Dipasquale, L.	291
Djinni, I.	79
Do, H.	132
Dobrolecki, L.E.	132
Dobrovolskaia, M.A.	59
Doiron, B.	226
Dominciano, L.C.C.	308
Don, E.S.	60
Donaji, J.I.	274
Dong, Q.	271
Dong, X.	270
Dostert, G.	77
Douay, L.	209
Doweidar, M.H.	227
Drewniak, L.	305
Drndić, M.	61
Du, L.	61
Dudhia, N.	138
Duhr, S.	102
Dulcey, A.E.	62
Duncan, K.W.	62
Duplan, E.	17
Durán, I.D.A.	171
Durmaz, B.	314
Dutta, M.	119
Dutta, P.	114
Dziadek, J.	248, 279
Dziedziczak, K.	279

E

Eder, J.	63
Edoh, N.	228
Edoh, N.L.	285
Edri-Brami, M.	245
Egashira, R.	286
Egesi, C.N.	228, 285
Ehtesham, M.	171
Ejaz, A.	63
Ekhaïse, F.O.	152
Eksangsri, T.	286
El-Adawi, H.	286, 287
El-Gaaly, G.A.	236
Elhadidi, A.	247
Elie, H.	65
Ellis, M.	228
Elmacı, C.	167
El-Meligi, A.A.	64
Elobeid, M.M.	275
EL-Refai, A.A.	239
Elshami, F.M.	185
Elsoud, M.M.A.	217
El-Wahab, E.W.A.	229
Encinales, L.	206
Encinales, L.	6
Endo, A.	90
Ene., A.C.	253
Epure, D.G.	291
Epure, L.I.	291
Ercan, G.	315
Erdas, O.	51
Ergün, B.	153
Eris, T.	268
Erös-Honti, Z.	265
Esselstyn, C.B.	229
Estrada-Chávez, C.	287
Eyunni, S.V.K.	185

F

Fachin, A.L.	280
Fæste, C.K.	153, 154
Falcon, R.	285
Falodun, A.	283
Fan, Y.	131
Fares, F.	230
Farid, S.	119
Farshad, H.	160
Fattah, A.F.A.	286
Fattah, A.F.A.	287
Federlin, J.D.	126
Feige, E.	67
Feldman, M.	175
Felipe, M.d.G.A.	314
Feng, X.	88
Feng, X.-r.	87
Feng, Y.	267
Feng, Y.-S.	86
Fenling-Liu,	323
Feris, E.J.	6, 206
Fernandez-Vina, M.	206
Fernandez-Vina, M.	6
Fernandes, K.L.M.	282
Ferreira, L.E.	289
Ferreira, R.S.	231
Ferrer, M.	62
Fischer, A.	95
Fischer, R.	232

Fontana, A.	291
Font-Réaulx, E.d.	149
Forte, J.	284
Förtsch, T.	95
Foster, P.	234
Fox, R.G.	134
Frączek, T.	154
Frame, N.	289
França, S.C.	289
Francius, G.	86
Franco, L.A.	155, 181
Franco, M.E.	280
Francois, P.	65
Franklin, N.S.	123
Fratini, M.	224
Freedman, S.	65
Freitas, J.A.	179
Freitas, T.E.	184
Freitas, V.S.de.	150
Fridman, M.	175
Fröhling, A.	309
Frysiak, M.	186
Fu, P.(Patrick)	233
Fu, X.	73
Fuentes, D.C.	155
Fujii, H.	96
Fukao, Y.	262
Fukutomi, Y.	250
Fulgosi, H.	290
Furtmann, N.	182

G

Gaafar, M.R.	247
Gabashvily, A.N.	219
Gaertner, F.C.	115
Gağala, I.	248, 279
Gaidau, C.	291
Gaitán, R.	155
Gajović, S.	306
Gandler, S.	135
Ganella, D.E.	165
Gao, H.	65
Gao, J.	194
Gao, R.	54
Gao, R.	66
Gao, Y.	194
García, A.M.C.	292
Garippa, R.J.	66
Gautier, C.A.	17
Gavilá, J.T.	311
Gaziz, B.	190
Gelperina, S.E.	219
Genç, L.	111
Genç, L.	151
Genç, L.	156
Gen-Xi, Z.	320
Georges, A.	65
Gerard, B.	90
Gerszten, R.	63
Getz, L.E.	320
Geudens, N.	92
Ghafoor, F.	293
Ghafouriyani/Ghafourian, M.	141
Ghaskadbi, S.S.	178
Ghatge, M.	108
Ghazy, A.I.	275
Gheorghe, B.	46
Ghosh, D.	290

Giardina, C.	143
Gibbs, E.	177
Gidea, M.	291
Gil, D.M.	291
Gilbertson, S.R.	132
Gillet, G.	163
Gillet, G.	77
Ginsburg, I.	175
Girish, H.N.	294
Girish, S.	110
Gobilik, J.	224
Goger, F.	161
Göger, F.	167, 168
Goharrizi, M.A.S.B.	292
Goiran, T.	17
Goldfine, A.	63
Golubić, M.	229
Gómez, O.M.	292
Gong, Y.	163
Gonzalez, E.M.	131
González-Nilo, F.D.	171
Gopal, R.	103
Gorbunov, E.A.	60
Gorin, N.C.	209
Goss, R.	264
Goto, S.	262
Gowrish, .	321
Graf, M.	121
Granada, S.D.G.	292
Granados, J.	6, 206
Grassi, G.	197
Grechenko, V.V.	60
Green, A.	212, 255
Greene, M.	210
Greer, M.	218
Greif, D.M.	260
Greig, N.H.	13, 21, 32, 56, 72, 188
Griffiths, P.C.	150
Gu, S.	271
Guilhon-Simplicio, F.	282
Gul, R.	293
Gull, I.	293
Gulzhamal, A.Z.	274
Gulzhan, K.K.	274
Gunathilake, V.K.	156
Gündüz, C.	314
Guo, J.	273
Guo, S.	170
Gursky, O.	289
Gütschow, M.	158, 182
Guzel, M.	67

H

Habaki, H.	286
Habenschus, M.D.	173
Hacham, Y.	217
Hadir, L.	210
Hafeez, F.Y.	216
Hahn, L.	95
Hajirezaci, M.-R.	292
Hamaguchi, N.	308
Hamel, C.	236
Hamid, F.B.	318
Hamulakova, S.	81
Handunnetti, S.M.	29
Hanif, S.	198
Hanif-Ur-Rehman	184
Hansen, C.	233

Hao, M.-H.	90
Haque, M.M.	277
Haque, Z.	171
Harats, D.	67
Harn, H.-J.	148
Harris, D.G.	46
Harrison, D.A.	211
Harrison, J.P.	211
Hart, M.	61
Hartley, R.M.	134
Hasançebi, S.	45
Hashiguchi, M.	307
Hashimoto, M.	157
Hassan, W.	68
Haughey, M.	52
Haun, W.J.	225
Häußler, D.	158
Hawkrigde, A.M.	68
Hayashi, H.	250
Hayashida, K.	97
He, J.	195
He, S.	62
He, T.	196
Hebb, M.O.	234
Hebb, M.O.	69
Hedayatizadeh-Omran, A.	159
Hegde, V.M.K.	294
Heimann, K.	312
Heinigen, A.V.	310
Helmrath, M.A.	234
Hendriks, R.	210
Hernández, O.R.	292
Hernández-Valencia, A.	280
Herrera, J.S.	292
Herz, T.	69
Hezave, S.J.H.	160
Hijnen, M.	229
Hiroayama, S.	97
Hodgetts, K.	106
Hoffer, B.J.	21, 188
Hoffman, E.	11
Hofrichter, J.	144, 160
Holliday, L.S.	70
Holson, E.	71
Hong, G.	243
Hong, Y.-Q.	238
Hongtrakul, V.	294
Hongyan, Q.	323
Hop, C.	110
Hosny, S.	71
Hossain, M.A.	318
Hoying, J.B.	211
Hrabinova, M.	81
Hsiao, T.-H.	61
Hsieh, J.	148
Hsu, S.-L.	146
Hu, J.	270
Hu, R.	270
Hu, W.	223
Hu, W.	53
Hu, X.	62
Hu, Z.	62
Huang, C.	261
Huang, C.-Y.F.	146
Huang, M.-H.	148
Huang, X.	13, 72, 106
Huang, Y.-J.	302, 303
Huang, Z.	73
Huerlimann, R.	312

Huitron-Resendiz, S.....	140
Hung, I.....	192
Hung, W.-C.....	170
Huo, L.....	132
Hür, D.....	151
Hür, E.....	111
Hussain, H.....	74
Hussain, N.....	148
Hussain, R.A.H.N.....	216
Hussain, S.....	256
Hwua, J.R.....	199

I

Ibrahim, M.M.....	236
Icgen, B.....	295
Idress, A.....	130
Idris, Z.....	195
Iijima, Y.....	250
İldeşer, A.C.....	111
Ilya, D.....	274
Imrich, J.....	81
Inagaki, S.....	307
Inceer, H.....	168
Ioannidis, H.....	192
Iqbal, J.....	148
Iqbal, M.A.....	279
Irfan, N.....	148
Iscan, G.....	161, 167
Islam, J.M.M.....	261
Ismahane, B.....	250
Ismail, A.H.B.....	195
Ismail, M.....	138
Issa, D.F.....	228
Ivanova, L.....	153, 154
Iwade, Y.....	75
Iwai, T.....	96
Iwase, H.....	46

J

Jabbari, E.....	161
Jabbari, E.....	22
Jacob, J.....	309
Jacobsen, H.J.....	216
Jacquot, A.....	86
Jager, R.D.....	123
Jaleel, S.....	130
Jallal, D.....	250
Jamal, Z.....	162
Janas, R.....	269
Janovec, L.....	81
Jayaraman, S.....	289
Jayasinghe, C.D.....	75
Jazestani, J.....	236
Jemioła-Rzeminska, M.....	264
Jeong, J.C.....	242, 297
Ji, L.....	237
Jia, Z.....	244
Jiali, Z.....	53
Jian, X.....	163
Jiang, H.-Y.....	238
Jiang, T.....	223
Jiang, Z.-G.....	23
Jian-Hua, Z.....	124
Jie, Y.....	53
Jin, J.Y.....	110
Jinchang, L.....	267
Jingjing, S.....	323

Jinli, T.....	267
Jin-Yu, W.....	320
Jirillo, E.....	24
Jobling, S.A.....	239
Joshi, A.....	76
Jossinet, F.....	229
Jr, M.S.....	180
Jung, J.....	315
Jung, S.....	315
Jusoh, S.A.....	200

K

Kachan, L.....	217
Kachole, M.....	254
Kaci, M.....	163
Kaci, M.....	77
Kadokawa, J.-i.....	77
Kadri, R.....	140
Kaftanovskaya, E.M.....	107
Kahn, E.....	78
Kahyaoglu, Ş.G.....	156
Kakutsi, O.....	138
Kamel, H.F.M.....	239
Kaminuma, O.....	250
Kanda, T.....	96
Kaneki, M.....	296
Kang, H.-G.....	164
Kanthikeel, S.P.....	107
Kaplan, A.....	217
Kaproń, B.....	187
Kara, N.T.....	45
Karabay, A.Z.....	144
Karr, C.....	90
Karuturi, R.....	46, 136
Kashman, Y.....	175
Katakura, M.....	157
Kato, K.....	157
Kato, S.....	157
Katou, K.....	307
Katrlík, J.....	317
Kaushansky, N.....	79
Kayano, S.-I.....	240
Kazmi, S.N.Z.....	68
Ke, Q.....	297
Keaney, G.F.....	90
Kecha, M.....	79
Kelley, S.....	80
Kenigsberg, S.....	240
Kennedy, E.J.....	80
Kerkhoff, C.....	144
Kerkhoff, C.....	160
Kersavond, T.V.....	92
Khademhosseini, A.....	12
Khademhosseini, A.....	43
Khalil, N.I.M.....	319
Khalil, R.A.....	24
Khan, A.....	130
Khan, M.A.....	275
Khan, S.....	276, 241
Khater, M.K.....	113
Khensri, C.....	294
Khursheed, R.....	164
Kikuzaki, H.....	240
Kim, E.L.....	241
Kim, H.....	243, 301
Kim, H.-G.....	223, 302
Kim, H.S.....	297
Kim, J.....	318

Kim, J.H.	165
Kim, J.Y.	105
Kim, S.	106
Kim, S.H.	242
Kim, Y.	243, 301
Kim, Y.-W.	223, 302
Kinoshita, A.	250
Kira, K.	90
Kirou, E.	106
Kirtikumar, P.M.	166
Klochko, V.V.	105
Kogan, A.	213
Kolate, E.	248
Kolocouris, A.	192
Kolta, M.G.	172
Korabecny, J.	81
Korayem, H.E.	71
Kóródi, G.	313
Korzinkin, M.	209
Kosari, F.	49
Kose, Y.B.	161, 167
Kosi, N.	306
Kouyama, S.	250
Kramer, J.H.	16
Kristian, P.	81
Kronfli, A.	46
Krzystek, L.	300
Kubicki, N.	46
Kuca, K.	81
Kucharek, K.	305
Küçük, S.	168
Kudabaev, E.	190
Kulig, K.	182
Kulus, D.	298
Kumar, A.	260
Kumar, P.	55, 262
Kumar, R.	12
Kumar, V.	117
Kuo, P.-F.	170
Kuret, J.	25
Kurkcuoglu, M.	168
Kwak, S.-S.	242, 297
Kwan, H.-S.	299
Kwong, P.D.	194

L

Lahiri, D.K.	13, 21, 32, 56, 72, 188
Laila, M.	276
Lajara-Romance, J.M.	311
Lall, N.	81
Lan, Y.(E.)	119
Lataillade, J.J.	209
Latha, K.P.	299
Latowski, D.	264
Lavis, I.	77, 163
Lawrence, A.J.	165
Lawson, K.	268
Lawton, R.B.	123
Le, P.T.	83
Leboulch, P.	264
Leburton, J.-P.	25
Ledakowicz, S.	300
Lee, C.	240
Lee, H.-J.	301
Lee, H.-S.	242, 297
Lee, H.-Y.	302, 303
Lee, J.	164, 243, 301
Lee, J.-H.	223, 302

Lee, L.	90
Lee, S.	243, 301
Lee, S.H.	223, 243, 302
Lee, S.H.I.	308, 309
Lee, T.-F.	302, 303
Lee-Kardashyan, L.	165
Legro, N.	240
Lemere, C.A.	83
Leong, H.	234
Lerin, C.	63
Leroudier, N.	17
Levy, C.W.	139
Levy-Lahad, E.	17
Lewis, M.T.	132
Leys, D.	139
Lezhnina, K.	209
Li, B.	128
Li, C.	110
Li, E.	63
Li, F.	132
Li, J.	225
Li, J.-D.	82
Li, J.T.	132
Li, L.	177
Li, M.-M.	238
Li, Q.	106
Li, R.	84
Li, S.	245
Li, W.	85, 131, 244, 245, 268
Li, Xu	244
Li, Y. P.	322
Li, Y. X.	322
Li, Y.	21, 167, 168
Liang, J.	73
Liang, M.	63
Liang, T.J.	62
Liang, W.-y.	87
Liao, C.-L.	301
Liao, J.-T.	301
Librach, C.	240
Lichtenstein, R.G.	245
Lichtensztejn, Z.	93
Liechty, K.W.	270
Lighvan, Z.M.	135
Lim, H.-J.	164
Lim, H.-K.	246
Lim, J.-S.	223, 302
Lin, C.-H.	170
Lin, G.	61
Lin, H.-Y.	169
Lin, M.-F.	283
Lin, S.-Z.	148
Lin, Y.-L.	301
Lin, Y.-P.	86
Linard, C.	209
Linder, M.	86, 139
Linhardt, R.J.	14, 87
Linscombe, S.	267
Linscombe, S.D.	269
Liou, M.-H.	302, 303
Liu, C.	295
Liu, H.-S.	304
Liu, J.	89
Liu, M.	53, 88
Liu, M.-I.	87
Liu, P.	246
Liu, Q.	212
Liu, T.	26, 88, 168
Liu, X.	90, 271

Liu, Y.	53, 97, 105, 195, 273
Liu, Z.-R.	89
Ljuma-Skupnjak, L.	290
Lloris, J.M.B.	281
Lloris-Carsii, J.M.	311
Lloyd, A.J.	195
Loeb, P.G.M.	29
Loeffler, F.F.	96
Loh, K.-C.	304
Lombardo, F.	91
Lomza, P.	305
Long, J.	56
Long, J.M.	13
Los, D.A.	324
Lovas, V.	265
Lowe, J.T.	90
Lowe, T.L.	26
Lu, D.	110
Lu, G.	194
Lu, P.-J.	170
Lu, P.-J.	193
Luikinga, S.	165
Lum, B.	110
Lun-Shan.	323
Luo, S.	225
Luo, T.	90
Lv, S.	194
Lyu, J.	295

M

Ma, J.-X.	212
Ma, X.	61
Mabuya, Z.	138
Maciotta, N.P.P.	312
Mackie, P.	240
Madder, A.	92
Madhaiyan, M.	237
Madsen, H.B.	165
Mady, R.F.	247
Mady, R.F.	247
Maftai, C.-A.	115
Mahfouz, T.M.	145
Mahmood, H.	279
Mahon, D.	26
Mailler, E.	229
Majid, G.	180
Majumder, R.	91
Mak, I.T.	16
Mak, J.	229
Makki, M.A.	171
Malinovskaya, Y.	219
Maloney, B.	13
Mamun, A.A.	157
Mancini, I.	79
Manfredsson, F.P.	106
Mankiewicz-Boczek, J.D.	248, 279
Mapalagamage, M.S.	29
Marcaurelle, L.A.	90
Mardones, O.F.	305
Mareosva, P.	81
Mari, Z.	131
Marie-Claire, F.K.	65
Marik, P.	306
Marins, M.	280
Marquet, R.	229
Márquez-Miranda, V.O.	171
Martins, J.C.	92
Marugan, J.J.	62

Maruszewski, B.	269
Masroor, M.S.	171
Mastrogiacomo, M.	224
Mathieu, N.	209
Mathis, L.	225
Matityahu, I.	217
Matsumoto, Y.	92
Matusinsky, P.	306
Maurya, D.K.	178
Mayes, M.	123
Mazzio, E.A.	185
Mbamalu, O.N.	138
McAllister, C.	134
McInnes, C.	27
Mclean, K.J.	139
McManus, K.J.	93
Mechanick, J.I.	27
Mehmood, S.	293
Mehregan, D.R.	106
Mehta, A.Y.	46, 191
Melicherčik, P.	174
Melnikov, P.A.	219
Mengyuan, G.	267
Meriem, K.	250
Meron-Sudai, S.	175
Meshik, X.	119
Messeha, S.S.	172, 185
Meziani, S.	234, 248
Mi, L.	53
Micale, N.	197
Michaux, C.	249
Migdadi, H.M.	275
Miguel, M.D.	282
Miguel, O.G.	282
Mikami, H.	307
Mikuła, A.	298
Miljuš, G.	317
Miller, J.	46
Mingchen, Z.	267
Min-Zhang.	323
Miranda-Hernández, D.U.	287
Mishra, P.J.	93
Mitani, T.	240
Mitra, A.K.	94
Mitrečić, D.	306
Mitrokhin, D.	190
Mitsui, C.	250
Mitzner, S.	144, 160
Miyagi, Y.	307
Mizui, Y.	90
Modaresi, M.	94
Modica-Napolitano, J.S.	95
Mohamad, F.H.	195
Mohamad, H.	195
Mohammadreza, V.	160
Mohammed, A.E.	275
Mohite, S.L.	219
Moioli, B.	312
Moldakulova, E.	190
Molinari, B.	123
Moloney, M.G.	14
Monsef, D.A.A.	247
Montagna, G.	173
Mooberry, S.	61
Mora-Gutierrez, A.	249
Morales, G.	171
Moreira, F.L.	173
Mori, A.	250
Morocco, J.	210

Moss, J.	52
Motaleb, M.A.	131
Mothy, M.	209
Mousavi, S.J.	227
Mukadam, S.	110
Mukherjee, S.	119
Müller, K.	155
Munir, M.S.	171
Munro, A.W.	139
Murad, F.	2, 202
Musmade, P.	142
Mustafa, A.	96
Mutiti, S.	284
Muzi, F.	173

N

N'Da, C.I.	174
Nadeem, M.	276
Nagase, H.	96, 307
Naim, M.M.	71
Naimi, D.	250
Nair, M.	107
Nakabayashi, R.	262
Nakamura, S.	307, 308
Nakamura, Y.	307
Nakata, E.	96
Nakatani, N.	240
Naor, D.	252
Nardulli, A.M.	49
Narmoneva, D.	213
Naseem, S.	216
Nasir, B.	148
Nawrot, I.	269
Nazarewski, S.	269
N'Da, D.D.	184
Nedic, M.Š.O.	317
Nepovimova, E.	81
Nesterov-Müller, A.	95
Nestor, C.	292
Nešuta, O.	174
Neto, A.C.	175
Neumann, S.	28
Nevalainen, M.	90
Nho, K.	56
Niculesu, M.	291
Nie, G.	170
Nieh, M.-P.	97
Nikiforova, M.V.	60
Nikolovska-Coleska, Z.	98
Niu, L.	195
Njoku, H.	228
Nkeh-Chungag, B.N.	175
Nkeh-Chungag, B.N.	177
Nnadi, U.V.	253
Noor, N.B.M.	195
Noordien, N.	138
Nowak, M.	203
Nowak, M.	3
Nuijten, M.	252
Nüsslin, F.	115
Nwachukwu, N.	253
Nyalambisa, M.	175

O

O'Shea, M.W.	90
Ocampo, Y.C.	155
Ocampo, Y.C.	181

Odeku, O.A.	98
Ofek, I.	175
Ogata, Y.	262
Ogbokiri,	228
Ogino, S.	3, 203
Ohtomo, M.	250
Ohtomo, T.	250
Ohtomo-Abe, A.	250
Okada, M.T.S.	212
Okamoto, N.	307
Oku, T.	307, 308
Okwu, Gloria N.	253
Okwuonu, I.	253, 285
Oliveira, A.R.M.de.	173
Oliveira, C.A.F.	308, 309
Oliveira, C.d.	69
Oliver, D.	217
Oloyede, G.K.	176
Oluwafemi, S.O.	175
Onay, H.	314
Onocha, P.A.	176
Onur, G.	295
Onyeka, J.	285
Oparah, A.C.	138
Orlando, I.	254
Orsi, R.d.O.	231
Oshikata, C.	250
Osman, H.	195
Ostad, S.N.	135
Ostapenko, A.	190
Ouyang, C.	270
Owens, L.	312
Oyediji, A.O.	175
Oyediji, A.O.	177
Oyediji, A.O.	99
Oyediji, O.O.	175
Oyediji, O.O.	177
Özkinay, F.	314
Ozyurek, O.	310

P

Pacheco, N.C.	171
Pacheco-Rodriguez, G.	52
Padmaraju, V.	32
Padrón, C.A.	281
Padrón-Sanz, D.L.-M.C.	311
Pagadala, N.S.	177
Paillart, J.-C.	229
Palenzuela, D.	28
Palmieri, L.	254
Palsson, B.	4, 204
Panat, N.A.	178
Panchal, B.	254
Panday, S.P.	255
Pandey, K.	114
Paneth, A.	186
Paneth, P.	154
Paoli, S.D.	20
Pappolla, M.A.	32
Parekh, B.	26
Parenteau, N.L.	214
Parikh, A.	108
Parikh, D.	119
Park, A.	164
Park, E.S.	90
Park, S.	297
Park, S.-C.	242
Park, T.	223, 243, 301, 302

Park, T.-H.	310
Parrondo, R.	28
Parveen, S.	256
Patel, S.D.	100
Pati, D.	132
Patti, M.-E.	63
Pavelka, S.	100, 178
Pazera, A.	300
Pei, G.	120
Peng, Y.	86
Penndrof, A.K.	175
Pérez-Coello, M.S.	210
Perez-Pineiro, R.	177
Perez-Stable, C.	28
Perino, S.A.	90
Perizat, U.S.	274
Perla, A.	173
Perpète, E.A.	249
Perreault, L.	123
Pertsemlidis, A.	61
Pessoa, A.Jr.	314
Petrie, J.	255, 263
Petrosino, V.	224
Petzer, A.	174
Petzer, J.P.	174
Philippis, R.D.	291
Pi, Y.	270
Pick, C.G.	188
Pick, C.G.	21
Pierson, R.N.	46
Pierson, R.N.	46
Pietro, A.D.	10
Pietro, R.C.L.R.	179
Pilla, F.	312
Pimenta, D.C.	231
Piperno, A.	197
Pleat, M.J.	228
Plech, T.	187
Ploeg, L.V.der.	123
Podkowa, A.	182
Pokhil, S.E.	60
Ponert, M.	182
Popov, R.	96
Pozas, A.d.I.	28
Prabhakaran, J.	189
Prajapati, S.	90
Prata, A.M.R.	314
Pratap, P.	119
Praveen, P.	304
Premawansa, S.	29
Prieto-Moure, B.	311
Pucetaite, M.	316
Pulina, G.	312
Pupulin, Á.R.T.	180
Puri, A.	30, 97
Putanyawiwat, P.	295

Q

Qi, R.	101, 147
Qian, J.	26
Qiang, W.	124
Qing-can, F.	320
Qiu, Y.	73
Quach, K.	240
Qureshi, J.	180

Qureshi, M.S.	171
---------------	-----

R

Rabbani, T.	180
Rachmany, L.	187
Radonjic, N.	278
Rajarathnam, K.	102
Rajashekar,	321
Rak, A.	102
Rali, S.	177
Ramanathan, A.	103
Ramirez-Cerda, E.L.	287
Raneen, M.	162
Ranginwala, S.	119
Ranjan, A.	115
Raoof, A.A.	103
Rapacz, A.	182
Rashnonejad, A.	314, 315
Rasiuk, M.	186
Rasool, F.	256
Ratjen, L.	171
Ratnasooriya, W.D.	156
Ravi, T.K.	103
Rawat, D.S.	104
Raymond, A.	107
Raza, S.	130
Razafitianamaharavo, A.	86
Razaghi, A.	312
Razaque, G.	180
Refuerzo, J.S.	104
Reiner, T.	28
Ren, M.	256
Renbaum, P.	17
Rescifina, A.	197
Reva, O.N.	105
Rewers, M.	298
Ribas, C.R.	257
Ridder, B.	96
Rikk, J.	313
Rita, T.	65
Rivera, D.E.	154, 181
Rivera, J.C.	171
Roat, C.	257
Robajac, D.	317
Roberts, A.J.	140
Robson, S.C.	46
Rodrigues, R.A.F.	150, 192
Rodrigues, R.C.L.B.	314
Rogers, J.T.	13, 72, 106
Rolt, A.	62
Rones, Z.	175
Rosa, E.A.R.	257
Rosa, R.T.	257
Rosbo, N.K.d	224
Rosenberg, Y.J.	258
Rosim, R.E.	308
Rosner, K.	106
Rosner, T.	106
Rowe, W.J.	214
Roy, U.	107
Ruan, D.	244
Rubovitch, V.	188
Rufai, B.S.	262
Russo, M.A.	31
Rymovicz, A.U.M.	257
Ryu, S.B.	315

S

- Saal, H. 123
 Sablinskas, V. 316
 Sachs, M.S. 273
 Saddler, J.N. 299
 Sadvakasova, A.K. 324
 Saeed, H. 148
 Safari, N. 40
 Safo, M.K. 108
 Sahab, A.F. 217
 Saini, R.K. 177
 Saito, D. 96
 Saito, K. 262, 258
 Sajesh, B.V. 93
 Sakagami, M. 108
 Sakai, J. 97
 Sakamoto, H. 262
 Sakurai, N. 262
 Salas, R. 155
 Sałat, K. 109, 181
 Saleem, S. 241
 Saleem, Z. 148
 Saliev, T. 316
 Sambamurti, K. 13, 21, 32, 56, 188
 Sambrani, S. 259
 Samineni, D. 110
 Sanchez, D.A. 281
 Sanchez-Gonzalez, L. 140
 Sanchini, A. 291
 Sandur, S.K. 178
 Sangadkit, W. 321
 Sangsri, B. 295
 Santos, C.A.M. 111
 Saraav, I. 114
 Sarsar, V. 317
 Sarsekeyeva, F.K. 324
 Sarwat, A. 171
 Sato, K. 286
 Savaroğlu, G. 111
 Saykin, A.J. 56
 Scala, A. 197
 Schaller, S. 264
 Schirmeister, T. 197
 Schlüter, O. 309
 Schmid, S. 69, 234
 Schmitz, J. 182
 Schonbrunn, E. 112
 Sebban, L.E. 252
 Šedivá, A. 317
 Sedlmayr, M. 96
 Segura, C.L. 292
 Seitak, A. 316
 Sekine, Y. 258
 Sekiya, K. 250
 Sellnow, R. 106
 Selow, M.C. 257
 Selwal, K.K. 317
 Selwal, M.K. 317
 Semont, A. 209
 Sempert, K. 144, 160
 Sen, S. 8
 Seok, A. 163
 Serhan, C.N. 4, 204
 Serwecińska, L. 248, 279
 Sévalle, J. 17
 Seydanova, A. 190
 Seyid, C. 315
 Shachar, I.B. 135
 Shah, S.W.A. 113
 Shahrin, R.O. 183
 Shalaby, S. 113
 Shalaeva, M. 33
 Shan, M. 90
 Shao, J. 259
 Shao, K. 256
 Sharma, A. 260, 318
 Sharma, G.J. 113
 Sharma, M. 114
 Sharma, S. 114, 260, 318
 Sharp, P.A. 205
 Sharp, P.A. 5
 Shchepinov, M. 123
 Sheikh, A. 213
 Sheikh, A.Q. 260
 Shekhar, H.U. 261
 Shen, B.-Q. 110
 Shen, J. 86
 Shen, W. 223
 Sheng, Y. 198
 Shengof, I. 245
 Shepelin, D. 209
 Shi, J. 261
 Shi, K. 115
 Shi, Y. 72, 245
 Shido, O. 157
 Shim, J. 49
 Shimura, F. 308
 Shin, C.-G. 318
 Shinde, B. 259
 Shiratake, K. 262
 Shishehbor, F. 141
 Shushen-Yang, 323
 Shwetha, C. 299
 Siebert, H.-C. 215
 Sievert, E. 46
 Signore, S. 173
 Silva, A.D.De. 29
 Silva, D.d.O. 33, 184
 Silver, P.A. 5, 205
 Simmon, J.M. 209
 Sine, J. 98
 Singh, A. 142
 Singh, J. 262
 Singh, K. 142
 Singh, R.K. 115
 Singh, S. 212, 255, 262, 263
 Singh, S.K. 115
 Sinnaeve, D. 92
 Skolnick, J. 15
 Smit, F.J. 184
 Smith, J. 116
 Smith, P.G. 90
 Smyth, R.P. 229
 Sobecky, P.A. 263
 Sobhia, M.E. 117
 Soldatkina, M.A. 105
 Solhaug, A. 154
 Soliman, K.F. 172
 Soliman, K.F.A. 185
 Solt, L.S. 140
 Son, B.W. 185, 186
 Song, L. 194
 Song, W. 13, 34
 Songca, S.P. 175
 Soni, J.S. 138
 Soni, S. 264
 Sorrechia, R. 179

Sousa, I.P.de	150
Southall, N.	62
Spanò, R.	224
Spilovska, K.	81
Spiru, L.	319
Spitzer, D.	34
Spurney, C.F.	16
Stączek, P.	186
Steinberg, D.	175
Stella, A.	312
Stepan, E.	291
Sterea, S.A.	319
Stern, J.N.H.	6, 206
Stern, S.	118
Stern, S.T.	118
Stoddard, T.	225
Stroscio, M.A.	119
Strzalka, K.	264
Strzelczyk, A.	186, 187
Stuckey, J.	123
Stuehr, D.J.	277
Stutz, S.	134
Subramanian, V.	120
Sulong, N.A.	319
Sun, W.	46, 101
Süslüer, S.	314
Suzuki, M.	262
Svobodova-Leisova, L.	306
Swain, S.P.	199
Szabó, K.	265
Szabo, Z.	131
Szarvas, T.	313
Szklarek, S.	279

T

Taghian, T.	213
Taha, M.	286, 287
Tahir, B.	148
Tajiri, S.	286
Takahashi, T.	97
Takato, M.	258
Taki, M.	262
Taleb-Contini, S.H.	289
Tamarat, R.	209
Tamargo, I.	21, 187
Tamayol, A.	140
Tan, S.	245
Tanabe, K.	307, 308
Tanabe, Y.	157
Tanaka, H.	307
Tandikul, N.	188
Tang, M.	147
Taniguchi, M.	250
Tao, Z.	320
Tarasov, S.A.	60
Tarroum, M.	276
Tartaglia, E.	173
Tati, G.	173
Tayag, T.J.	320
Teherán, V.	155
Tehrany, E.A.	163
Tepe, J.J.	35
Thayerc, D.	98
Thipayarat, A.	321
Thompson, A.A.	264
Thomson,	284
Tian, S.	120
Tian, X.-F.	120

Tiftikcioglu, Y.	315
Ting, H.-M.	303
Tivitmahaisoon, P.	90
Tiwari, V.	210
Tohidfar, M.	292
Tokimatsu, T.	262
Tomlinson, S.	134
Tong, R.-s.	128
Topno, I.	189
Torres, I.P.G.	292
Torzewski, J.	121
Tripathy, M.	200
Trippe, C.	123
Trofimov, A.	265
Tsuburai, T.	250
Tu, W.	261
Tuntipaiboonatana, R.	294
Tuntland, T.	36
Turaga, R.C.	89
Turkoz, S.V.E.	315
Turuspekova, S.T.	190
Tvaruzek, L.	306
Tweedie, D.	21, 187
Tzalik, S.	245
Tztzoglaki, C.	192

U

Uanseoje, S.	152
Uccelli, A.	224
Uchikoba, F.	258
Udagama, P.	75
Udagama, P.V.	156
Udugamasooriya, D.G.	122
Ueno, Y.	286
Uhlig, S.	153
Ujowundu, C.O.	253
Ulmer, J.B.	16
Uluer, M.C.	46
Uribe, C.	292
Userbayeva, A.A.	324
Utomo, H.	267
Utomo, H.S.	269

V

Vagdevi, H.M.	321
Vahdati, K.	292
Valiante, N.M.	122
Vanderburg, C.R.	72
Vanhercke, T.	255
Vanhove, B.	46
Vasantharaju, S.G.	190
Vasmatzis, G.	49
Vasudev, M.C.	123
Vaupel, P.	115
Velicka, M.	316
Velot, E.	77
Venitz, J.	108
Verdam, M.C.d.S.	282
Verespy, S.	136, 191
Verma, M.	115
Vila-Lameiro, P.	210
Viotti, J.	17
Vivet-Boudou, V.	229
Vleeschouwer, M.D.	92
Voelkel, N.F.	108
Vojta, L.	290
Voswinkel, J.	209

Voytas, D.	225
Vranesic, M.	131
Vukičević, S.	290
Vyas, P.	240

W

Wagner, S.B.	305
Walters, M.C.	264
Wang, A.Q.	62
Wang, C.	147
Wang, C.-W.	283
Wang, D.	270
Wang, G.-R.	238
Wang, J.	127, 168, 196
Wang, J.Y.	90, 283, 322
Wang, L.	88
Wang, Q.	188
Wang, R.	54, 130
Wang, R.F.	124, 191
Wang, S.	124
Wang, T.	268
Wang, W.	36
Wang, X.	147, 267, 268
Wang, X.-J.	322
Wang, Y.	132, 168
Wang, Y.Z.	322
Wang, Z.	297
Wang, Z.-Q.	120
Ward, S.G.	125
Warmuth, M.	90
Wasim, L.	56
Watanabe, Y.	97
Watarai, K.	250
Weber, K.	121
Webster, T.J.	37
Weffort-Santos, A.M.	126
Weglicki, W.B.	16
Weiss, I.E.	175
Weissig, V.	37
Wenefrida, I.	267, 269
Whoddal, J.	46
Wild, C.	134
Wilhelm, C.	264
Wilkens, J.J.	115
Wirth, J.	198
Wishart, D.S.	177
Wolozin, B.	126
Wong, D.F.	54
Wong, E.	69
Wong, H.	110
Wong, J.H.	195
Wong, K.	195
Wong, K.-S.	299
Wood, C.	212
Wood, W.	125
Wookey, P.J.	127
Wozniwicz, B.M.	269
Wozniwicz, M.	269
Wright, A.K.	192
Wu, H.-P.	52
Wu, K.-M.	90
Wu, L.-C.	146
Wu, M.X.	128
Wu, T.	119

Wu, X.	163
-------------	-----

X

Xiang-Qian, Z.	320
Xiao, H.-T.	128
Xiao, J.	62
Xiaorong, C.	53
Xie, S.	53
Xiping-Deng,	323
Xu, G.	108
Xu, H.	69, 234
Xu, J.	194, 270
Xu, K.(R.)	119
Xu, Q.	129
Xu, S.	52
Xu, W.	270, 271
Xu, X.	62
Xudong, C.	323

Y

Yamaguchi, M.	250
Yamakawa, T.	97
Yamane, L.T.	193
Yan, B.-X.	212
Yan, Z.	244
Yang, A.	66
Yang, C.-N.	193
Yang, H.	270, 271
Yang, J.	129
Yang, L.	323
Yang, S.	271
Yang, Y.	194, 196
Yang, Y.-S.	164
Yao, Z.	163
Yasmeen, N.	216
Ye, A.Q.	38
Ye, M.	84
Yeh, S.-A.	303
Yeliseev, A.A.	272
Yen, L.-C.	301
Yen, S.-Y.	148
Yi, S.-X.	86
Yilmaz, F.	295
Yonath, A.	206
Yonath, A.	6
Yones, S.E.	71
Yoon, J.	217
Younis, M.	180
Younus, M.	130
Youssef, N.	286, 287
Yu, D.-k.	128
Yu, H.	130
Yu, L.	90
Yu, R.	194
Yu, Z.	223
Yun, K.	185, 186
Yun, Z.	124
Yunis, E.J.	6, 206
Yunjun, Y.	244
Yu-ping, G.U.	320
Yusoff, A.A.M.	195

Z

Zaborowski, A.	248, 279
Zafar, M.	130
Zaghary, W.A.	131

Zainal, N.A.	200	Zhao, W.	323
Zakaria, A.A.	319	Zhao, Z.	61
Zaki, H.M.	200	Zhavoronkov, A.	209
Zakia, S.	210	Zhen, G.	267
Zalewski, M.	248, 279	Zheng, G.Z.	90
Záamorová, M.	317	Zheng, J.	133
Zapata, Y.M.	292	Zheng, M.Y.	272
Zaręba, P.	182	Zheng, W.	62
Zarmouh, N.O.	172, 184	Zhengnan, H.	244
Zarnani, A.-.	40	Zhong, J.	233
Zayadan, B.K.	324	Zhong, L.	198
Zbukvic, I.C.	165	Zhou, J.	134
Zecevic, N.	278	Zhou, M.	270, 273
Zedan, H.	135	Zhou, Q.	26, 120
Zelioli, I.A.M.	150	Zhou, W.	223
Zgheib, C.	270	Zhou, X.	223
Zhang, F.	225	Zhou, X.-R.	212
Zhang, G.	66, 53	Zhou, Y.	38, 54, 66, 132, 271
Zhang, H.	90, 194	Zhu, A.H.	191
Zhang, H.-M.	238	Zhu, J.	194
Zhang, J.	88, 131, 195	Zhu, Q.	209
Zhang, L.-j.	128	Zhu, X.-y.	87
Zhang, M.	66	Zhuravel, E.V.	105
Zhang, N.	132	Zi, J.	194
Zhang, Q.	163	Zidan, J.	135
Zhang, S.	132	Ziegler, S.I.	115
Zhang, T.	46	Zilkha-Falb, R.	79
Zhang, Y.	108, 223, 323	Zimmermann, O.	121
Zhang, Z.-L.	87	Zulkefeli, M.	200
Zhao, G.	147	Zuñiga, J.	6, 206
Zhao, J.	53, 162	Zuo, C.	132

CUMULATIVE SUBJECT INDEX

A

AA-induced platelet aggregation 88
 Aberrant crypts foci (ACF) 251
 Abiotic stress 216, 236, 296, 323, 324
 A β precursor protein (APP) 13, 32, 34, 57, 72, 105, 106
 Acclimatization 285, 286
 AChE 17, 81, 110, 174, 261, 262
 Achyrocline satureioides 192
 Acid mine drainage (AMD) 32, 285
 Acinetobacter 295, 296
Acmea oleracea 149, 150, 192
 Actin binding activity 70
 Active pharmaceutical ingredients (API) 34, 147
 Acute toxicity 75, 113, 138, 253, 282
 Adherens junction (AJ) 144
 Adipose tissue 28, 31, 32, 178, 269
 Afforestation 265
 AGEs 239
 AgNPs 317, 318
 Agricultural pollutions 280
 Agrobacterium transformation 253
 AIDs 125, 126, 173, 262, 298, 317
 Allosterism 136, 190, 191
 Alzheimer's disease 13, 17, 18, 19, 21, 23, 32, 34, 52, 54, 56, 72, 83, 105, 126, 174, 188, 252
 Alzheimer's disease neuroimaging initiative (ADNI) 54, 57
 Amazonian rainforest 282
 AMD metals 285
 Amino acids 32, 44, 124, 158, 265, 269, 291, 296
 AMPs 174, 276
 Amyotrophic lateral sclerosis 70, 126, 268
 Anabolic activity 18
 Anaerobic digestion 233, 301
 Analgesic activity 282
 Anaplastic lymphoma kinase (ALK) 36, 83
 Angiotensin 137, 170
Annual ryegrass 275, 276
 Anthocyanin 242, 262
 Antibacterial activity 14, 15, 68, 79, 89, 105, 115, 116, 186, 187, 224, 225, 276
 Antibiotic sensitivity 277, 278
 Antibody therapy 127
 Anticancer activity 154, 155, 183, 184, 287
 Anticancer drugs 10, 23, 33, 64, 151, 168, 169, 184
 Antidiabetic activities 143
 Anti-hyperglycemic activity 299
 Anti-inflammatory activities 17, 138, 174, 175, 176, 189, 217, 252, 283
 Anti-inflammatory effects 138, 189, 296
 Antimicrobial activities 68, 99, 176, 187, 287, 318
 Antimicrobial effect 166, 276
 Antioxidant activity 45, 48, 113, 159, 161, 167, 176, 240, 299, 321
 Antioxidant activity of prunes 240
 Antioxidants 48, 72, 82, 99, 105, 149, 161, 166, 176, 178, 192, 242, 248, 257, 265, 281, 299, 305, 311, 321, 322
 Antiproliferative activity 33, 128, 133, 169, 184
 Antipsychotic drugs 71
 Antiretroviral therapy 180
 Antitubercular activities 321
 Antitumor agents 184
 Antitumor effects 93
 Apocynaceae 110
 Apoptosis 10, 17, 29, 37, 56, 89, 92, 93, 95, 98, 106, 110, 113, 120, 132, 142, 143, 144, 148, 172, 184, 213, 238, 316, 318, 319
 Aptamers 119
 Arabidopsis 263, 284, 297, 315

Arbuscular mycorrhizal fungi (AMF) 236, 260
 Arca inflate 194
 Arginine 158, 313
Aristotelia chilensis 305, 306
 AR protein 29
 AR-regulatory DUBs 29
 Artemisinin 60
 Artificial neural networks 148, 258
Aspergillus flavus 159, 217
Astragalus chrysochlorus 45
 Atherosclerosis development 296
 Atomoxe 102, 103
 Atomoxetine hydrochloride 102, 103
 Atorvastatin 157
 ATP 8, 27, 50, 51, 275
 ATPase 256
 Autohydrolysis 310
 Autoimmune diseases 41, 42, 269

B

Bacillus licheniformis 280, 281
 Bamboo shoots 195
 Bariatric surgery 27
 Benign lesions 123, 124, 191
 Betulinic acid (BA) 29, 205, 276
 Binding modes 81, 135, 136, 183, 261, 262
 Bioactive strains 115, 116
 Biochemical methane production (BMP) 300, 301
 Biodegradable nanogels 55, 56
 Biodiesel production 236, 237, 244, 304, 324
 Biofuels 233, 236, 237, 304
 Biogas production 300, 301
 Biogas slurry 318
 Biological effects of nitric oxide 2, 202
 Biological fluids 240, 316
 Biomass 236, 256, 257, 260, 285, 300, 324
 Biomaterials 124, 283
 Biopesticide 36, 318
 Biopharmaceutics 150
 Biophysical methods 102
 Bioproducts 231, 255, 263
 Bioreactor 223, 224, 248, 280, 304, 305, 320
 Bioremediation 260, 324
 Biosensors 123, 319
 Biotic stress 228, 255
 Biotransformation 208, 257, 279
 Bithiazole 40, 135
 Bleomycin 58, 135
 Blood brain barrier (BBB) 70, 188
 Blood cancer cells 316
 Blood glucose 152, 165, 308
 Blood proteins 95
 Body fat 1, 164, 201
 Body mass index (BMI) 24, 164, 303
 Bog iron ores 305
 Bone disease 70
 Bone imaging 130
 Bone metastatic tumor 191
 Bone scan (BS) 191
 Bovine heparins 87
 Brain absorptions 94, 95
 Brain tumour 127, 147
 Breast cancer (BC) 162, 195, 208, 212, 303, 308
 Breath hydrogen excretion 308
Byrsonima duckeana 282

C

Caffeic acid (CA) 108, 109, 196, 200
 Cancer biomarker discovery 50
 Cancer cells 8, 12, 13, 56, 98, 106, 121, 133, 142, 170, 172, 177, 322
 Cancer chemotherapy 95, 96
 Cancer-related proteins 11
 Cancer stem cells (CSCs) 13, 21, 127, 170
Candida albicans 257, 286, 287
Candida rugosa lipase (CRL) 244
 Candidate genes 320
 Cannabinoid receptor 272
 Canonical discriminant analysis (CDA) 196, 312
 Carbonized sorbents 274
 Carbon rice husks (CRH) 274
 Carboxytherapy 173
 Cardiac glycosides 121
 Cardiac toxicity 238
 Cardiovascular diseases 37, 87, 88, 94, 101, 121, 160, 179, 229, 239, 248, 251, 252
Carica papaya 75, 76
 β -carotene hydroxylase 242
 Carotenoid 242
 Cassava 228, 285, 286
 Cassava bacterial blight (CBB) 228
 Cassava green mite (CGM) 228
 Cassava mosaic disease (CMD) 228
 Cathepsin 153, 182, 183, 198
 CD3+ T-lymphocyte 40
 CD34+ stem cells 71, 72
 CD spectropolarimetry 178
 Cell body model 258
 Cellular immunity 7, 207
 Cellular signaling 2, 202, 255
 Cellulose 148, 192, 238, 299, 310
 Centipede extraction 120
 Cereal diseases 306
 Chalcone 10, 184, 296
 Charge-reversal 196
 Chemical oxygen demand (COD) 233, 302
 Chemical probes 119, 143, 146
 Chemoenzymatic heparin analog (CHA) 46
 Chemoenzymatic synthesis 14, 89, 90
 Chemokines 101, 125
 Chemotherapy 64, 98, 117
 Childhood obesity 24
 Chilo partellus insects 254
 Chitosan 164, 192, 271
 Chloroplast genome sequence 310, 311
 4-chromanone derivatives 173, 174
 Chromatin remodeling complexes 12, 13
 Chronic kidney disease (CKD) 160
 Chrysanthemum 298
 Circadian rhythm 102, 103, 139, 140
 Circulating tumor cells (CTCs) 52
 Cochlea 302, 303
 Codon usage 273
 COD removal 233
 Cognitive deficits 52, 109
 Cold plasma 309
 Coliforms 321
 Colitis 120, 143, 144, 210, 250, 251
 Colon cancer 33, 128, 142, 143, 222, 317
 Colorectal cancer cells 93
 Compound danshen dripping pills (CDDP) 85, 120
 Compound treated animals 166
 Computational approach 91, 136
 Conformational change 190, 198

Converting enzyme inhibitors 137
 Copolymers 42, 124, 196, 197
 Core β -ladder domain 301, 302
 Coronary artery disease (CAD) 88, 196, 197, 228
 C-reactive protein (CRP) 121, 251
 CREB binding protein (CREBBP) 146
 Crop breeding 272, 273
 Crude extract 45, 68, 156, 166, 250, 251, 299
 Cryopreservation 277, 298
Crypt foci 251
 Curcuminoids 84
 Cyclic GMP 2, 202
 Cyclic GMP research 2, 202
 Cyclic lipopeptides (CLPs) 92
Cynodon dactylon 224, 225
Cyperus rotundus extract 253
 Cytokines 156, 218, 281, 283, 294
 Cytotoxic effects 144, 155, 156, 172
 Cytotoxicity 41, 62, 77, 98, 99, 128, 136, 178, 196, 197, 219, 279
 Cytotoxic therapies 242

D

DatSCAN 131
 DDE activity 265
 Dementia 13, 19, 25, 83
 Dendrimers 151, 155, 168, 170, 171
 Dengue severity 29, 30
 Denitrification 248, 279, 280
 Denitrifying barrier 279, 280
 Dental implants 221
 6-deoxypladienolide 90
 DHA levels 157, 263
 Diabetes 1, 9, 17, 27, 44, 58, 67, 94, 101, 123, 137, 143, 152, 164, 167, 180, 201, 222, 226, 239, 297, 307, 317, 320
 Diabetic nephropathy 239
 Diabetic stroke 166
 Diabetic wound healing impairment 271
 Diadinoxanthin de-epoxidase (DDE) 264, 265
 Diastolic blood pressure (DBP) 137, 164
 Diesel oil 295, 296, 304
 Dietary fibre materials 308
 Dietary supplements 144, 164, 165, 248
 Differential scanning calorimetry (DSC) 147, 151, 156, 271
 2, 2-diphenylpicrylhydrazyl 176
 Diltiazem hydrochloride 180, 181
 Dimeric cyclen 200
 Disaccharidase 307
 Discovery studio 105, 183
 Distal colon 251
 DNA barcodes 295
 DNA binding 135, 178
 DNA damage 106, 136, 178, 316
 DNA markers 295
 Domain interactions 198
 Doubled haploids (DH) 272
 Doxorubicin 30, 118, 196, 238
 Drought stresses 216, 296
 Drug delivery strategies 59, 64
 Drug discovery process 14, 17, 35, 65, 103
 Drug interactions (DIs) 110
 Drug therapy problem (DTP) 137, 138
 Dry matter content (DMC) 228, 275, 301
 DUB activity 29
 Duchenne muscular dystrophy (DMD) 11, 12
 Dynamic multi-bed scanning 123, 124
 Dyspnea 215

E

Efficient microbes 260
 Elastin 280
 Electroacupuncture group 53
 Electroacupuncture pretreatment 53, 54
 ELISA 22, 30, 53, 60, 86, 129, 156, 181, 220, 244, 288, 312
 ElogD 33
Emblica officinalis (EO) 189
 Embryogenesis 272, 273, 276
 Embryonic stem cells 20, 315
 Emphysema 108, 109
 Enantiomeric composition 281
 Endemic 45, 160, 167, 168
 Endomycorrhiza 265
 Endophytes 115, 237, 257
 Endothelial cells 67, 101, 188, 270, 323
 Endothelial colony-forming cells (ECFCs) 161
 Endothelial dysfunction 24, 214, 305, 306
 Endothelial progenitor cells (EPCs) 22, 161
 Enniatin B 153
 eNOSoxy 277
 Enoxacin 70
 Enrofloxacin 129
 Enstity functional calculations 79
 Enz combination 29
 Enzymatic hydrolysis 94, 310
 Enzyme activities 100, 178, 179, 277
 EO extract 189
 EPA 157, 263
 Epidermal growth factor receptor (EGFR) 16, 80
 EP Premium methocel 180, 181
 Equisetin 14, 15
Escherichia coli 115, 68, 187, 224, 225, 277, 278, 293, 321
 Esophageal cancer 170
 Esophageal squamous cell carcinoma (ESCC) 170
 Essential oils (EOs) 161, 166, 167, 168, 174, 175, 176, 179, 289
 Ethanol production 274
 Ethno medicine 176
 Ethnopharmacology 155, 289
 Ethylene glycol 55, 168, 196
 Etiologies of obesity 1, 201
 Etoposide 55, 56
 Exemestane 208, 279
 Experimental autoimmune encephalomyelitis (EAE) 40, 42, 79, 224
 Expression of inflammatory cytokines 244
 Extracellular membrane protein 50
 Extracellular vesicles (EVs) 240
Ex-vitro adaptation 285

F

Farnesylation inhibitor 297
 Fasting blood sugar (FBS) 143
 Fatty acid metabolism 157
 Fatty acids 236, 255, 263, 318, 324
 FDG scans 54
 Fear-motivated task 109
 Fermentation 35, 185, 186, 249, 280, 281, 314, 318
 Field-effect transistors 119
 Flaviviral NS1 fusion proteins 302
 Flavonoids 10, 45, 68, 140, 141, 177, 178, 240, 283
 Flow-limited oxygen-dependent (FLOD) 115
 Fluoroquinolones 187
Foeniculum vulgare 174, 175
 Follicle-stimulating hormone (FSH) 130, 230
 Food processing waste (FPW) 233
 Foreign body reaction (FBR) 38

Fourier transform infrared (FTIR) 56, 296, 317, 318
 Free radical scavenging 159, 176, 177, 178
 Fresh root yield (FRY) 228
 Functional dyspepsia (FD) 53
 Fungal endophytes 115, 116
 Fungal morphology 224, 300

G

$\alpha 1\beta 2\gamma 2$ GABA A receptor 195
 Gag precursor 229
 Gastric adenocarcinoma cancer 268
 Gastritis 86
 Gastrointestinal infections 289
 GAT1 inhibitor 109
 GBM cells 69, 147
 Gene copy number 313
 Gene targeting 314, 315
 Gene therapy 67, 246, 264, 311
 Genetically modified (GM) 46, 67, 285, 286, 292
 Genetic code 6, 106, 206
 Genetic stability 216, 277
 Genome-wide association studies (GWAS) 3, 71, 203
 Genomic RNA 44, 229
 Genotypes 228, 233, 263, 285, 312
 Gentamycin 278
 Genuine pathogens 6, 206
 GFAP immunoeexpression 71, 72
 Glial cells 278
 Glioblastoma 69, 75
 Glioblastoma-initiating cells (GICs) 242
 Glioma cells 219, 242
Gloriosa superba 294
 1,3;1,4- β -D-glucan (betaglucan) 238
 α -glucosidase 9, 17
 Glucosyltransferase 307
 Glutathione 217, 218
 Glycans 26, 245, 268, 317
 Glycosylation 26, 77, 317
 G-protein-coupled receptors (GPCRs) 4, 101, 134, 272
 Grammatophyllum 319, 320
 Grape culture cells 262
Graptopetalum paraguayense (GP) 145
 Graves' disease (GD) 28
 Green chemistry 150
 GTase 307
 Gut associated lymphoid tissue (GALT) 107

H

Haliclona (Soestella) sp. 156
 Halogenated bioactive compounds 185
 Harvest index 228
 HD patients 239
 Heart failure therapy 121
 Heavy metals 68, 89, 260, 284
 HeLa cells 45, 56, 57, 169
Helix aspersa 250, 251
Helix aspersa crude extract (HACE) 251
 Hemodialysis patients 239
 Hemostasis 20, 91, 190, 191
 Heparin 14, 20, 46, 87, 89, 90
 Heparin binding domains (HBDs) 33, 191
 Hepatitis 17, 62, 199, 294
 Hepatitis C virus (HCV) 62, 199, 294
 Herbal tea 161, 167
 Herbicide resistance 276
 HFU emulsions 77, 163
 HGG cell lines 127

High beta-carotene 228
 High copy number events 253
 High-density lipoproteins (HDL) 164, 165, 253, 289
 High fat fed rats (HFFR) 305
 High glucose-treated endothelial cells 101
 Highly active antiretroviral therapy (HAART) 154, 180
 High-performance liquid chromatographic (HPLC) 56, 138, 142, 151, 156, 190, 257, 293
 High pressure fermentation 281
 High pressure homogenization (HPH) 163
 High prevalence of bTB 287, 288
Himatanthus lancifolius 110
 Histone deacetylase inhibitors (HDACi) 56
 HIV-1 11, 44, 107, 193, 229, 258
Holarrhena antidysenterica 299
 Homologous recombination 314, 315
 Host and non-host PIs 254
 HS mimetics 46
 Human glioma cells 183, 184
 Human hepatocellular carcinoma 99, 323
 Human immunodeficiency virus (HIV) 16, 17, 44, 107, 125, 154, 199, 232
 Human liver microsomes 172
 Human tissues 4, 100, 212
 Human umbilical vein endothelial cells (HUVECs) 101, 188, 270
 Humoral immunity 7, 207
 Huntington's disease (HD) 23, 239
 Hybrid detection 295
 Hydrangea plant 285
 Hydraulic retention time (HRT) 233, 304
 Hydraulics 302
 Hydrocarbons 233, 259, 263, 295, 296, 299, 324
 Hydrodistillation 174, 176
 Hydrogels 12, 26, 140, 220
 Hydrogenated resistant glucan (HRG) 308
 Hydrogen production 233
 Hydrolysis 50, 243, 299
 Hydrophilicity 270
 4-hydroxybenzoic acid 195
 Hydroxypyrrone tautomerism 79
 Hypertension 24, 137, 266
 Hypothalamic phospholipids 189, 190
 Hypoxia 23, 31, 32, 75, 96, 100, 108, 114, 115, 261
 Hypoxia-ischemia 96, 100

I

ICCB-longwood 116
 IgG antibodies 245, 246
 Immobilization 274, 305
 Immune response 20, 47, 96, 97, 122, 125, 161, 219, 288
 Immune system 59, 73, 75, 95, 107, 110, 122, 125, 156, 251, 269
 Immunoglobulins 58, 268
 Immunomodulation 156
 Immunopathologies 140, 141
 Immunostimulation 76, 156
 Immunosuppressant drugs 228
 Immunotherapy 57, 79, 171, 244, 269
 Immunotoxin 127
 Implanted tumor 120
In vitro anti-tumor activity 194
In vitro metabolism 153
In vitro seed germination 319, 320
In vivo biocompatibility 270
 Indomethacin 104, 138, 189, 282, 283
 Induced apoptosis 93, 144, 145
 Induced bed reactors (IBR) 233
 Inflammatory bowel disease (IBD) 12, 41, 42, 143, 234
 Inflammatory cytokines 24, 76, 153, 244, 311

Inflammatory protein expressions 101
 Inflammatory response 38, 161, 244, 252, 270, 297
 Inhibitory activity 81, 110, 111, 187
 Inhibitory molecule 261
 Inhibitory synaptic models 258
 Insulin 9, 57, 63, 67, 152
 Insulin therapy 152
 Intensity-modulated radiation therapy (IMRT) 302, 303
 Interdomain electron transfer (IET) 198
 Intervertebral disk degeneration (IDD) 244
 Intracellular drug delivery 196, 197
 Intrauterine development 266
 Iodothyronine deiodinases (IDs) 100, 178
 Iron chelators 105, 169
 Irradiation 82, 209, 210, 261, 262, 287, 301
 Isoniazid 117, 262

J

Japanese encephalitis virus (JEV) 301, 302
Jatropha curcas 237
 JEV/EV-71 recombinants 302

K

Karanja deoiled cake 318
 Kelly model 115
 Keratin 220, 280, 315
 Kruppel-like factors (KLF) 64, 65
 KYNA treatment 278

L

Labeo rohita 256
 LAB extracts 286, 287
 Laccase activity 300
 Laccase biosynthesis 300
 Laccase-mediator systems (LMSs) 299
 Lactic acid bacteria 250, 286
 LAM cells 52
 Lamiaceae 160, 167, 321
 L-Arginin 2, 202, 305, 313
 Larynx carcinoma 64, 65
 Lauric acid 195
 LDL level 165, 261
 LDR pretreatment 238
 Lectin-based protein microarray method 317
 Left ventricular mean pressure (LVMP) 88
 Leptin 178
 Lignin precipitates 310
 Linear regression 123, 132
 Line probe assay (LPA) 262, 263
 Lipid peroxidation 48, 122, 165, 177, 281, 299
 Lipid signaling 315
 Liposomes 30, 86, 93, 151, 155, 219
 Liquid nitrogen 277, 298
 L-lysine 196, 313
Listeria monocytogenes 309
 Loss of heterozygosity 52
 Low-BMI breast cancer patients 303
 Low copy number events 253
 Low density lipoprotein (LDL) 164, 253, 305
 Low doses radiation (LDR) 238
 Low molecular weight heparins (LMWHs) 14, 89
 Lung cancer 36, 57, 65, 83, 120, 133, 193
 Lung carcinomas 64, 65
 Lung treatment 108, 109
 Lymphangioliomyomatosis 52
 Lymphocyte function 125

Lymphocytes 6, 47, 75, 110, 125, 178, 206, 210, 240, 247, 250, 269
 Lymphoid organs 73, 251
 Lysine 51, 275, 313
 Lysosomal destabilization 153

M

Macrophage polarization 161
 Magnetic nanoparticles 113
 Magnetic resonance imaging 191
 Malaria 16, 36, 60, 74, 179, 184, 197, 232
 Malignancy therapy 196, 197
 Marine-derived microorganisms 185, 186
 Marine Streptomyces 79
Mariscus alternifolius 176
 Marshmallow 95
 Mast cells 96, 100
 Mature leaf concentrate 75, 76
 MCAO 307
 Medicinal plants 138, 176, 184, 210, 299
 Melanoma 57, 90, 106
 Melatonin 180, 268
 Melittin 276
 Mesenchymal stem cell (MSC) 22, 161, 209, 224, 270, 271
 Metabolic abnormalities 180
 Metabolic syndrome model 157
 Metabolite intermediate complex (MIC) 79, 99, 159, 160, 172, 175, 176, 179, 186, 225, 276, 287, 322
 Metabolites 79, 84, 172, 208, 217, 218, 318
 Metallogenic information extraction 256
 Metastasis 12, 13, 28, 43, 44, 124, 170, 194, 195, 323
 Methanol extract 161, 166, 321
 Methionine 32, 217, 296
 Metyrapone 165, 166
 Micelles 196, 197
 MIC range 159, 160, 166
 Microalgae 304, 324
 Microalgae cultivation 304, 305, 324
 Microarray 57, 75, 114, 170, 209, 263, 296, 317
 Microbial biofilms 174, 309
 Microbial cells 274
 Micro-electro-mechanical systems (MEMS) 258, 259
 Microelements 251
 Microfilaments 70
 Microparticle 4, 300
 microRNA 5, 13, 57, 61, 62, 70, 94, 116, 255, 268, 270, 271
 microScale thermophoresis (MST) 47, 102
 Middle cerebral artery occlusion 166
 Mild cognitive impairment (MCI) 54, 57
 Milk-based peptides 250
 Mineralized cell sheet 161
 Mini-mental state exam (MMSE) 54
 MiR-29a expression 271
 miRNAs 13, 57, 94, 255, 270, 311
 Mitogen-activated protein-kinases (MAPK) 149
 Mitotic inhibitors 142, 143
 Mobile phone radiation 277, 278
 Molecular beacons 119
 Molecular diagnostics 94, 243, 306
 Molecular dynamic simulation 198
 Molecular pathological epidemiology (MPE) 3, 203
 Monosodium glutamate 71, 72
 Motor function 71, 190
 Moxibustion 86
 mRNA expression 157, 162
 MSC injections 209, 210
 MSC therapy 209, 210
 MSC treatment 270, 271

Mucoadhesive buccal tablet 148
 Mulgeum site 223, 302
 Multiple sclerosis (MS) 12, 40, 42, 79, 120, 143, 148, 149, 154, 176, 224, 252, 317
 Multivariable logistic regression 303
 Mycobacterial vectors 96, 97
Mycobacterium tuberculosis 114, 117, 125, 139, 262
 Myocardial infarction (MI) 87, 88, 101
 Myocardial ischemia 53, 54
 Myocardial Ischemia and reperfusion injury (MIRI) 53, 54

N

Na⁺/H⁺ antiporter 216
 Nanobiocomposites 274
 Nano-cocrystals 34, 35
 Nanodiscs 98, 277
 Nanofiber 161, 271
 Nanofunctionalization 140
 Nanogels 22, 55, 56
 Nano lipid particles (NLPs) 151, 155, 156
 Nanoliposomes 118, 140
 Nanomaterials 37, 133, 169
 Nanomedicine 58, 59, 118, 215, 283
 Nanoparticles tracking analysis (NTA) 163, 240
 Nanotechnology platforms 117, 118
 Nanotechnology reformulation 118
 Native silk fibroin powder (NSFP) 270
 Native Strains 236
 Neiguan 53, 54
 Neovessels 211, 212
 NEP expression 57
 Neural stem cells (NSCs) 268, 306, 307
 Neuroblastoma 61, 131, 172
 Neurodegeneration 13, 21, 23, 25, 126, 166
 Neurodegenerative diseases (NDs) 23, 25, 52, 105, 126, 224
 Neuropathic pain relief drugs 152
 Neuroprotective drugs 23, 268
 Neuropsychiatric diseases 134
 Neutrophil-activating chemokines (NACs) 101
 NF- κ B expression 40
 Nicotiflorin 45
 Nitrates removal 279, 280
 Nitric oxide 2, 30, 110, 125, 126, 177, 202
 Nitric oxide research 2, 202
 Nitric oxide synthase (NOS) 198, 305
 Nitrogen compounds (NC) 54, 248, 279
 Nitrogen contaminants 248
 Nitrogen-fixation 237
 Nitrogen source 314
 Non-apoptotic cell death 153
 Non-nucleoside reverse transcriptase inhibitors (NNRTIs) 154
 Non-saccharide GAG mimetics (NSGMs) 20, 21
 Non-treatment groups 253
 Normal tissue complication probability (NTCP) 302, 303
 Normal white blood cells 316
 NR1 expression 278
 NTFs 234
 Nuclear magnetic resonance (NMR) 92, 128, 143, 145, 151, 156, 176, 177, 215, 272
 Nuclear medicine 66, 114, 123, 124, 132, 191
 Nutritional compounds 141
 Nutritional quality 269, 314
 Nutritious food product 269
 Nymphaea 295

O

Obesity research 1, 201

Oleanolic acid (OA) 176, 177
 Organic sanitizers 309
 Organ transplants 228
 Orphan drugs 11
 Outer mitochondrial membrane 43, 44
 Ovarian cancer 134, 213
 Oxidative stress 23, 80, 81, 159, 177, 193, 218, 238, 248
 Oxidized low-density lipoproteins 239
 Oxygen radical absorbance capacity (ORAC) 240
 Oxygen transfer rate (OTR) 280, 281

P

Paecilomyces lilacinus 318
 PAF receptor expression 101
 Palmitic acid 195, 236
 Pancreatic beta cells 226
 PAOT skin technology 248
 Parametric imaging 38, 39, 123, 124
 Parkinson's disease (PD) 4, 17, 21, 23, 66, 131, 149, 185, 204, 234
 PBPK model 110
 PCa cells 29
 Peanut allergy 142
 Pentobarbitone-induced sleep test 138
 Peptides 17, 25, 33, 72, 80, 121, 123, 170, 171, 174, 225, 230, 249, 252
 Peracetic acid 308, 309
 Petroleum bioremediation 296
 Phantom vibration syndrome 171
 Pharmaceutical care 137, 138
 Pharmac-MPE paradigms 3, 203
 Pharmacophoric models 183
 Phenolic profile 167
 Phenothiazines 19, 20
 Phenotypic screening 35, 36, 63
 Phenylpropanoid pathway 45
 Phormidium fragile 275
 Phosphatase 125
 Phosphate buffered saline (PBS) 141, 220, 251
 Phosphate solubilizing bacteria (PSB) 236
 Photopolymerization 271
 Photosynthesis 233, 275
 PH potential 196, 197
 PH-responsiveness 197
 Physicochemical characterization 59, 133
 Physiologically-based pharmacokinetic (PBPK) 110
 Phytochemical analysis 138, 152, 289
 Phytochemical screening 68, 115, 116
 Phytohormone 255, 277
 Phytohormone signaling 255
Pichia pastoris 244, 245, 299, 312
 PIC micelles 197
 Pim-1 kinase 183
 Plant extracts 81, 172, 225
 Plant growth 217, 236, 237
 Plant growth promoting bacteria 237
 Plant growth promoting rhizobacteria (PGPR) 236
 Plant species 138, 265, 284
 Plant tissue culture 320
 Plasma 16, 47, 49, 69, 84, 123, 157, 173, 267, 268, 289
 Plasma membrane 8, 289
 Plasma microRNAs 268
 Plasmid vectors 97
Plasmodium falciparum 60, 74, 184
 Platelet cytotoxicity 29, 30
 Platelet cytotoxicity levels 30
 Platelet-rich fibrin (PRF) 283
 Point mutation 3, 203, 276
 Polyglutamic acid 280, 281

Polyion complex micelle 197
 Polymeric micelles 151, 155
 Polymers 20, 99, 148, 180, 192, 227, 281
 Polypeptide 124, 196
 Polysaccharides 26, 45, 58, 77, 222
 Polyurethane 270
 Positive predictive value (PPV) 191
 Postnatal wounds 220, 221
 Practical miniaturized technique 321
 Preventive medicine 265
 PRF gel 283
 Proanthocyanidin polymer 145
 Proanthocyanidins 175
 Production of biofuels 233, 304
 Prognosis 94, 170
 Prostatin 212, 213
 Protease inhibitors 94, 254
 Proteasome inhibitors 35
 Protective effect 86, 238
 Protein arginine deiminases (PADs) 119, 120
 Protein farnesylation 296
 Protein function 273
 Protein glycosylation 317
 Protein grafting 22
 Protein-polymer core-shell nanoparticles 188
 Protein-protein interactions 27
 Protein S (PS) 2, 15, 22, 29, 58, 65, 91, 121, 150, 177, 202, 213, 280, 287, 293, 322
 Proton-coupled oligopeptide transporter 1 162, 163
 Prototype foamy virus (PFV) 318, 319
 PRP therapy 189, 190
Psoralea corylifolia (PC) 53, 54, 145, 184
 Pulmonary hypertension (PH) 180, 204, 260, 261
 Pulmonary lesions 123, 124
 Pulsatile 102, 103
 Purkinje cells 71

Q

Quantitative structure-activity relationship (QSAR) 65, 183
 Quantum dots (QDs) 58, 119, 129
 Quinone-derivatives (QD) 154, 155

R

Radial-arm water maze (RAWM) 109
 Radial glial cells (RGCs) 278
 Radical scavenging activity (RSA) 281
 Radiotherapy 75, 209
 Ramularia leaf spot (RLS) 78, 306
 Rapamycin 52
 Rapid colony enumeration 321
 Rational biological design (RBD) 214
 Rational protein design 89
 R&D productivity 103
 Reactive nitrogen species (RNS) 29
 Reactive oxygen species (ROS) 29, 30, 47, 48, 56, 122, 153, 159, 173, 178, 213, 238, 247, 316
 Real-time fluorescent quantitative PCR 268
 Receptor binding assay 293
 Recombinant growth hormone 293
 Red blood cells (RBC) 107, 283
 Reducing power 159, 321
 Regions of interest (RoI) 4, 54, 66, 131, 204
 Regulating protein expression 273
 Regulatory development 232
 Rehabilitation 189, 190
 Renal disease 239
 Residue-residue interaction 198

Resistant glucan 308
 Response surface methodology (RSM) 314
 Retrotransposons 295
 Reutericyclin 14, 15
 Rheumatoid arthritis (RA) 120, 252, 294, 317
 Rhizome extract 113
 Ribosomes 6, 206
 Rice bran extract (RBE) 314
 RNA binding proteins (RBPs) 126
 RNA splicing 90
 Rotating tube technique (RTT) 150
 Ruthenium metallodrugs 33, 184

S

Salinity and alkalinity pollutants 260
 Salinity and osmotic stress 292
Salix caprea 112, 113
 Salt stress 216, 260, 297
 Sanitizers 308, 309
 Scopolamine-treated control mice 109
 SCoT marker 277
 SDS-PAGE 241, 293
 Seafloor polymetallic sulfide 256
 Seed oil 212, 236, 244, 263
 Selective androgen receptor modulators (SARMs) 18
 Selective pressure 312, 313
 Semiquantitative evaluation 131
 Separase 131, 132
 Separase overexpression 131, 132
 Sepin-1 131, 132
 Serum LDH levels 29, 30
 Serum nitrite 30, 165
 SHIP-1 125
 SHR-cp rats 157
 Sick cell disease (SCD) 107
 Sideritis 160, 161
 Silk fibroin 270
 Silver colloids 316
 Silver precursor 317, 318
 Single emission computed tomography 191
 Sinomenine 194, 195
 Sinomenine hydrochloride 194, 195
 siRNA 5, 43, 116, 205
 Sleep do 171
 Small molecule immune potentiators (SMIPs) 122
 Small RNA 5, 240, 255
 Smectite 143, 144
 SMN protein 314
 SMN protein expression 314
 Smooth muscle cells (SMCs) 260, 261
 SNPs 305, 320
 Sodium alginate 99, 148, 298, 307
 Soestella 156
 Solanaceae 138
Solanum commersonii 310, 311
Solanum nigrum 310, 311
Solanum tuberosum 310, 311
 Solid lipid nanoparticles (SLNs) 151, 155
 Solubilization efficiency 301
 Somatic embryo 276, 292
 Soyasapogenol B 217
 Soybean saponin 217
 Spatial memory 109, 165
 Specialized proresolving mediators (SPM) 4, 204
 Spilanthal 149, 150, 192
 Spinal cord 40, 224, 246
 Spinal cord injury 23, 189, 190
 Spinal muscular atrophy (SMA) 205, 314, 315

Spirulina 261
 Sponge crude extract (SCE) 156
 Spray flash evaporation (SFE) 34, 35
 SR clones 250
 Standardized uptake value (SUVs) 66, 132
 Standard uptake values ratios (SUVr) 54, 66
Staphylococcus aureus 79, 105, 186, 187, 309
 Stem cell therapy 209, 211, 269, 315
 Stepwise discriminant analysis (SDA) 312
 Steroid resistant (SR) 250
 Stress adaptation 255
 Stress granules (SGs) 126
 Stress response 324
 Stroke 23, 50, 67, 91, 165, 166, 266, 307
 Structure-activity relationship 9, 10, 15, 17, 36, 49, 62, 87, 183, 187, 261
 Subtilisin1 280
 Subtilisin3 280
 Sugarcane bagasse hemicellulosic hydrolysate (SBHH) 314
 Suicidal gene therapy 113
 Sun protection factor (SPF) 82
 Supercritical extraction 150
 Supplementation of atorvastatin 157
 Supramolecular chemistry 200
 Surface-enhanced raman scattering (SERS) 316
 Survival motor neuron (SMN) 314, 315
 Sustainable development 242
 SUVmax 123
Symphytum tuberosum 310, 311
 Symptomatic radiation pneumonitis (SRP) 303
 Synapses 83, 84, 145, 307
 Systolic blood pressure (SBP) 137
Syzygium aromaticum 176, 177

T

Tachycardia 214, 215
 TAK-085 supplementation 157
 Target-based drug discovery 36
 Target enzyme 158, 190, 198
 Technetium-99m 130
 Temporal release 22
 Th1/Th2 cytokines 40, 156
 Therapeutic hypothermia 96, 100
 Therapeutic mAbs 11
 Therapeutic monoclonal antibodies 11, 26
 Therapeutic strategies 68, 82, 142, 148, 261
 Therapeutic targets 17, 19, 46, 52, 75, 82, 93, 124, 146, 148
 Thiosemicarbazides 186
 Thrombin 46, 136, 191
 Thrombophilia 91
 Thrombosis 20, 91, 191
 Thymus 73, 167, 251, 269, 291
 Thyroid hormones 28, 100, 178, 179
 Thyroid-stimulating hormone (TSH) 28, 230
 Tinnitus 302, 303
 TLR2 40
 Toll like receptors 40
 Topoisomerase inhibitors 56, 186, 187
 Total carotene content (TCC) 228
 Total cholesterol 157, 164, 165, 253
 Total phenolic content (TPC) 166, 167, 281
 Toxic effects 73, 74, 231, 313
 TP administration 73
 Transcriptional co-regulation 312, 313
 Transcription factor 31, 64, 101, 108, 139, 193, 255, 296
 Transcutaneous applications 225

Transdisciplinarity 218
 Transduced CD34+ cells 264
 Transfected cells 314, 323
 Transformation 13, 45, 110, 251, 253, 272, 292, 300, 313
 Transformation flavodoxin gene 292
 Transgene copy number 253
 Transgenic cassava 253
 Transgenic mice 72, 268
 Transgenic pea 216
 Transgenic plants 216
 Translational research 19, 47, 231, 232
 Traumatic brain injury (TBI) 21, 187, 188
 Trehalose surfactant 92, 93
Trichophyton rubrum 179, 280
 Tricosane 176
 Triple negative breast cancer (TNBC) 57, 131, 133
Triticum aestivum L. 324
 1,2,4-triazole-3-thiones 187
 Tropical ginger 113, 114
 Troxerutin 177, 178
 TSHR antagonist 28
 Tuberculin 6, 7, 206, 207
 Tuberculin skin test (TST) 6, 7, 206, 207, 288
 Tuberculosis 7, 16, 96, 97, 114, 117, 125, 126, 139, 186, 207, 262, 277, 287, 321
 Tumor angiogenesis 323
 Tumor cells 13, 64, 75, 92, 93, 110, 168, 178, 251
 Tumor endothelial cell (TECs) 323
 Tumor markers 268
 Tumor microenvironment 115, 121
 Tumors 8, 13, 56, 64, 69, 84, 114, 121, 124, 132, 133, 162, 163, 196, 209, 323
 Tyrosine kinase inhibitors (TKI) 16

U

Uleine 110, 111, 125, 126
 Ultra-low molecular weight heparin (ULMWH) 14, 89
 Unified Parkinson's disease rating scale 131
 Up-regulated genes 296
 Urban trees 265
 Uric acid 316
 Uterine fibroid 113

V

Vascular endothelial growth factor (VEGF) 22, 31, 108, 134, 219
 Vascular graft 270
 Vascular normalization 75, 194, 195
 Vascular smooth muscle cells (VSMCs) 146
 Vasculogenesis 22
 Vaseline gauze 315
 V-ATPase 70
 Vector backbone (VBB) 253
 Vector copy number (VCN) 264
 Viniferin 257
 Violaxanthin de-epoxidase (VDE) 264, 265
 Virtual library 136
 Virtual screening 177
 Vitamin 34, 242, 265
 Vitamin A deficiency (VAD) 34
 Voltage-dependent anion channels (VDACs) 43, 44
 Vorinostat 55, 56

W

Walnut gene transformation 292
 Waste activated sludge 301
 Waterlily 295
 Water qualities 223, 302
 Wheat glume 296
 Wheat straw 310
 White adipose tissue 178
 Whole cell biocatalyst 217
 Whole cell catalyst 244
Withania somnifera 138
 Wound healing 45, 220, 276, 305, 315

X

Xenopus oocytes 195
 Xenotransplantation 46
 X-ray diffraction (XRD) 92, 271, 317, 318
 X-ray synchrotron phase contrast tomography (XSPCT) 224
 Xylitol 314
 Xylitol production 314

Y

Yeast fermentation 314

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