Conference 2015:

Drug Discovery and Development in the Post-Genomic Era

May 26-28, 2015
Chelsea Hotel, Toronto, ON, Canada

A joint conference of:

Canadian Society for Pharmaceutical Sciences
Canadian Chapter of Controlled Release Society

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Fred Haynes, Ph.D., President

Conference Program

TUESDAY, MAY 26, 2015	
Time	
7:00 AM	***Registration***
8:00 AM - 5:00 PM	**VACCINE INNOVATION CONFERENCE** *(One-day Conference)* (Mountbatten Salon A)
3:00 to 5:00 PM	**CSPS SPECIAL SESSION: The Future of Pharmaceutical Sciences** *Chair*: Mo Jamali, University of Alberta (Mountbatten Salon B)
	Speakers: **Kishor Wasan, University of Saskatchewan** The Future of Pharmaceutical Sciences Research: Background and Current Status **Rav Kumar, GlaxoSmithKline** New Models for Partnership and Externalization of Pharmaceutical R&D **Parimal Nathwani, MaRS Innovation** Commercializing Academic Innovations in Life Sciences - What Works and What Doesn't? **Sam White CDRD** A Unique Model Paving the Path to Commercialization **Pierre Charest, NSERC** The Natural Science and Engineering Research Council of Canada (NSERC) Support for Pharmaceutical Sciences: Recent Trends **Phil Sherman, Canadian Institutes of Health Research (CIHR)** Update on Reforms at CIHR **PANEL DISCUSSION**
5:00 to 7:30 PM	***Joint Welcome Reception*** (Mountbatten Court) **SPONSORED BY: COREALIS Pharma**

WEDNESDAY, MAY 27 – MORNING						
7:30 to 9:00 AM	***Trainee Mentoring Breakfast*** **BREAKFAST SPONSORED BY: AGILENT TECHNOLOGIES** *Chair:* Todd Hoare, McMaster University (Rosetti Room - 3rd Floor) ***Registration and Poster Set-Up - Continental Breakfast***					
9:00 to 10:00 AM	**PLENARY: Shana Kelley, University of Toronto** New Technologies for Ultrasensitive Analysis of Clinically-relevant Biomolecules *Chair:* Carolyn Cummins, University of Toronto (Mountbatten Salon A & B)					
10:00-10:30	***Refreshment and networking break, Poster viewing and Exhibitors*** (Mountbatten Lane & Court)					
10:30 - 12:00 noon	**SESSION 1: The Evolving Business of Pharmaceuticals** *Chair*: Ted Witek, University of Toronto (Scott Room - 3rd Floor)		**SESSION 2: Analysis of Peptide and Protein Drug Targets by LC/MS/MS** *Chair*: Carolyn Cummins, University of Toronto (Wren Room - 3rd Floor)		**SESSION 3: Mucosal Drug Delivery** *Co-Chairs*: Emmanuel Ho, University of Manitoba, and Marta Cerruti, McGill University (Rosetti Room- 3rd Floor)	
10:30	**Ted Witek University of Toronto**	Why Pharma Needs a Strategy for the Second Half	**Anne-Claude Gingras Res. Institute, Mt. Sinai Hospital**	Spatio-temporal Regulation of Cellular Signaling	**Olivia Merkel Wayne State University**	Targeted siRNA Delivery to T Cells in the Lung as Novel Asthma Therapy
11:00	**Brett Skinner, Rx&D**	What are the Latest Policy Metrics and Why do they Matter?	**Christoph Borchers, University of Victoria**	LC-MS/MS: For Precise and Multiplex Protein Quantitation using MRM and for Structural Characterization of Protein Therapeutics using HDX, Sub-zero LC and ETD	**Justin Hanes Johns Hopkins University**	Mucus Penetrating Nanoparticles
11:30	**Steve Gyorffy Boehringer Ingelheim Pharma**	The Truth about Genomics and Drug Development	**Laura McIntosh Caprion Proteome**	Quantitative Proteomics Approaches for Personalized Medicine	**Haeshin Lee KAIST, Korea**	Adhesive Paintable Biomaterials Inspired by Marine Mussels
12:00-12:10	**Manuela G. Neuman, University of Toronto** (Selected abstract for oral presentation)	Immunogenetic Basis of Platelet - Inhibitors Induced Hypersensitivity - Lymphocyte Toxicity Assay	**Ryan Lillico, University of Manitoba** (Selected abstract for oral presentation)	HDAC Inhibitor Induced Changes in Histone Methylation	**Yufei Chen, University of Manitoba** (Selected abstract for oral presentation)	Evaluating the Drug Release and In Vivo Biocompatibility of an Intravaginal Implantable Device in a Rabbit Model
12:10 – 12:40	**CSPS Leadership Award Lecture: Neal Davies, University of Manitoba** 30 Years of Coffee, Beer and Serendipity in Pharmacy Research *Chair*: Frank Burczynski, University of Manitoba (Mountbatten Salon A & B)					

WEDNESDAY, MAY 27 – AFTERNOON						
12:40 to 2:30 PM	***Lunch at Annual General Meetings*** (**CSPS** - Mountbatten Salon A; **CC-CRS** - Baker Room) ***Networking and poster viewing*** (Mountbatten Lane & Court)					
2:30 -5:10 PM	**SESSION 4: New Methodologies of Genome Wide Target Validation** *Chair*: Stephane Angers, University of Toronto (Scott Room- 3rd Floor)		**SESSION 5: Regulatory Updates and Developments** *Chair*: Manon Belisle, Teva Canada (Wren Room- 3rd Floor)		**SESSION 6: Antibody-based Therapeutics** *Chair*: Marie Roumi, National Research Council Canada (Rosetti Room- 3rd Floor)	
2:30	**Corey Nislow University of British Columbia**	Genomics for Precision Drug Therapy in the Community Pharmacy: "DNA-guided Drug Dosing"	**Fakhreddin Jamali University of Alberta**	CSPS Workshop on Subsequent Entry Biologics (SEBs): Summary, Lessons Learned, and Conclusions	**Joseph Balthasar, State University of New York at Buffalo**	Application of PK/PD Modeling to Guide the Development of New Antibody-based Therapies
3:00	**Panagis Filippakopoulos University of Oxford, UK**	Control of Gene Expression by Selective and Non-selective Direct Inhibition of Bromodomain-acetyl-lysine Readout	**Agnes Klein Health Canada**	Controversies in Biosimilars	**Danica Stanimirovic, National Research Council**	Conquering the Barriers - Development of Bi-specific Antibodies to Treat CNS Diseases
3:30-3:40	**Ma'en Obeidat, UBC** (Selected abstract for oral presentation)	Systems Genetics Approach Identifies Nicotine Receptor Antagonist as a Potential Therapeutic Target in COPD	Discussion		**Simmyung Yook, University of Toronto** (Selected abstract for oral presentation)	^{177}Lu-labeled and Dual-receptor Targeted Radiation Nanomedicine for Simultaneous Targeting of HER2 and EGFR on Breast Cancer Cells
3:40-4:10	***Refreshment and networking break, poster viewing and exhibitors*** (Mountbatten Lane & Court)					
4:10	**Stephane Angers University of Toronto**	Deciphering Growth Factor Signalling Mechanisms in Cancer using Functional Genomics and Proteomics	**Nasrullah Undre Astellas Pharma EMEA, Chertsey, United Kingdom**	Clinical and Bioequivalence Consideration for Introducing Alternative Formulations of Critical Dose Drugs	**Ilia Tikhomirov, Formation Biologics Inc.**	Development of Novel Antibody-drug Conjugates in Oncology
4:40	**Randall Platt, Biological Engineering - MIT**	CRISPR-Cas9 for in vivo Genome Editing and Disease Modeling	**John Chapdelaine, Pharmascience**	3Rs of GLP Certification for Bioanalysis: Reasons, Results, and Reactions	**Heman Chao, Helix BioPharma Corp.**	Development of a Camelid Antibody Enzyme Conjugate for the Treatment of Non-small Cell Lung Cancer
5:10 - 6:00	***Networking and Poster Viewing*** (Mountbatten Lane & Court)					
	Conference Gala and Awards Dinner (Mountbatten Salon) **6:30 PM Cash Bar; 7:00 PM Dinner**					

THURSDAY, MAY 28 – MORNING						
8:00 to 9:00 AM	***Registration and Poster Set-up*** ***Continental Breakfast*** **BREAKFAST SPONSORED BY: AGILENT TECHNOLOGIES**					
9:00 to 10:00 AM	**PLENARY: Richard Hargreaves, BIOGEN IDEC.** Imaging in CNS Drug Discovery and Development *Chair:* Robert Young, Simon Fraser University (Mountbatten Salon A & B)					
10:00-10:30	***Refreshment and networking break, poster viewing and exhibitors*** (Mountbatten Lane & Court)					
10:30 - 12:10	**SESSION 7: Imaging in Drug Delivery** *Co-Chairs*: Christine Allen, University of Toronto, and Jinzi Zheng, University Health Network (Rosetti Room- 3rd Floor)		**SESSION 8: Nuclear Receptors in Drug Discovery** *Chair*: Reina Bendayan, University of Toronto (Scott Room- 3rd Floor)		**SPONSORED BY: COLORCON** **SESSION 9: IV-IVC Modeling and Simulation as a Tool to Facilitate Drug Development and Marketing** *Co-Chairs*: Elisabeth Kovacs, Apotex, and Raimar Loebenberg, U Alberta (Wren Room- 3rd Floor)	
10:30	**Helen Lee Merrimack Pharmaceuticals**	Tumor Deposition Imaging as a Predictive Biomarker for Personalized Nanomedicine	**Richard Kim Western University**	Regulation of Drug Metabolizing Enzymes and Transporters - Implications in Personalized Medicine	**Jasmina Novakovic Apotex**	Modeling and Simulation in Development and Life-Cycle Management of a Generic Drug Products
11:00	**Willem Mulder Mount Sinai School of Medicine**	Imaging and Nanomedicine in Inflammatory Atherosclerosis	**Wen Xie, University of Pittsburgh**	Xenobiotic Nuclear Receptors as Novel Therapeutic Targets in Obesity and Diabetes	**Isadore Kanfer Rhodes University, South Africa**	Methods for Bioequivalence of Topical Products for Local Action and IVIVC
11:30	**Twan Lammers, Aachen University, Germany**	Nanomedicines and Theranostics	**Henry Krause, University of Toronto**	A Zebrafish Based Platform for Nuclear Receptor and Cofactor Drug Discovery	**Raimar Loebenberg University of Alberta**	How to Develop Clinically Relevant Dissolution Methods
12:00-12:10	**Shyam Garg, University of Alberta** (Selected abstract for oral presentation)	Traceable Nanocarriers for Targeted Therapy of Primary and Metastatic Breast Cancer	**Jasmine Williams-Dautovich, University of Toronto** (Selected abstract for oral presentation)	LXR Antagonism Attenuates Glucocorticoid-induced Osteoclast Activation in a Mouse Model of Cushing's Syndrome	**Jodi Dickstein, Janssen Pharmaceuticals** (Selected abstract for oral presentation)	Appropriateness of Traditional Bioequivalence Metrics to Infer Therapeutic Equivalence for Follow-on Long Acting Injectables with Complex Pharmacokinetic Profiles
12:10 – 12:40	**CSPS Lifetime Achievement Award Lecture: Roger Williams** 0.5×10^2: Looking Back and Forward *Chair:* Raimar Loebenberg (Mountbatten Salon A & B)					

THURSDAY, MAY 28 – AFTERNOON				
12:40 to 2:30 PM	***Lunch Break, Networking, Poster Viewing, and Exhibitors*** (Mountbatten Lane & Court)			
2:30 to 5:10 PM	**SESSION 10: Pharmacogenomics in Drug Development** *Chair*: Micheline Piquette-Miller, University of Toronto (Wren Room- 3rd Floor)		**SESSION 11: Bioavailability of Novel Dosage Forms** *Co-Chairs*: Adrien Musuku, Pharmascience, and Michael Doschak, University of Alberta (Rosetti Room- 3rd Floor)	
2:30	**Shiew Mei Huang Center for Drug Evaluation & Research (CDER), FDA**	Application of Pharmacogenomics in Drug Development, Regulatory Review and Clinical Practice	**Michael Doschak, University of Alberta**	Advances in Peptide Delivery Formulation to Impart Bone Specificity
3:00	**Allen Roses Duke University/ Zinfandel Pharmaceuticals**	Short Tandems Repeats [STRs] are more Informative for Disease Diagnosis and Translation than SNP Associations	**Adrien Musuku Pharmascience**	Updates on the Bioanalytical Sector
3:30	***Refreshment and networking break, poster viewing, and exhibitors*** (Mountbatten Lane & Court)			
4:00-4:10	**Stephanie Dumas, Université de Montréal** (Selected abstract for oral presentation)	Preliminary Results on the Impact of Genetic Factors on Gastrointestinal Bleeding in a Prospective Cohort of New Warfarin Users	**Petro Czupiel, University of Toronto** (Selected abstract for oral presentation)	Targeted Delivery of siRNA by Polymeric Nanomicelles
4:10	**Edward Sellers University of Toronto, DL Global Partners**	The Post-Genomic Era: Two Steps Forward and One Step Back?	**Krishna Bhandari Ashland Specialty Ingredients, Ashland Inc.**	Role of Excipients in Design of Solid Amorphous Drug Dispersions Using Hot Melt Extrusion and Spray Drying for the Delivery of Poorly Water-soluble Drugs
4:40	**James Elliott Thermo Fisher Scientific**	Clinical Pharmacogenetics: A Case Study in Personalized Medicine	**Barbara Davit, Merck**	Bioequivalence and Pharmaceutical Equivalence Criteria Recommended by US-FDA for Drugs Acting Locally Within GI Tract
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Tuesday, May 26

The Future of Pharmaceutical Sciences

Pharmaceutical Sciences in Canada

Fakhreddin Jamali, Professor, Faculty of Pharmacy & Pharmaceutical Sciences, Edmonton, AB, Canada

Pharmaceutical sciences are a group of interdisciplinary study areas addressing the discovery, design, action, disposition, delivery, chemistry (organic, inorganic, physical, biochemical, analytical and chemometrics), biology (anatomy and physiology, biochemistry, immunology, cell biology, and molecular biology), epidemiology, statistics, regulatory affairs, mathematics, physics, and chemical engineering, as they apply to the study of drugs. Such activities, therefore, expand beyond the traditional pharmacy schools. However, it appears that the development side of pharmaceutical sciences is often left behind by funding agencies, creating a shortage of highly trained personnel in the areas.

Fakhreddin Jamali

Dr. Jamali (Doctor of Pharmacy, University of Tehran; MSc, pharmaceutics and PhD, pharmacokinetics, University of British Columbia) is a professor at the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. He joined the faculty at the University of Alberta in 1981. His research interests include effect of pathophysiological changes on the action and disposition of drugs, stereochemical aspects of drugs action and disposition, basic and clinical pharmacology of anti-rheumatic, analgesic and cardiovascular drugs, and toxicology of nonsteroidal anti-inflammatory drugs. He has trained 39 PhDs and published over 220 refereed articles (H-Score, 41). http://scholar.google.ca/citations?user=8LIRXpkAAAAJ&hl=en.

Dr. Jamali has been an invited speaker at many conferences. He has been involved as a principal investigator with the Centre of Excellence for Gastrointestinal Inflammation and Immunity Research and also a Theme Leader of the Canadian Arthritis Network (Networks of Centres of Excellence). For his academic achievements and research, he has been appointed as a Fellow of the Canadian Society for Pharmaceutical Sciences (CSPS), the American Association of Pharmaceutical Sciences, and the American College of Clinical Pharmacology. He has received the Killam Professorship, McKeen Cattell Memorial Award of the American College of Clinical Pharmacology, the McCalla Professorship of the University of Alberta, the McNeil Award of Association of Faculties of Pharmacy of Canada, and the CSPS Research Leadership Award. For his service to the public, he has been honored with the Alberta Centennial Medal and the Alberta Pharmacy Centennial Award of Distinction. Dr. Jamali has served as an expert witness as well as consultant and/or a member of the board of directors of many pharmaceutical houses. He has been a member of the Health Canada's Expert Advisory Committee on Bioavailability and Bioequivalence, and the Expert Advisory Panel on Nonsteroidal Anti-inflammatory Drugs. He is the founding president of CSPS and editor of J. Pharm. & Pharm. Sci. (www.cspsCanada.org). He teaches pharmacokinetics and has been involved in pharmacy curriculum development.

The Future of Pharmaceutical Sciences Research: Background and Current Status

Kishor Wasan, Dean, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK

Over the last 10 years the funding of pharmaceutical sciences research and research within pharmacy schools in Canada from the major granting agencies (i.e. CIHR and NSERC) appears to have significantly decreased. This presentation will present data to support this claim and provide possible suggestions and solutions on how to enhance funding for pharmaceutical sciences research and research within pharmacy schools in Canada.

Kishor Wasan

Dr. Wasan was appointed Dean of the College of Pharmacy and Nutrition in August 2014.

He has published over 200 peer-reviewed

articles and 240 abstracts in the area of lipid-based drug delivery and lipoprotein-drug interactions. Dr. Wasan completed his undergraduate degree in Pharmacy at the University of Texas at Austin and his Ph.D. at the University of Texas Medical Center in Houston Texas at MD Anderson Cancer Center in Cellular and Molecular Pharmacology. After completing a postdoctoral fellowship in Cell Biology at the Cleveland Clinic, Dr. Wasan joined the Faculty of Pharmaceutical Sciences at UBC until 2014.

Dr. Wasan was one of the recipients of the 1993 American Association of Pharmaceutical Scientists (AAPS) Graduate Student Awards for Excellence in Graduate Research in Drug Delivery, the 2001 AAPS New Investigator Award/Grant in Pharmaceutics and Pharmaceutics Technologies, the 2002 Association of Faculties of Pharmacy of Canada New Investigator Research Award and was named an AAPS fellow in 2006. In addition, Dr. Wasan was awarded a Canadian Institutes of Health Research University-Industry Research Chair in Pharmaceutical Development (2003-2008), was named a University Distinguished Scholar in April 2004, received the 2007 AAPS Award for Outstanding Research in Lipid-Based Drug Delivery, and the 2008 AFPC-Pfizer Research Career Award. In 2009, Dr. Wasan was named CIHR/iCo Therapeutics Research Chair in Drug Delivery for Neglected Global Diseases and in 2010, Dr. Wasan was named a Fellow of the Canadian Academy of Health Sciences. In 2011, Dr. Wasan was award the Canadian Society of Pharmaceutical Sciences Leadership award for outstanding contributions to Pharmaceutical Sciences in Canada. Dr. Wasan has received support from the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), and from several charitable foundations and pharmaceutical companies.

New Models for Partnership and Externalization of Pharmaceutical R&D

Rav Kumar, Vice-President - R&D Operations/ Business Development, GSK Canada

[No abstract]

Rav Kumar

Dr. Rav Kumar leads GlaxoSmithKline's Canadian R&D/Business Development organization which includes Clinical Research, Regulatory Affairs, External R&D Alliances and New Business Development as well as GSK's Established Products portfolio in Canada.

Rav is a pharmacy graduate with a PhD in pharmaceutical sciences (from Bath University, UK) involving research into controlled delivery of drugs such as insulin. He has more than 25 years of global drug development experience having worked for start-up and multinational pharmaceutical companies in the United Kingdom, France and North America.

Rav has been very involved in the Canadian Pharmaceutical R&D "Ecosystem" over the past decade including serving as President of the Canadian Society for Pharmaceutical Sciences (CSPS), Vice-Chair of Regulatory and Medical/Clinical Committees at Rx&D and Chair of several DIA Canada conferences.

Rav was instrumental in bringing together academia, industry and government for the "Canadian Clinical Trials Summit" held in Ottawa, to develop an action plan for improving Canada's competitiveness in this area. This resulted in the following report: *To Your Health and Prosperity – An Action Plan to Help Attract More Clinical Trials to Canada* as well as the recent creation of the Canadian Clinical Trials Co-ordinating Centre (CCTCC) – a collaborative effort of CIHR, Rx&D, and ACAHO/CHA.

Dr Kumar has served on various boards and councils including: Member of steering committee for CHIR/SPOR (Strategy for Patient Oriented Research); Lesley Dan Faculty of Pharmacy Advancement Board; Board of CQDM (Quebec Consortium for Drug Discovery); University of Toronto Mississauga Campus Council; Board of Hillfield Strathallan College, Hamilton.

Rav has guided GSK into important public private partnerships and collaborations for drug discovery with CDRD, CQDM and MaRS Innovation as well as the GSK $50M Life Sciences Innovation Fund. These have resulted in numerous investments in the Canadian Life Sciences ecosystem.

He also serves as President of SAPNA – a non-profit organization dedicated to improving the health and Health awareness of South Asian Canadians.

He has given talks at numerous conferences, events and academic institutions on topics such as Building Trust in the Pharmaceutical Industry, New Models for Drug Discovery and Development and Careers in life sciences and was the recipient of "2014 Leadership in Canadian Pharmaceutical Sciences" Award".

Commercializing Academic Innovations in Life Sciences - What Works and What Doesn't?

Parimal Nathwani, Vice President, Life Sciences, MaRS Innovation, Toronto, ON

[No abstract]

Parimal Nathwani

Parimal Nathwani received his M.Sc. from the University of British Columbia and his M.B.A. from Simon Fraser University.

He has a decade of experience in various aspects of the biotechnology industry, including finance, intellectual property management, business development and operations; he has been actively involved in preparing business plans, forming start-up companies, raising early-stage capital, managing intellectual-property portfolios and out-licensing initiatives.

Most recently, Parimal was a healthcare analyst with a boutique investment bank, where he conducted due diligence on publicly-traded biotechnology companies in Canada and the U.S. for an institutional investor client base and advised companies on financing strategies. He has also held the position of technology transfer manager in the life sciences at one of Canada's leading technology transfer offices.

A Unique Model Paving the Path to Commercialization

Sam White, Ph.D. M.Pharm., Manager, Project Search and Evaluation/Foundation Partnerships, The Centre for Drug Research and Development (CDRD), Vancouver, BC

As getting new drugs and other therapeutic products to the market becomes more and more challenging and the associated costs and risks increase, it is important to find unique new partnership models that enable innovative ways of collaborating to advance early stage discoveries. Such collaboration will make it possible for the various players — from academic institutions and government, to translational research organizations to foundations and industry around the world — to find new ways to effectively remove the risk from new technologies, to leverage resources and to fill the gaps in the development continuum, ultimately bringing these innovative discoveries to patients.

The Centre for Drug Research and Development (CDRD) is Canada's national non-for-profit drug development and commercialization centre, enabling academic investigators from across Canada and internationally to de-risk, advance and translate their innovative discoveries in the health science into therapeutics that can impact the lives of patients. CDRD is one of only a handful of fully integrated translational research organizations in the world—and the only one in Canada—with the in-house infrastructure and expertise to undertake all aspects of pre-clinical drug development and commercialization.

By providing expertise and infrastructure to enable researchers from leading health research institutions to advance promising early-stage drug candidates, and through its unique partnerships with Academia, Industry, Government and Foundations, the CDRD is able to help de-risk discoveries stemming from publicly-funded health research and transform them into viable investment opportunities for the private sector. The model is successfully bridging the commercialization gap between academia and industry, and translating research discoveries into new therapies for patients while also maximizing the return on government's investment in basic health research from both an economic and societal perspective.

Sam White

Dr. White leads the Project Search and Evaluation Team at The Centre for Drug Research and Development (CDRD), Canada's national, not-for profit drug development and commercialization centre, responsible for the identification and evaluation of new technologies, raising non-dilutive funding for projects and chairs CDRD's Foundations Working Group. To date he has evaluated several hundred new technologies from CDRD's global network of affiliated institutions and secured over $20 million to enable the advancement of drug development projects.

Before joining CDRD in 2010, Dr. White worked for H2O Venture Partners LLP, a private equity, technology investment firm based in Oxford, UK. Dr. White holds a Masters degree in Pharmacy (1st Class Hons.), completed his clinical training in 2005 and obtained his PhD in 2009 from the Welsh School of Pharmacy, Cardiff University, UK.

The Natural Science and Engineering Research Council of Canada (NSERC) Support for Pharmaceutical Sciences: Recent Trends

Pierre Charest, Vice-President, Research Grants and Scholarships Directorate, NSERC

[Abstract not available]

Pierre Charest

Pierre Charest joined NSERC in August 2011 and currently serves as Vice-President, Research Grants and Scholarships Directorate. He previously held the position of Associate Vice-President of Corporate Planning and Policy at NSERC.

Prior to his time at NSERC, Pierre worked at Health Canada, where he was Director General of the Science Policy Directorate. Pierre also held the positions of Associate Vice-President of the Science Branch of the Canadian Food Inspection Agency and Director General of Health Canada's Biologics and Genetic Therapies Directorate and Office of Biotechnology and Science.

Pierre started his career in the public service in 1989 as a research scientist at the Canadian Forest Service of Natural Resources Canada, where he occupied a number of positions with increasing responsibility. He also had appointments as adjunct professor at the University of Victoria and as honorary research associate at the University of New Brunswick.

Before joining NSERC, Pierre served as chair and member of several peer review committees for NSERC, the Canadian Institute of Health Research, the Canada Foundation for Innovation and Genome Canada. He has had a productive research career as author or co-author of 77 scientific publications and presenter of 80 scientific communications and over 25 invited lectures.

Pierre holds a B.Sc.A. and an M.Sc. in Agronomy from Université Laval, graduate certificates in Health System Leadership and Executive Coaching from Royal Roads University, and a Ph. D. in Molecular Biology from Carleton University.

Update on Reforms at CIHR

Philip M. Sherman, MD, FRCPC, Scientific Director, Institute of Nutrition, Metabolism and Diabetes, Canadian Institutes of Health Research (CIHR)

[Abstract not available]

Philip Sherman

Philip Sherman is Professor of Paediatrics, Microbiology, & Dentistry at the Hospital for Sick Children, University of Toronto where he has been on faculty since 1984. Phil completed medical school at the University of Calgary and training in pediatrics at the University of California, San Francisco. Training in gastroenterology and research was completed at the Hospital for Sick Children and the Walter Reed Army Institute of Research in Washington, DC.

Phil is a Past-President of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and a Past-President of the Canadian Association of Gastroenterology. He is the recipient of a Canada Research Chair (tier 1) in Gastrointestinal Disease. His research program is funded by support currently provided by the Crohn's and Colitis Foundation of Canada and the Canadian Institutes of Health Research (CIHR). His research interests focus on epithelial cell signal transduction responses to pathogenic, commensal, and probiotic bacteria.

In 2010, Phil was awarded the prestigious Shwachman Award from the North American Society for Pediatric Gastroenterology and Nutrition (NASPGHAN). The award recognizes significant and lifelong scientific or educational contributions to the field of pediatric gastroenterology, and awardees must also hold a record of advocacy for child digestive diseases and demonstrate exemplary service to the field.

Phil assumed the position of Scientific Director of the Canadian Institutes of Health Research (CIHR) Institute of Nutrition, Metabolism and Diabetes in January, 2009.

Wednesday, May 27

Plenary Lecture 1

New Technologies for Ultrasensitive Analysis of Clinically-relevant Biomolecules

Shana O. Kelley, Departments of Pharmaceutical Sciences, Chemistry, Biomedical Engineering and Biochemistry, University of Toronto, Toronto, ON

The analysis of panels of molecular biomarkers offers valuable diagnostic and prognostic information for clinical decision making. Robust, practical platforms that detect low levels of biomolecules (< 1000 copies) are urgently needed to advance medical care by diagnosing and predicting the progression of cancer and other disease states. Electrochemical methods providing low cost and direct biomarker read-out have attracted a great deal of attention for this application, but have, to date, failed to provide clinically-relevant sensitivity. We exploit controlled nanostructuring of electrode surfaces to promote surface accessibility and enhance capture rate and efficiency to solve this long-standing problem, and showed that the nanoscale morphologies of electrode surfaces control their sensitivities.[1] In addition, we have worked towards integrating nanomaterials-based electrodes into a chip-based platform to facilitate multiplexed analysis in a robust, practical format.[2] Recently, we have developed assay that are able to detect nucleic acids, proteins and small molecules, with universally high sensitivity levels.[3,4] Our efforts to use these components to detect markers in clinical samples to develop tests for infectious disease diagnosis, oncological management and transplant medicine will be featured in this lecture.[5-10]

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2. B. Lam, R.D. Holmes, L. Live, A. Sage, E. H. Sargent, S.O. Kelley, *Nature Communications,* **2013**, *4*, 2001.
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8. B. Lam, Z. Fang, E.H. Sargent, S.O. Kelley, *Analytical Chemistry*, **2012**, *84*, 21
9. J.D. Besant, J. Das, E.H. Sargent, S.O. Kelley, *ACS Nano*, **2013**, 7, 8183.
10. E. Vasilyeva, Z. Fang, M. Minden, E.H. Sargent, S.O. Kelley, *Angewandte Chemie*, **2011**, *50*, 4137.

Shana Kelley

Dr. Shana Kelley is a Distinguished Professor of Chemistry, Biochemistry, Pharmaceutical Sciences, and Biomedical Engineering at the University of Toronto. Dr. Kelley received her Ph.D. from the California Institute of Technology and was a NIH postdoctoral fellow at the Scripps Research Institute.

The Kelley research group works in a variety of areas spanning bioanalytical chemistry, chemical biology and nanotechnology. Shana's group has developed novel electrochemical sensors that enable ultrasensitive nucleic acids detection for clinical diagnostics, and has also investigated a new set of chemical probes that interact with intracellular nucleic acids. The Kelley labs also use nucleic acids as building blocks for complex nanomaterials assembly. Dr. Kelley's work has been recognized with a variety of distinctions, including being named one of "Canada's Top 40 under 40", a NSERC E.W.R. Steacie Fellow, and the 2011 Steacie Prize. She has also been recognized with the Pittsburgh Conference Achievement Award, an Alfred P. Sloan Research Fellowship, a Camille Dreyfus Teacher-Scholar award, a NSF CAREER Award, a Dreyfus New Faculty Award, and was also named a "Top 100 Innovator" by MIT's Technology Review. She is a founder of two molecular diagnostics companies, GeneOhm Sciences (acquired by Becton Dickinson in 2005) and Xagenic Inc.

Wednesday, May 27

SESSION 1:

The Evolving Business of Pharmaceuticals

Why Pharma Needs a Strategy for the Second Half

Theodore J. Witek, Jr, DrPH

The pharmaceutical industry has developed the frameworks and competencies to successfully bridge the first valley of death between biological concept and drug. The now popular concept of translational science was, in many ways, advanced most successfully in drug development. Bridging the second valley of death, i.e., drug to integrated health and happiness, however, has been more a tightrope than bridge. While competencies such as value demonstration have been developed at a reasonable pace, the understanding of evolving health systems, organizations and their inhibiting inertia, integration into systems and the human behaviour that organize and mange them have remained immature for current needs. Deliberate attention, including identification, development, and deployment of competencies to these issues will uncover the true value of the innovations that the pharmaceutical industry brings to health care.

Theodore J. Witek, Jr

Dr. Ted Witek is a health care advisor and scholar based in Toronto and Lisbon. He currently serves as Senior Vice President, Corporate Partnerships, Clinical and Medical Affairs at Theravance, Inc. in San Francisco. Prior to joining Theravance, Dr. Witek, served as President and Chief Executive Officer, Boehringer Ingelheim in Canada from 2008-2014, and in Portugal from 2004-2008. Joining Boehringer in 1992, Dr. Witek held a number of positions of increasing responsibility, during which time he led the global clinical development and launch of several respiratory products, most notably Spiriva®. He also led the Respiratory and Immunology clinical research groups in the United States. In 2001, he moved to Germany to lead the operating team for Spiriva® where he also served as the Boehringer Ingelheim Co-chair of the Joint Operating Committee with Pfizer in their global alliance. During his tenure in Canada, Dr. Witek served on the Board of Directors at Rx&D, Canada's National Association for Research-Based Pharmaceutical Companies, chairing its Heath Technology Assessment Committee and Public Affairs Committee. He also served over ten years on the Drug/Device Discovery and Development Committee of the American Thoracic Society, serving as Chairman from 2010 to 2012. He is currently appointed by the Ministries of Heath and Innovation to the Ontario Heath Innovation Council. He is an advisor to the "Design for Health" Graduate Program at The Ontario College of Art and Design University and has been appointed Adjunct Professor at The University of Toronto.

Dr. Witek holds a Doctor of Public Health degree from Columbia University, a Master of Public Health from Yale University, and a Master of Business Administration from Henley Management College in London. He has authored over 100 manuscripts and chapters in the field of respiratory public health and drug development, including the textbook *Pharmacology and Therapeutics in Respiratory Care*. In addition to his expertise in drug development in obstructive airway disease, he has been a major contributor to the field of cough, cold and allergy, publishing many novel methodologies in the areas of sleep and psychomotor performance as well as laser Doppler velocimetry in assessing nasal blood flow and acoustic reflection in assessing nasal volume.

On a personal note, he is an accomplished photographer who publishes and exhibits widely.

What are the Latest Policy Metrics and Why do they Matter?

Brett J. Skinner, Rx&D

Dr. Skinner will present recent policy metrics relevant to the innovative pharmaceutical industry in Canada including access to new medicines, spending, prices, development costs, R&D, clinical trials, industry economic impact and the value of pharmaceutical innovation.

Brett J Skinner

Dr. Brett J Skinner is Executive Director, Health and Economic Policy at Canada's Research-Based Pharmaceutical Companies (Rx&D). Dr. Skinner is also the Founder and CEO of Canadian Health Policy Institute (CHPI). Dr. Skinner has a B.A. from the University of Windsor, an M.A. through joint studies between the University of Windsor and Wayne State University (Detroit), and a Ph.D. from the University of Western Ontario where he has lectured in both the Faculty of Health Sciences and the Department of Political Science. Dr. Skinner has specialized in the study of health policy and economics since 2001. He is the principal author or co-author of one book and over 50 major health policy research papers, book chapters, conference papers and numerous articles published through think-tanks in Canada and the United States, and in several academic journals including Economic Affairs, Pharmacoeconomics, and Alimentary Pharmacology & Therapeutics. He has also authored opinion-editorials appearing in major daily newspapers and popular magazines across Canada and the United States and has presented his research at conferences and events in Canada, the United States, Israel and South Africa. Dr. Skinner has provided expert testimony about his research in several Canadian legal cases, and he has testified before the House of Commons Standing Committee on Health in Ottawa. He has also briefed bi-partisan Congressional policy staff at the U.S. Capitol in Washington, D.C.

The Truth about Genomics and Drug Development

Steve Gyorffy, Ph.D., Therapeutic Director Oncology, Boehringer Ingelheim Canada Ltd

The introduction of targeted therapies has changed the way that many cancers are now treated and has opened the era for personalized medicine. Targeted small molecule inhibitors or monoclonal antibodies have improved the outcomes of many patients diagnosed with cancer. The identification of "actionable biomarkers" within tumors has translated to successes in non-small cell lung cancers with EGFR mutations, HER-2 expression in breast and BCR-ABL in chronic myeloid leukemia. However, the development of resistance to the targeted agent inevitably leads to progression of the disease. This is compounded by the extensive genomic heterogeneity within the tumor itself allowing subpopulations of cells to be unaffected by the treatment.

The development of new innovative therapies for the unmet medical need in oncology is therefore urgently required. Despite the advances in genomics and the vast genomic information available, the timelines required to develop new agents takes many years with the vast majority of preclinical compounds never achieving regulatory approval due to failure usually in late stage development. Furthermore the identification of an appropriate robust biomarker for an actionable mutation is critical to ensure that the right targeted drug is given to the right patient. Implementation of proper, timely testing of tumor samples from newly diagnosed patients may require additional training for multi-disciplinary teams involved in the patients care. In addition, many targeted therapies have been shown in the clinic to have adverse effects different than those seen with standard treatments like traditional chemotherapy.

The challenges to the development of new drugs in the era of genomics and personalized medicine will be explored in this presentation.

Steve Gyorffy

Dr. Steve Gyorffy is the Director of Oncology at Boehringer Ingelheim Canada Ltd. He obtained his Ph.D. in Health Sciences at McMaster University in the Molecular Immunology and Virology program with Dr. Jack Gauldie. The focus of his research was on the combination of immune and anti-angiogenic therapies in breast cancer models. He then completed his postdoctoral fellowship at Harvard Medical School with Drs Judah Folkman and Robert D'Amato working on the mechanisms of tumor angiogenesis. In 2003, Dr. Gyorffy joined Boehringer Ingelheim Canada as a clinical scientist and has worked on the clinical development of many oncology compounds from Phase I to Phase III registration trials.

Selected Abstract for Oral Presentation

Immunogenetic Basis of Platelet - Inhibitors Induced Hypersensitivity - Lymphocyte Toxicity Assay (Abstract # 66)

Manuela G. Neuman, University of Toronto

Wednesday, May 27

SESSION 2:

Analysis of Peptide and Protein Drug Targets by LC/MS/MS

.

Spatio-Temporal Regulation of Signaling Pathways

Anne-Claude Gingras, Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital

Kinases and phosphatases coordinate critical cellular decisions, including whether to grow and divide, to differentiate into a specific cell type, or to die. They must respond to environmental cues and transmit precise signals. Deregulation of the phosphorylation balance is implicated in multiple diseases, including cancer and neurodegenerative diseases. This deregulation can involve mutations directly in the kinase or phosphatase proteins, changes in their splicing patterns, or may involve expression modulation; all these events can lead to network rewiring. Since kinases and phosphatases frequently associate with regulators, scaffolding molecules and substrates, a possible outcome of response to a cue, or a mutation or splicing alteration (besides modulation of catalytic activity) is a change in these physical interactions.

In the past several years, we have developed proteomics methods to monitor these regulated interactions for key signaling molecules. These include a coupling of affinity purification (AP) with Selected Reaction Monitoring or with the Data Independent Acquisition (DIA) approach SWATH. With our collaborators, we have also been developing software tools to perform identification from DIA/SWATH data, leading in a more efficient utilization of the instrument time, while increasing sensitivity in the detection of the regulated interactions.

We have harnessed the AP-SWATH approach to probe the dysregulation of kinases and phosphatase interactions induced by mutations, and to analyze the consequences of pharmacological treatment on the interactions established by signaling proteins. We will discuss these approaches in the context of cancer and vascular disease

Anne-Claude Gingras

Anne-Claude Gingras received her PhD in 2001 from the Sonenberg lab at McGill University for her studies on translational control, and in particular on the regulation of translation initiation by the PI3K-Akt-mTOR pathway. She did her postdoctoral training in the laboratory of Ruedi Aebersold at the Institute for Systems Biology in Seattle where she developed approaches using mass spectrometry to identify protein-protein interactions amongst signalling molecules. She joined the Lunenfeld-Tanenbaum Research Institute in 2005 where she is now a Senior Investigator, a Canada Research Chair in Functional Proteomics and the Lea Reichmann Chair in Cancer Proteomics. She was cross-appointed in the department of Molecular Genetics at the University of Toronto in 2006 where she is currently an Associate Professor. Her lab focuses on the study of signalling pathways using systematic approaches and the development of quantitative proteomics technologies, both experimental and computational.

In collaboration with Alexey Nesvizhskii (U Michigan) and Mike Tyers (IRIC, U Montreal), she has developed a series of software tools to store, track and analyse mass spectrometry data, including powerful tools to identify true interactors from background contaminants and to visualize the data. More recently, she has been adapting her workflows for identifying dynamic protein-protein interactions to data independent mass spectrometry acquisition. She also continues her studies on the characterization of the STRIPAK complex, a signalling complex containing kinase and phosphatase activities, which is linked to both vascular diseases (Cerebral Cavernous Malformations through CCM3) and cancer (through an association with the Hippo kinases).

LC-MS/MS: For Precise and Multiplex Protein Quantitation using MRM and for Structural Characterization of Protein Therapeutics using HDX, Sub-zero LC and ETD

Christoph H. Borchers, Ph.D., Full Professor, University of Victoria, Director, UVic Genome BC Proteomics Centre

Precise and accurate protein quantitation is essential for screening biomarkers for risk stratification, disease prognostication, and therapeutic monitoring. The most promising analytical strategy for quantifying unverified biomarkers therein relies on targeted MRM/MS with isotopically labeled standards. Using that general strategy, we have developed a number of highly reproducible and multiplexed panels for quantifying candidate protein disease biomarkers in biofluids (plasma, cerebrospinal, and urine) and dried blood spots (DBS). The methods collectively utilize a bottom-up sample prep workflow with a complex mixture of our in-house synthesized peptide standards (for normalization) and standard-flow LC-MRM/MS analysis (for heightened robustness and sensitivity). In these workflows, data analysis and results interpretation were facilitated by our developed software tool – Qualis-SIS. To date, we have robustly quantified 192, 136, 130, and 97 endogenous proteins (all spanning at least 5 orders of magnitude in concentration) in human plasma, urine, cerebrospinal fluid, and DBS, respectively.

Accurate and comprehensive structural characterization of protein therapeutics is essential for in-depth understanding of their functions and the development of biosimilars. However, this remains challenging for traditional structural methods including X-ray crystallography and NMR. Amide hydrogen/deuterium exchange (HDX) monitored by MS is now established as a powerful technique for measuring protein higher-order structural changes. Although the enzyme digestion-based "bottom-up" approach is most commonly used, it only gives peptide-level information and incomplete sequence coverage, and suffers from high level of back-exchange (10-50%). The "top-down" approach, which analyzes intact proteins directly using electron-based dissociation, is becoming an important alternative and has several advantages. However, the commonly used top-down strategies are direct-infusion based, and thus could not be used for studying proteins under physiological conditions -- the very conditions which are often very important for preserving a protein's native structure and function. In this presentation, we show how top-down ECD/ETD, HDX, and subzero temperature HPLC can be combined and used to overcome these limitations and characterize the higher-order structure of protein therapeutics including monoclonal antibodies at close to single-residue resolution.

Christoph Borchers

Dr. Borchers received his B.S., M.S. and Ph.D. from the University of Konstanz, Germany. After his post-doctoral training and employment as a staff scientist at NIEHS/NIH/RTP, in North Carolina, he became the director of the UNC-Duke Proteomics Facility and held a faculty position at the UNC Medical School in Chapel Hill, NC (2001-2006). Since then, Dr. Borchers has been employed at the University of Victoria (UVic), Canada and holds the current positions of Professor in the Department of Biochemistry and Microbiology and the Don and Eleanor Rix BC Leadership Chair in Biomedical and Environmental Proteomics. He is also the Director of the UVic – Genome BC Proteomics Centre, which is one out of five Genome Canada funded Science & Technology Innovation Centres and the only one devoted to proteomics. Dr. Borchers is also appointed as Professor at McGill University in the Department of Oncology, Montreal, QC and received there the Segal Chair in Molecular Oncology at the Jewish General Hospital of the McGill University.

His research is centred around the improvement, development and application of proteomics technologies with a major focus on techniques for quantitative targeted proteomics for clinical diagnostics. Multiplexed LC-MRM-MS approaches and the immuno-MALDI (iMALDI) technique are of particular interest. Another focus of his research is on technology development and application of the combined approach of protein chemistry and mass spectrometry for structural proteomics. Dr. Borchers has published over 200 peer-reviewed papers in scientific journals and is the founder and CSO of two companies, Creative Molecules. Inc. and MRM Proteomics Inc. He is also involved in promoting proteomic research and education through his function as HUPO International Council Member, Scientific Director of the BC Proteomics Network and Vice-President, External of the Canadian National Proteomics Network.

Quantitative Proteomics Approaches for Personalized Medicine

Laura M. McIntosh, Vice President, Translational Research, Caprion Proteome

The discovery and validation of novel blood based biomarkers of resistance or response to therapy is a critical component for the advancement of personalized medicine. Liquid chromatography mass spectrometry (LC-MS/MS) has enormous potential for the identification of protein candidates in biological samples. Two examples will be highlighted, hepatic C virus (HCV) and metastatic colorectal cancer (mCRC), in which novel predictive biomarkers have been discovered and validated using LC/MS-MS. First, an unbiased label-free, gel-free, quantitative mass spectrometry approach was used to profile proteins present in serum/plasma from patients undergoing treatment for HCV (peg-interferon and ribavirin) or for mCRC (XELOX or FOLFOX + Bevaciaumab). Using the profiling data, signatures composed of pre-treatment protein levels were identified that correlated to sustained viral response for HCV or to progression free survival for mCRC. Subsequently, the response was validated in an independent cohort of subjects using an orthogonal, targeted multiple reaction monitoring (MRM) mass spectrometry approach. Proteins identified as differentially-expressed between responders and non-responders to HCV treatment were quantified, revealing 15 proteins that were significantly differentially expressed. Many proteins (7/15) were involved in focal adhesion, suggesting that up-regulation of the focal adhesion pathway is part of an early host response to HCV infection. This proteomics approach allows the prediction of treatment response and has the potential to guide treatment regimes and lead to new avenues for combination therapy.

Laura McIntosh

Laura is Vice President, Translational Research at Caprion and is responsible for the scientific management of multiple client programs. She brings significant industrial experience in drug and device development, including the design, management and regulatory aspects of preclinical and clinical studies. Before joining Caprion, Laura was President and General Manager of Osprey Pharmaceuticals, where she oversaw the development of a platform of therapeutic proteins for the modulation of inflammation. Her previous experience also includes positions at ART Advanced Research Technologies, where she developed a novel optical molecular imaging device, and Argose Inc, where she was involved in the development of a non-invasive glucose monitoring technology. Laura earned a Ph.D. in Cell Biology from the University of Manitoba, followed by a post-doctoral fellowship at the National Research Council of Canada.

Selected Abstract for Oral Presentation

HDAC Inhibitor Induced Changes in Histone Methylation (Abstract # 48)

Ryan Lillico, University of Manitoba

Wednesday, May 27

SESSION 3:

Mucosal Drug Delivery

Targeted siRNA Delivery to T Cells in the Lung as Novel Asthma Therapy

Yuran Xie, Na Hyung Kim, Archana Thakur, Lawrence Lum, David JP Bassett, Olivia Merkel*
Department of Pharmaceutics, Department of Oncology, Wayne State University and Barbara Ann Karmanos Cancer Institute, Detroit, MI

According to the World Health Organization, 235 million people worldwide currently suffer from asthma. Inhalable, targeted, cell-specific RNA interference (RNAi)-based therapies could improve patients' ability to control asthma. However, clinical translation of RNAi has been thwarted mainly by a lack of biocompatible carriers for short interfering RNA (siRNA) that are inhalable, overcome extracellular and intracellular barriers, and that improve the currently inefficient targeting of siRNA to the desired organs and cell types. This project describes the design of inhalable bioconjugate nanocarriers that are capable of efficiently delivering siRNA to the cytoplasm of activated T cells by overcoming the natural barriers in the lung. More specifically, we have ***1)*** *designed biocompatible TfR-targeted nanocarriers that deliver siRNA specifically to ATCs;* ***2)*** *identified siRNA sequences that efficiently downregulate GATA-3 on RNA and protein level; and* ***3)*** *targeted ATCs in the lung.* In the next step in improving the efficacy of T cell targeted siRNA-based therapies which have great promise in the treatment of asthma, experiments on therapeutically reducing inflammation after pulmonary delivery in a mouse model of allergic asthma for maximum gene knockdown and therapeutic effects are currently underway.

Olivia Merkel

Dr. Olivia Merkel is an Assistant Professor of Pharmaceutics and an Associate Faculty Member of Oncology at Wayne State University, Detroit, MI, USA. She is also a Scientific Member of the Molecular Therapeutics Program and Faculty in the Cancer Biology Graduate Program at Barbara Ann Karmanos Cancer Institute in Detroit, MI. She became a Registered Pharmacist in 2005. In 2006, she received a MS in Pharmaceutics from Martin-Luther-Universität Halle-Wittenberg, and a PhD in Pharmaceutics from Philipps-Universität Marburg, Germany, in 2009. She received several awards, including a Promotion to Young Researchers awarded by the German Lung Foundation, the Young Pharmaceutical Investigator Award granted by the European Federation for Pharmaceutical Science, the Young Investigator Award by the College of Pharmacy at Wayne State, Invitation to the Lindau Nobel Laureates Meeting, the Carl-Wilhelm-Scheele-Award and the award for the best PhD thesis at Philipps-Universität Marburg. Currently Prof. Merkel's research focuses on targeted siRNA delivery in cancer and inflammatory diseases.

Mucus Penetrating Nanoparticles

Justin Hanes, Ph.D., Center for Nanomedicine, Johns Hopkins University School of Medicine

The controlled delivery of bioactive molecules to target tissues can significantly improve drug effectiveness while reducing side effects by concentrating medicine at selected sites in the body. Mucus layers coat and protect nearly all entry points into the body that are not coated by skin. Until recently, human mucus was thought to be nearly impenetrable to drug delivery particles even as small as 59 nm in diameter. Particles that become trapped in mucus are typically rapidly cleared from the organ of interest, usually within minutes to a few hours. Thus, while the barrier properties of mucus provide outstanding protection against infection and other potentially toxic substances, they have also thwarted efforts to achieve uniform and sustained drug and gene delivery to mucosal surfaces. This talk will focus on our work to understand the length-scale dependent and adhesion-mediated barrier properties of mucosal fluids, and how this knowledge has guided the development of polymeric

nanoparticulate carriers capable of improved drug and gene delivery to the respiratory tract, female reproductive tract, gastrointestinal tract, surface of the eye, and other mucosal tissues.

Justin Hanes

Justin Hanes is the Lewis J. Ort Professor and Director of the Center for Nanomedicine at the Johns Hopkins University School of Medicine. He holds faculty appointments in Biomedical Engineering, Chemical & Biomolecular Engineering, Environmental Health Sciences, Neurosurgery, Oncology, Pharmacology & Molecular Sciences, and a primary appointment in Ophthalmology. He directs a research program at the interface of biomaterials, biophysics, drug delivery and translational medicine at Johns Hopkins. He is a founder and member of the board of directors of Kala Pharmaceuticals, a company commercializing his laboratory's "mucus penetrating particle" nanotechnology, and he is founder, CEO and Chair of the Board of Directors of GrayBug, a private company developing advanced drug delivery systems with a special focus on the treatment of diseases that affect vision. He also serves on the scientific advisory board for Genentech in the Drug Delivery Division. Justin received a B.S. in Chemical Engineering from UCLA in 1991 and Ph.D. in Chemical Engineering from MIT in 1996. He did postdoctoral training in Oncology and Neurosurgery at the Johns Hopkins University School of Medicine in 1996-1998.

Adhesive Paintable Biomaterials Inspired by Marine Mussels

Haeshin Lee, PhD, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST)

Adhesive proteins of marine mussels contain amino acids with side chain chemistry of catechol and amine. Synthetic mimic of catecholamine polymers exhibited robust, wet-resistant adhesion. One of representative example is poly(dopamine) which functionalizes virtually any material surfaces (H. Lee et al. Science 2007), and recently poly(norepinephrine) coating has been an alternative surface chemistry (S. Hong et al. Angew. Chemie. Int. Ed. 2013). However, the chemical structures of the mussel-inspired surface chemistries have been poorly characterized due to intrinsic complexity of indole polymerization. Thus, polymers with defined chemical structures yet maintaining wet-resistant, material-independent adhesion should be useful alternatives for biomedical applications. This talk, two catecholamine adhesive polymers of chitosan-catechol and poly(ethylenimine)-catechol will be introduced, and novel paintable, attachable medical applications of the polymers will be explained.

Haeshin Lee

Professor Haeshin Lee studied at KAIST where he received his B.S. degree in Biological Sciences between in 1996. He received his Ph.D. degree at Biomedical Engineering Department, Northwestern University in 2007. He started his professional carrier from 2008 at Department of Chemistry, KAIST. His research interests have been bio-inspired materials particularly for adhesive materials that have been applied to biomedical adhesives, drug delivery systems, and energy storage devices. He is a currently director of Center for Nature-inspired Technology (CNiT). Some special recognitions and awards are the followings: POSCO Chung-Am award (2011), KAIST Pioneer Award, (2012), NASA inventor award (2008), Top science stories Discover Magazine (2008), Future young scientist award from the Ministry of Education, Science and Technology (2007), Excellence in Teaching Award, KAIST (2010, 2011), and KAIST Award for Excellence in Collaboration (2013). His total citation number is about 8000, and h-index is 36.

Selected Abstract for Oral Presentation

Evaluating the Drug Release and In Vivo Biocompatibility of an Intravaginal Implantable Device in a Rabbit Model (Abstract # 36)

Yufei Chen, University of Manitoba

Wednesday, May 27

CSPS Award of Leadership in Canadian Pharmaceutical Sciences Lecture

30 Years of Coffee, Beer and Serendipity in Pharmacy Research

Neal M. Davies, Professor and Dean of Pharmacy, University of Manitoba, Winnipeg, MB

The formulation and delivery of pharmacy research is much more complex than publication numbers and citations, impact factors, extramural grants, and a high H-index. Serendipity and hard work are not excipients but often the key ingredients in providing leadership in pharmaceutical research. The University of Manitoba College of Pharmacy is being developed at the nexus of Engagement in the Practice of Pharmacy and the Development of Life Sustaining and Life –Saving Pharmaceutical Research. We are committed to excellence in the integration of Pharmaceutical Care and Research. The research within the College of Pharmacy spans the pharmaceutical, clinical and social sciences, and encompasses the discovery, design, synthesis, mechanism of action, delivery and the fate of drugs, to the dispensing of pharmaceuticals and health care advice, clinical patient-care and optimization of pharmacotherapy and pharmacoeconomic and pharmacoepidemiological aspects of pharmacy and health services management and policy. The College of Pharmacy has maintained levels of expertise surrounding pharmaceuticals that are valuable at all stages from discovery, pharmacology, development, production, delivery, distribution, utilization, and dissemination of related knowledge. The College of Pharmacy is unique in the detailed, comprehensive understanding of physical, chemical and biological interactions and their outcome on pharmacotherapy. The team at the College of Pharmacy at the University of Manitoba is continuing to develop an outstanding national and international reputation for research and scholarship in an "intermodal" Pharmacy program under the "gloves" of effective leadership

Neal M. Davies

Dr. Neal M. Davies received a BSc. in Pharmacy and a PhD in Pharmaceutical Sciences from the University of Alberta in Edmonton, Canada. He then went on to complete post-doctoral training in pharmacology and toxicology at the University of Calgary, Faculty of Medicine in the Department of Pharmacology & Therapeutics. He has held academic positions at the University of Sydney Faculty of Pharmacy and was Chair of the Institutional Animal Care and Use Committee, and Director of Professional and Undergraduate research and an Associate Professor at the College of Pharmacy at Washington State University before becoming Dean and Professor at the Faculty of Pharmacy at the University of Manitoba on September 1, 2011. Dr. Davies has authored over 300 publications including abstracts, journal articles and one book primarily focused on the pre-clinical and clinical pharmacokinetics, pharmaceutics, pharmacology, and toxicology of anti-inflammatory, anti-cancer drugs and natural products. He has over 6600 citations and an H-Index of 46. Dr. Davies has and continues to successfully train a variety of undergraduate, graduate students, and post-doctoral fellows in his laboratory. He has served on the United States Pharmacopeia Inhalation Advisory Panel and received an Extraordinary Contributions Award (2010) and the United States Gastroenterology Expert Panel and was awarded a USP Meritorious Service Award in 2011. Dr. Davies is a member of the Rho Chi Society and has acted as a consultant to a variety of pharmaceutical companies and is on the editorial board of a number of pharmaceutical journals. He has received funding from the Australian Research Council, the United States National Institutes of Health and US Department of Agriculture, the Medical Research Council of Canada and the pharmaceutical industry.

Wednesday, May 27

SESSION 4:

New Methodologies of Genome Wide Target Validation

Genomics for Precision Drug Therapy in the Community Pharmacy: "DNA-guided Drug Dosing"

Corey Nislow, University of British Columbia

Rapid, inexpensive, next-generation sequencing should revolutionize health care and empower patients and their healthcare providers by providing a comprehensive record of each individual's genetic disposition. The nature of the genome is such that this data, once collected, is a lifelong record. The access to, and application of, an individual's genetic information for disease diagnosis, prognosis, and therapy has not yet become a routinely acceptable approach in the community health care environment. The reasons for the slow adoption are several fold, paramount among them is the lack of a clear mandate from the health care community. We aim to illustrate how and why the community pharmacist is the ideal conduit through which genetic information can be acquired, assessed, and utilized to guide therapeutic decisions. We have developed Standard Operating Procedures (SOPs) for: i) patient recruitment, education and informed consent; ii) collection and documentation of patient saliva samples and therapeutic phenotyping by pharmacists in community pharmacies; iii) genetic analysis, processing of data, and information storage in a secure database; and iv) training of pharmacists in the retrieval and utilization of patient genetic information to optimize therapy. While data will initially be used with anticoagulant therapy, the WES data collected provides information relevant throughout a patient's lifetime for interventions both medical or non-medical (e.g. affecting physiology such as diet or exercise) and has the potential to optimize patient health. This information can be used by any appropriately trained healthcare professional wherever the patient is receiving treatment. Importantly, this information exchange will be guided by training from skilled genetic counsellors.

Corey Nislow
For as long as he can remember, Corey Nislow has been fascinated by the ability to apply new tools to important biological questions. He completed a Bachelor of Arts in Developmental Biology at New College (Sarasota, Florida), and a Doctor of Philosophy in Cell and Molecular Biology at the University of Colorado (Boulder, Colorado) and was an American Cancer Society Postdoctoral Fellow. He served as group leader in two Biotechnology Companies (MJ Research and Cytokinetics, Inc. in the San Francisco Bay Area) and as a Senior Genome Scientist at Stanford University. As an Associate Professor at the University of Toronto he was the founder and Director of the Donnelly Sequencing Centre. At the University of British Columbia he founded the UBC-Seq Centre and is spearheading the effort to bring genomics to the community pharmacy.

Control of Gene Expression by Selective and Non-selective Direct Inhibition of Bromodomain-acetyl-lysine Readout

Panagis Filippakopoulos, Structural Genomics Consortium & Ludwig Institute for Cancer Research, University of Oxford, UK

Bromodomains (BRDs) are evolutionary conserved protein interaction modules that specifically recognize ε- N-lysine acetylation (Kac) motifs, a key event in the reading process of epigenetic marks. They are of substantial biological interest, as components of transcription factor complexes and determinants of epigenetic memory. Importantly, inhibition of their ability to read Kac is possible: the Bromo and Extra Terminal (BET) sub family has recently been successfully targeted by several classes of small molecule inhibitors leading to pan-BET inhibitors that are capable of attenuating BET function. BET proteins have a modular architecture, including two N-terminal BRD modules and an extra

terminal (ET) domain. They recognize and bind to patterns of lysine acetylation found on histones and further act to recruit components of the transcriptional machinery via their modular architecture. Inhibition of their Kac readout function results in dissociation from chromatin, thus controlling the transcription of key oncogenes and anti-apoptotic proteins. With several chemical scaffolds on track in clinical trials, there is a need to generate well characterized, highly specific and potent, cell permeable chemical tools that can be used to validate the underlying biology of these protein targets in order to successfully target them in diverse clinical settings. I will be discussing the efforts to develop promiscuous as well as selective inhibitors targeting the BET sub-family of bromodomains, leading to highly potent tool compounds that are now available in the public domain. I will also be discussing the impact of these efforts in our understanding of bromodomain biology, leading to better follow-up in the clinic.

Panagis Filippakopoulos

Panagis obtained his BSc. in Chemistry from the Aristotelian University of Thessaloniki (Greece). He carried out his doctoral studies in Inorganic Chemistry at the University of Michigan and received his PhD in 2004. He moved to Oxford University in the UK at the Structural Genomics Consortium (SGC) where he focused on protein crystallography and biophysics, first as a postdoctoral scientist until 2007, then as a Team Leader until 2011. In December 2011 he received a Research Career Development Fellowship Award from the Wellcome Trust and took a post as a Principal Investigator at the Nuffield Department of Medicine in Oxford University. Since November 2012 he is also an affiliate of the Ludwig Institute for Cancer Research (LICR) in Oxford and since April 2015 an Associate Professor at the Nuffield Department of Medicine at Oxford. His research interests focus on structural comparisons of entire protein families and the discovery of shared and distinct mechanisms that determine substrate recognition and protein regulation, as well as the structure-guided design of specific inhibitors that modulate the function of proteins involved in epigenetic signaling, aiming to target them in cancer.

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Selected Abstract for Oral Presentation

Systems Genetics Approach Identifies Nicotine Receptor Antagonist as a Potential Therapeutic Target in COPD (Abstract # 70)

Ma’en Obeidat, UBC, University of British Columbia

Deciphering Growth Factor Signalling Mechanisms in Cancer using Functional Genomics and Proteomics

Stephane Angers, Departments of Pharmaceutical Sciences & Biochemistry, University of Toronto, Canada

A recurrent set of growth factor signalling pathways that fulfill fundamental roles during embryonic development are dysregulated in human cancers and contribute to tumour initiation and progression. Identification of tumour specific mutations and epigenetic changes that lead to hyperactivation of these signalling pathways is required to develop tailored medicines. A precise understanding of the molecular mechanisms of the signalling events in normal and disease conditions is also needed to fully leverage the promise of targeted therapies. My laboratory studies the signalling events controlled by the Wnt and Hedghog families of secreted growth factor. We are using proteomic and functional genomic approaches to further understand signalling mechanisms. Our recent efforts include the development and implementation of whole-genome CRISP-Cas9 sgRNA libraries enabling loss of function and synthetic lethality screens to identify novel targets in cancers where Wnt signalling is dysregulated.

Stephane Angers

Dr. Stephane Angers is an expert in the fields of Signal Transduction and Cancer biology. After his undergraduate training in Biochemistry at McGill University, he obtained a Ph.D. in Biochemistry in 2002 from the Université de Montréal under the guidance of Dr. Michel Bouvier. His thesis work led to the development and application of light energy transfer methodologies for the study of protein-protein interaction and signal transduction that are now widely used. From 2002-2006 he was a Howard Hughes Post-Doctoral Fellow at the University of Washington in Seattle under the

supervision of Dr. Randall T. Moon, where he made important contributions to the area of cell-cell communication. Since October 2006, Dr. Angers is a Canada Research Chair in the Department of Pharmaceutical Sciences at the Faculty of Pharmacy of the University of Toronto. His research program aims to explore the functional architecture of signal transduction activated by the Wnt and Hedgehog families of growth factors and by the large family of G protein Coupled Receptors. For this, he exploits mass spectrometry and genome-wide functional screening approaches to identify novel proteins regulating these signalling pathways and characterizes their functional and cellular roles. His findings have been published in high impact journals such as *Science, Nature, Nature Cell Biology and Journal of Cell Biology*.

CRISPR-Cas9 for in vivo Genome Editing and Disease Modeling

Randall J. Platt, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

CRISPR-Cas9 is a versatile genome editing technology for studying the function of genetic elements. While it has been widely adopted for use in cell lines and single cell embryos its use in vivo in mammals has been limited. Directly editing genes in vivo has the potential to complement or in some cases replace traditional transgenic animal models, whereby rather than deriving unique animal strains for every desired mutation the mutations can be created directly in the cell types of interest. Moreover, with CRISPR-Cas9 the ability to multiplex and create different mutations in multiple genes is possible, opening up an avenue for studying multigenic biological and disease processes. To broadly enable the application of CRISPR-Cas9 in vivo, we established a Cre-dependent CRISPR-Cas9 knockin mouse and demonstrated genome editing using adeno-associated virus (AAV)-, lentivirus-, and particle-mediated delivery of guide RNA in neurons, immune cells and endothelial cells. Using these mice, we simultaneously modeled the dynamics of KRAS, p53, and LKB1, the top three significantly mutated genes in lung adenocarcinoma. Delivery of a single AAV vector in the lung generated loss-of-function mutations in p53 and Lkb1, as well as homology-directed repair-mediated Kras(G12D) mutations, leading to macroscopic tumors of adenocarcinoma pathology. Together, these results suggest that CRISPR-Cas9 mice empower a wide range of biological and disease modeling applications.

Randall J. Platt

Randall Platt is a graduate student in Biological Engineering at MIT and the Broad Institute. Platt works with advisor Feng Zhang to develop and apply genome editing technologies (TALENs and CRISPR) towards elucidating the genetic and molecular basis of disease. His research empowers genome editing applications directly in vivo, which enables the rapid creation of versatile disease models and provides a novel platform for discovering new disease-associated genes and testing therapeutics. At MIT he is an NSF, McGovern, and Afeyan Fellow. He obtained a bachelor's degree from the University of Utah in biomedical engineering and chemistry, and as a Whitaker Fellow he obtained a master's degree from the Imperial College London in material science.

Wednesday, May 27

SESSION 5:

Regulatory Updates and Developments

Subsequent Entry Biologics in Canada: Current State of the Sciences

Fakhreddin Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, Edmonton, Alberta, Canada

On December 10th 2014, Canadian Society for Pharmaceutical Sciences organized a workshop on the current state of sciences of Subsequent Entry Biologics (SEBs). The day-long workshop provided an opportunity to discuss recent regulatory developments and a wide range of scientific issues related to SEBs. The workshop was attended by about 150 participants from Health Canada, academics, industry representatives and patient group representatives. The report of the workshop is since published (J Pharm Pharm Sci, 18 (2): 177-183, 2015). The aim of this presentation is to highlight and discuss the outcomes of the workshop, and layout the next step forward.

[See Bio on page 19]

Controversies in Biosimilars

Agnes V. Klein, MD, DPH, Director, Centre for the Evaluation of Radiopharmaceuticals and Biotherapeutic Products, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON

Biosimilars are a topical and, in many ways a controversial topic for discussion, in meetings and conferences that deal with the development, safety, efficacy and place of this new class of therapeutics, in the treatment of patients with a variety of diseases.

This talk will highlight some of the issues and some of the subjects that continue to be problematic, from the clinical perspective. The comparability exercises between a reference product and a biosimilar are inescapable considerations. However, the matters of extrapolation, the considerations and principles used as well as the use of a biosimilar in place of an original drug are still hotly debated.

The aim will be to show how these gaps can be bridged and to show that, despite lack of experience over a longer period of time the products are safe and effective.

One way to obviate concerns is for sponsors to meet with Health Canada to be well informed of the regulatory flexibilities that are available to them as they develop these important new products.

Agnes V. Klein

Agnes V. Klein, MD, DPH, is currently the Director, Centre for the Evaluation of Radiopharmaceuticals and Biotherapeutic Products in the Biologics and Genetic Therapies Directorate, Health Canada.

After receiving her medical degree from the University of Toronto, Dr. Klein trained in Endocrinology, Medical Biochemistry and Public and Community Health. After joining Health Canada, she has occupied many and varied positions, scientific and managerial. Amongst relevant accomplishments, Dr. Klein represented Health Canada on NCBHR, as founding member and NCEHR as well as chairing the Committee on Clinical Trials of the Council.

In 2000, Dr. Klein moved to Biologics where she actively participated in the inception of the new Directorate and its processes.

Dr. Klein was an active participant in the CIOMS document on Pharmacogenetics and Pharmacoeconomics as well as in the ICH process drafting of guidelines (ICH E15 and E16 on pharmacogenomics). In addition to her special interest in biomarkers, surrogate endpoints and the appropriate design of clinical trials, especially the issues related to small studies, Dr. Klein is interested in the regulatory and clinical issues regarding subsequent entry biologics (SEBs). This interest has included several presentations on biosimilars, as well as authorship and co-authorship of several articles on subjects related to their issues.

Dr. Klein has made numerous presentations to professional and regulatory groups on issues

surrounding the ethics of clinical trials and the integrity of clinical trial data. Dr. Klein has also presented on a wide-variety of new regulatory endeavours.

Dr. Klein is an active supporter for excellence in the development of medicines.

Clinical and Bioequivalence Consideration for Introducing Alternative Formulations of Critical Dose Drugs

Nasrullah Undre, PhD, Senior Director, Basic Science, Department of Medical Affairs, Astellas Pharma Europe, Middle East & Africa, London, UK

Critical dose drugs are defined by Health Canada as those drugs where comparatively small differences in dose or concentration lead to dose- and concentration-dependent, serious therapeutic failures and/or serious adverse drug reactions which may be persistent, irreversible, slowly reversible, or life threatening, which could result in inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability or incapacity, or death[1].

Medications meeting these criteria include warfarin, digoxin, and the immunosuppressive medications tacrolimus and cyclosporine. The administration and dosage of warfarin is individualized according to the patient's responsiveness to the drug, according to results of the patient's PT/INR. However, in the case of immunosuppressive drugs, tacrolimus and cyclosporine, therapeutic drug monitoring (TDM) is used as a surrogate for clinical response. This is based on an established relationship between systemic exposure, measured as area under the concentration time-profile (AUC), and safety and efficacy. A specific, validated, pharmacokinetic parameter (for tacrolimus; whole blood trough concentration) is used as a marker to maintain clinical exposure in a very narrow range.

Any formulation of orally administered alternative formulations must be capable of being used interchangeably with the reference products in clinical practice. Product substitution is permissible only if the alternative product is therapeutically equivalent to the reference product.

Bioequivalence guidelines for the approval of immunosuppressants vary across the world. Canadian guidelines require critical dose drugs to meet tighter bioequivalence acceptance limits of 90 to 112% for AUC, whereas other regulatory agencies allow the 80 – 125% acceptance interval. However, even when bioequivalence has been established, such evidence may only provide a presumption of therapeutic equivalence. Therapeutic equivalence ought to be supported by appropriately designed clinical studies to demonstrate equivalent clinical safety and efficacy, and failure to validate TDM concepts for alternative formulations through clinical studies may expose the patients to the graft rejection (resulting from inadequate exposure) or toxicity (due to over-exposure to the drug).

The pharmacokinetics (PK) of tacrolimus are influenced by the underlying pathophysiology of and the change in the clinical condition of the transplanted patient. Therefore, comparative PK ought to be studied in the relevant patient populations to ensure that the systemic exposure to tacrolimus or cyclosporine is maintained when individual patients are switched from one formulation to the other.

As TDM is critical to ensuring optimal clinical outcomes in patients, it is critical to validate the TDM for each new formulation through independent clinical studies. The threshold level cannot be uncritically extrapolated from one product to another.

Reference: Health Canada Guidance to Industry – Comparative Bioavailability Standards: Formulations Used for Systemic Effects, May 2012.

Nasrullah Undre

Nasrullah Undre is based at Astellas Pharma Europe, London, UK, and has been involved in research and development (Clinical Pharmacokinetics and Pharmacology) with Astellas since 1992.

Graduate studies at the University of Hertfordshire, UK followed by postgraduate studies at the Universities of Manchester and London.

During his early career he joined Guy's Hospital in London, Department of Clinical Physiology, as research associate His thesis was based on the study of the effects of anaesthetics on myocardial metabolism. He subsequently joined the pharmaceutical industry (GSK), as a principal scientist working extensively in the field of both pre-clinical and clinical pharmacokinetics and drug metabolism.

For over three decades, he has been involved in the development of several medicinal products. Areas of research include immunosuppressive agents, topical immunomodulatory agents, NSAIDs, antihypertensive agents, anti-emetics,

antidepressants and anti-infectives.

During his career at Astellas, he has been responsible for the development of Tacrolimus containing formulations (Prograf, Advagraf and Modigraf) for prophylaxis of allograft rejection; Protopic for topical treatment of Atopic Dermatitis as well as Micafungin (an antifungal agent of echinocandin class).

He is author or co-author of more than 100 publications in medical journals in the field of pharmacokinetics and pharmacology.

3Rs of GLP Certification for Bioanalysis: Reasons, Results, and Reactions

John Chapdelaine, Manager, Bioanalytical Laboratory, Pharmascience, Montreal, Canada

Canadian companies are under the jurisdiction of the Organisation for Economic Co-operation and Development (OECD), and as such require GLP certification from the Standards Council of Canada (SCC) for the analysis of non-clinical samples in regulated studies. Other regions of the world; such as the United States participate in FDA inspections; countries in Europe by Member States' GLP-monitoring authority inspections; and countries in Asia are required to satisfy ISO-15189:2012 (based on ISO9001 and ISO17025) medical laboratory certification. These approaches have a common goal in ensuring that the bioanalysis data generated is reliable and leads to safe products on the market. Inspections also provide an external point of view, with citations often improving internal processes and keeping updated with the latest industry standards.

Auditors from the SCC were scheduled to inspect the bioanalytical laboratory and procedures for a duration of two days. The Lead auditor and his accompanying Observer had good knowledge of the required GLP structure having previously worked in GLP facilities. Questions raised by the Lead auditor were related to archiving processes, quality assurance program, equipment calibration/ maintenance, and standard operating procedures. The audit findings required several changes to procedures to ensure compliance to GLP regulations, some clarifications to terminology, and a minor repair in the laboratory.

Although not yet certified at the time of this abstract, the nature of the findings has resulted in increased confidence from the company upper management team, business partners, and business units within the company. The bioanalytical staff appreciated the recognition that their work meets the high regulatory requirements associated with GLP regulations. Overall the experience was extremely positive since it will have certified the laboratory as meeting industry requirements and allowed the bioanalytical department to understand the benefits of working as a team to ensure strong GLP compliance, not only in preparation for the GLP inspection but on each and every study they are participate in.

John Chapdelaine

John Chapdelaine has over 15 years of experience in management of analytical and bioanalytical laboratory operations related to research and development, validation, and analysis for non-clinical (bioanalytical and dosing formulations), phase 1 & 2, and bioequivalence study samples. He joined Pharmascience in 2014 and is the manager of the bioanalytical laboratory for development, validation and sample analysis.

Prior to joining Pharmascience, he served as a manager of the analytical/bioanalytical laboratory at ITR Laboratories Canada in Baie D'Urfe, Quebec, Canada. Earlier, he was a Director of bioanalytical validation and scientific liaison at Warnex Bioanalytical in Laval, Quebec, Canada. He also collaborated and supervised research and development at Warnex Bioanalytical and MDS Pharma Services, St-Laurent (Montreal), Canada.

John has a B.Sc. degree in chemistry from Concordia University, Montreal, Canada.

Wednesday, May 27

SESSION 6:

Antibody-based Therapeutics

Application of PK/PD Modeling to Guide the Development of New Antibody-based Therapies

Joseph P. Balthasar, Ph.D., Professor, Associate Dean for Research, Director of the Center for Protein Therapeutics, University at Buffalo, Buffalo, New York

Pharmacokinetic and pharmacodynamic (PK/PD) modeling is widely employed in the pharmaceutical industry to facilitate drug development. Main applications of PK/PD modeling include: data summarization, cross-species prediction of pharmacokinetics and/or pharmacodynamics, clinical trial design, and therapeutic optimization / individualization. Although not yet fully developed in the pharmaceutical industry, there is growing interest in using PK/PD modeling as a tool to guide discovery efforts. Possible applications include: (a) Candidate Optimization: PK/PD simulation-based identification of ideal drug characteristics (e.g., ligand/receptor binding kinetics and affinity) to guide candidate selection and/or engineering efforts. (b) Target Identification: Use of simulation with mechanistic PK/PD models to evaluate the possible therapeutic benefit derived with inhibition or stimulation of physiological processes. (c) Drug Combinations: PK/PD simulations may allow a priori prediction of optimal drug combinations for increasing the safety and efficacy of pharmacotherapy. This presentation will provide a few examples of the use of PK/PD analyses and modeling to assist in the discovery and early development of antibody-based therapies. Possible advantages of model-based programs of drug discovery will be discussed.

Joseph Balthasar

Dr. Balthasar is Professor of Pharmaceutical Sciences and Associate Dean for Research in the School of Pharmacy and Pharmaceutical Sciences at the State University of New York at Buffalo. Dr. Balthasar received a B.S. in Pharmacy (1991) and a Ph.D. in Pharmaceutics (1996) from the University at Buffalo. He served as a Clinical Assistant Professor of Pharmaceutics at the University at Buffalo from 1996-1997 and, from 1997-1999, as an Assistant Professor of the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Dr. Balthasar rejoined the University at Buffalo as an Assistant Professor in 1999, and was promoted to Associate Professor in 2003 and to Full Professor in 2008. Dr. Balthasar serves as the Director of the Center for Protein Therapeutics. Dr. Balthasar's research utilizes pharmacokinetic and pharmacodynamic analyses to guide the development of new therapies. Current research focuses on physiologically-based modeling of monoclonal antibody disposition, the development of drug targeting strategies to improve the selectivity of cancer chemotherapy, and on the development of new immunotherapies for the treatment of humoral autoimmune diseases.

Conquering the Barriers - Development of Bi-specific Antibodies to Treat CNS Diseases

Danica Stanimirovic, MD, PhD, Director, Translational Bioscience Department, Human Health Therapeutics Portfolio, National Research Council of Canada, Ottawa

Monoclonal antibodies have become particularly successful as treatments for inflammatory diseases and cancer. However, this success has not extended to treating diseases of the central nervous system (CNS), at least in part, due to the presence of the blood brain barrier (BBB), which severely limits the access of antibodies into the CNS. Identification of ways to enable biologic drugs to cross the BBB has become a priority for the field and is essential if this class of drugs is to be used in the clinic. Pre-clinical research has identified receptor mediated transcytosis (RMT) pathways by which the antibody can cross the brain endothelial cell layer by harnessing the cellular uptake and transport of

natural ligands to receptors including transferrin receptor, insulin receptor and lipoprotein receptor related protein-1. Increased brain penetration of therapeutic antibodies can be achieved by engineering bi-specific antibodies in which one antibody binding specificity recognizes a BBB receptor that undergoes RMT and the second binding specificity recognizes a therapeutic target within the CNS. These bi-specific antibodies can be built using various antibody fragments as 'building blocks', including monomeric single-domain antibodies, the smallest antigen-binding fragments of immunoglobulins. The development of BBB-crossing bi-specific antibodies requires targeted antibody engineering to optimize multiple characteristics of both BBB-crossing- and therapeutic arms, as well as other antibody properties impacting pharmacokinetics and effector function. This talk will review recent advances and guiding principles for designing, engineering and evaluating BBB-crossing bi-specific antibodies using transferrin receptor antibodies and the single-domain antibody FC5, as BBB-crossing 'arms'. The principal obstacle for capitalizing on the future promise of CNS-active antibodies remains the scarcity of known, characterized RMT receptors which could be exploited for the development of BBB carriers. The talk will discuss novel screening approaches to identify and characterize new and improved BBB-crossing antibodies and RMT receptors.

Danica Stanimirovic

Dr. Danica Stanimirovic holds M.D. and PhD degree in Neurochemistry from the Faculty of Medicine, University of Belgrade. She was trained in cerebrovascular and stroke research at the Stroke Branch, NINDS, NIH. She subsequently established a program in neurobiology and brain delivery of therapeutics at the National Research Council (NRC) of Canada. As Director of the Translational Bioscience Department, NRC's Human Health Therapeutics Portfolio, she manages a portfolio of R&D projects in partnership with Canadian and international biotherapeutic companies aimed at de-risking and advancing biologics pipeline in preclinical development. Dr. Stanimirovic leads NRC's Strategic Program, Therapeutics beyond Brain Barriers, aimed at development of biologics for CNS indications. She is Adjunct Professor at the Department of Cellular and Molecular Medicine, University of Ottawa and founding member of the International Brain Barriers Society, and serves on NIH advisory panels and Scientific Advisory Boards of biotechnology companies. Dr. Stanimirovic is recipient of several Canadian and international awards. She is an author on 129 manuscripts, 25 book chapters and 12 patents in the field of brain vascular physiology and drug delivery across the blood-brain barrier.

Selected Abstract for Oral Presentation

^{177}Lu-labeled and Dual-receptor Targeted Radiation Nanomedicine for Simultaneous Targeting of HER2 and EGFR on Breast Cancer Cells (Abstract # 35)

Simmyung Yook, University of Toronto

Development of Novel Antibody-drug Conjugates in Oncology

Ilia A. Tikhomirov, Founder, AvidBiologics Inc.

AvidBiologics' lead therapeutic is AVID100, an antibody-drug conjugate currently undergoing IND-enabling development, with Phase I scheduled for 2016. During this presentation, design of safety and PK/PD studies to enable first-in-man trials will be discussed, including species, dose and dosing schedule selection.

Ilia Tikhomirov

Ilia A. Tikhomirov is an entrepreneur and founder of AvidBiologics Inc. (Avid), a Canadian biotechnology company developing oncology drugs. Ilia is also the inventor and co-inventor of several of antibody-drug conjugates (ADCs) that Avid is currently developing. Prior to Avid, Ilia occupied positions of increasing responsibility at YM BioSciences Inc. (YM), a publicly traded Canadian oncology drug development company, which was acquired by Gilead Sciences in a $500 million transaction. At YM, Ilia was involved in in-licensing, mergers and acquisitions (M&A) target analysis, strategic research, and business development. His findings at YM led to the development of the technology that is at the core of AvidBiologics' science.

Development of a Camelid Antibody Enzyme Conjugate for the Treatment of Non-small Cell Lung Cancer

Heman Chao, Helix BioPharma Corp

The anti-tumor activity of Jack bean urease was combined with the specificity of anti-CEACAM6 single domain antibody in the form of antibody-urease conjugate (L-DOS47). L-DOS47 was found to bind specifically to CEACAM6-expressing cancer cell lines and exerted potent cytotoxic effects *in vitro*. Cytotoxicity of L-DOS47 depends on the availability of urea *in situ* and susceptibility of targeted cells to ammonia toxicity. Silencing of the CEACAM6 gene in BxPC-3 cells helped to protect the cells from L-DOS47 effects, while CEACAM6 overexpression rendered transfected H23 cells susceptible to L-DOS47 cytotoxicity. Immunochemical staining of human normal and cancer tissues showed that L-DOS47 bound preferentially to lung adenocarcinoma, as well as to colon and pancreatic adenocarcinoma with positive but weaker staining. *In vivo* imaging studies showed that Cy5.5 fluorescent dye labelled L-DOS47 bound and accumulated at the tumor site for up to 72 hours. A metastasis study of lung adenocarcinoma A549 cells in mice also showed that L-DOS47 was effective in reducing cancer cell counts in lung at a concentration of 10 μg/mL. L-DOS47 is being investigated as a potential therapeutic agent in human Phase I clinical studies in the US and Poland for non-small cell lung cancer.

Heman Chao

Dr. Chao received his Ph.D. in biochemistry at Queen's University Kingston Canada in 1994. He completed a post-doctoral fellowship at the Protein Engineering Center of Excellence at the University of Alberta. Subsequently, he was awarded a "Natural Sciences and Engineering Research Council of Canada (NSERC)" industrial post-doctoral fellowship to work at Sensium Technologies Inc – a subsidiary of Helix BioPharma Corp. At Sensium, Dr. Chao was responsible for the development of a protein chip platform and managed a drug discovery program for Helix. In 2002 Dr. Chao became V.P. Technology for Helix and President of Sensium Technologies Inc. In Helix, Dr. Chao established a new anti-cancer drug discovery portfolio and became V.P Research in 2004. Dr. Chao became CSO for Helix BioPharma in 2008. Dr. Chao oversees company's drug discovery program; manages external research collaboration with companies, universities and institutes; and administer company's intellectual property portfolio. Dr. Chao has co-authored a number of scientific papers and is co-inventor of the Company's DOS47 technology.

Thursday, May 28

Plenary Lecture 2

Imaging in CNS Drug Discovery and Development

Richard Hargreaves, Vice-President, Research, Biogen

The use of molecular imaging biomarkers shortens cycle times for CNS drug development thereby increasing the efficiency and return on investment from research. Imaging biomarkers can select the molecules, doses and patients most likely to test therapeutic hypotheses by stopping those that have little chance of success and accelerating those with potential to achieve beneficial clinical outcomes. CNS imaging biomarkers have a unique place in the drug development for progressive neurodegenerative disorders as they can be used to detect and track the effects of disease modifying therapeutics.

Richard Hargreaves
Previous worldwide head of imaging and discovery neuroscience at Merck and Co. Currently Vice-President, Research at Biogen. Over 25 years of leadership experience in pharmaceutical research, drug discovery and development, translational medicine and medical imaging with an emphasis on molecular and functional CNS imaging.

Thursday, May 28

SESSION 7:

Imaging in Drug Delivery

Tumor Deposition Imaging as a Predictive Biomarker for Personalized Nanomedicine

Helen Lee, Merrimack Pharmaceuticals, Inc.

Nanomedicines such as liposomes accumulate in tumors via the enhanced permeability and retention effect, delivering its drug payload to tumor cells. Kinetic modeling and preclinical data support the hypothesis that effective drug delivery is one of the rate-limiting factors for effective chemotherapy in solid tumors. We believe that quantifying tumor deposition using noninvasive imaging may be useful for predicting which patients are likely to respond to liposomal therapeutics. In a translational clinical study, we utilized ^{64}Cu-labeled MM-302 (HER2-targeted liposomal doxorubicin) to demonstrate that liposomes accumulate in human tumor lesions, including liver, bone, and brain metastases, to highly variable extents as quantified on the PET images. Lesion uptake of ^{64}Cu-MM-302 range from 0.52 to 13.9 %i.d./kg (percentage of injected dose per kilogram of tumor) and 0.37 to 18.5 %i.d./kg at 24 and 48 hours post-injection, respectively. A ^{64}Cu-liposomal positron emission tomography (PET) agent, MM-DX-929, is being developed as a companion diagnostic imaging agent for several of our liposomal therapeutics. Preclinically, high MM-DX-929 deposition in tumor correlates with better response in xenograft models treated with MM-302, Doxil®, or nal-IRI (liposomal irinotecan, MM-398). In addition to PET, we are also investigating the utility of off-label use of ferumoxytol (FMX; Feraheme®, 30 nm superparamagnetic iron oxide nanoparticles) with enhanced magnetic resonance imaging (MRI) as a surrogate for tumor deposition of nal-IRI. In our pilot clinical study, individual lesion size change was shown to associate with tumor FMX-MRI signal (9 patients across 7 indications, 31 lesions). A multi-institution expansion of this study focusing on HER2-negative metastatic breast cancer patients receiving nal-IRI treatment is currently underway to confirm these findings and assess multi-site feasibility of quantitative FMX-MRI.

Helen Lee
Dr. Helen Lee is currently a Principal Scientist at Merrimack Pharmaceuticals. Started as an in vivo pharmacologist, she has transitioned into managing internal and external research activities to support clinical development of MM-398 (liposomal irinotecan), and developing an imaging companion diagnostic strategy for Merrimack's liposomal drugs pipeline. Her efforts include preclinical development and characterization of two liposomal PET agents (^{64}Cu-MM-302 and MM-DX-929), generate preclinical data to support the use of MR imaging and iron oxide particles as an alternative diagnostic strategy, as well as clinical implementation and clinical image/data analyses. Helen received her BASc in Biomedical Engineering, and her PhD from the Department of Pharmaceutical Sciences at the University of Toronto under the supervision of Dr. Christine Allen. Her areas of expertise include polymer micelle and liposome formulation, drug delivery, in vivo pharmacology, oncology drug development, PET and CT imaging.

Imaging and Nanomedicine in Inflammatory Atherosclerosis

Willem J. M. Mulder, Translational and Molecular Imaging Institute, Department of Radiology, Icahn School of Medicine at Mount Sinai, New York

Cardiovascular disease is the leading cause of morbidity and mortality worldwide with an estimated cost exceeding $500 billion per year in the US alone. Cardiovascular diseases are predominantly caused by atherosclerosis, a systemic disease, characterized by a chronic inflammation of the arterial wall with concomitant vascular lumen lipid deposits, known as plaques. Bioengineering

provides unique opportunities to better understand and manage atherosclerotic disease. Twenty-first century innovations in diagnosing and treating atherosclerosis are the result of integrating cardiovascular medicine, immunology, medical imaging, bioengineering, and (bio)chemical engineering. The field is entering a new era that merges the latest biological insights into inflammatory disease processes with targeted imaging and nanomedicine.

Ideally, nanotechnology will guide drug delivery to culprit cells and plaque, possibly with specifically engineered drug delivery particle properties, such as size, surface charge, and affinity ligands. Preclinical cardiovascular molecular imaging allows the in vivo study of such nanotherapeutics specifically directed toward immune system components that drive atherosclerotic plaque development and complication. At the same time, recent successes in multimodality imaging integration in multicenter trials pave the way for anti-atherosclerosis nanotherapy evaluation in human subjects.

Willem Mulder

Dr. Mulder founded the Nanomedicine Laboratory as part of Sinai's Translational and Molecular Imaging Institute (Nano-TMII). His research is funded by multiple NIH grants and a vidi grant from the Dutch Science Foundation (NWO). He currently leads a team of 8 postdocs, 2 technicians, and 10 graduate students. At Nano-TMII, nanomedicinal approaches are developed and applied towards molecular imaging and targeted therapy of disease. Dr. Mulder's lab develops libraries of nanoparticle probes that are screened for specificity of cancer and atherosclerosis related processes. Selected materials are subsequently loaded with drugs to intervene in these processes. This tailored approach allows precision medicine in a non-conventional setting and is synchronized with the latest immunological insights. This approach is also applied towards molecular imaging of cancer and atherosclerotic disease. Nanomaterials from immunological screens are selected and labeled for highly sensitive and quantitative PET imaging.

For the treatment of inflammatory atherosclerosis, Dr. Mulder established a program that is aimed at 1) applying nanomedicine to better understand, diagnose and treat cardiovascular disease in mouse models, 2) translating cardiovascular imaging and targeted nanotherapy to large animal models (rabbits and pigs), and 3) performing cardiovascular nanomedicine clinical trials.

The goal of Dr. Mulder's research activities is to further develop these approaches and translate them to the clinic in collaboration with international partners.

Nanomedicines and Theranostics

Twan Lammers, Department of Experimental Molecular Imaging, RWTH Aachen, Germany; Department of Controlled Drug Delivery, University of Twente, Enschede, Department of Pharmaceutics, Utrecht University, Utrecht, The Netherlands

Nanomedicines are 1-100(0) nm-sized carrier materials designed to improve drug delivery to pathological sites. By preventing (chemo-) therapeutic drugs from being excreted by the kidney and degraded by the liver, nanomedicines assist in improving the pharmacokinetics and the biodistribution of low-molecular-weight agents. Moreover, by delivering drugs more efficiently to pathological sites, and by attenuating their accumulation in potentially endangered healthy tissues, nanomedicines improve the therapeutic index of drug molecules, i.e. the balance between their efficacy and toxicity. In the present lecture, I will briefly introduce the basics of nanomedicine-mediated drug targeting, I will present several examples of nanomedicine formulations used in the clinic for treating cancer and inflammatory disorders, I will discuss means to improve EPR-mediated tumor targeting, and I will highlight several recent preclinical advances, dealing e.g. with targeted treatment of metastasis, and drug delivery across the blood-brain barrier. Finally, I will discuss the potential of nanotheranostics and image-guided drug delivery. By combining diagnostic and therapeutic agents within a single nanomedicine formulation, the biodistribution and target site accumulation of the carrier material can be visualized and quantified, and this information can be used pre-select patients, and to individualize and improve (chemo-) therapeutic treatments.

Twan Lammers

Twan Lammers obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008 and a PhD degree in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at the Institute for Experimental Molecular

Imaging and the Helmholtz Institute for Biomedical Engineering at RWTH Aachen. In 2014, he was promoted to full professor of Nanomedicine and Theranostics at RWTH Aachen. Since 2012, he has also worked as a part-time assistant professor at the Department of Targeted Therapeutics at the University of Twente. He has published over 100 research articles and reviews, and has received several awards. He is associate editor for Europe for the Journal of Controlled Release, and serves on the editorial board member of several other journals. His primary research interests include drug targeting to tumors, image-guided drug delivery and tumor-targeted combination therapies.

Selected Abstract for Oral Presentation

Traceable Nanocarriers for Targeted Therapy of Primary and Metastatic Breast Cancer (Abstract # 28)

Shyam Garg, University of Alberta

Thursday, May 28

SESSION 8:

Nuclear Receptors in Drug Discovery

Regulation of Drug Metabolizing Enzymes and Transporters - Implications in Personalized Medicine

Richard B. Kim, FRCPC, FACP, Professor & Chair, Division of Clinical Pharmacology; Director, Centre for Clinical & Therapeutics, Department of Medicine, Western University, London, ON, Canada

Currently, there is tremendous interest in the potential of genomics-based technologies to providing personalized or precision medicine. However, it is becoming clear that for personalized medicine to become truly clinically meaningful, we also need to better understand the mechanisms by which drug are cleared from the body. Specifically, we know that key drug metabolizing enzymes such as CYP3A4 as well as the drug efflux transporter P-glycoprotein (P-gp) are involved in the disposition of nearly 50% of all the drugs in clinical use today. Interestingly, although interpatient variation in CYP3A4 or P-gp activity can vary nearly 20-to-100 fold, commonly occurring genetic variation in CYP3A4 or P-gp do not appear to account for such interpatient variation. The marked variation is often mediated through the action of other transporters that alter cellular entry of substrate drugs as well as nuclear receptors that transcriptionally regulate key drug metabolizing enzymes and transporters. In this session, examples of drug transporter-nuclear receptor interplay as well as genetic variations in nuclear receptors and drug transporters will be highlighted. Importantly, clinical examples of unexpected drug response or toxicity that highlight the importance of such processes to individualization of drugs in clinical use such as warfarin, tamoxifen, and the recently approved direct acting oral anticoagulants will be provided.

Richard Kim

Dr. Kim received his medical degree from the University of Saskatchewan in 1987. After completing an internship and residency training in Internal Medicine, he went on to carry out fellowship training in Clinical Pharmacology at Vanderbilt University from 1991-1994, and then remained at Vanderbilt as a faculty member in Clinical Pharmacology where he rose to the rank to tenured full Professor by 2004. Since 2006, he has been Professor and Chair of the Division of Clinical Pharmacology, and holds the Wolfe Medical Research Chair in Pharmacogenomics at Western University. Areas of active research include in vitro and in vivo models of drug transporters, pharmacogenetics of drug metabolizing enzymes and transporters, as well as patient oriented clinical research. The goal is that of better understanding the molecular basis of interindividual differences in drug disposition and the application of such finding to the emerging field of Personalized Medicine. He is an elected Member of the American Society for Clinical Investigation (ASCI) and Fellow of the American Association of Pharmaceutical Scientists (AAPS) and Canadian Academy of Health Sciences (CAHS).

Xenobiotic Nuclear Receptors as Novel Therapeutic Targets in Obesity and Diabetes

Wen Xie, Center for Pharmacogenetics and Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA (wex6@pitt.edu)

We study nuclear receptor-mediated regulation of genes encoding drug metabolizing enzymes and transporters. The same enzyme and transporter systems are also responsible for the detoxification and homeostasis of numerous endogenous substances or endobiotics. As such, the nuclear receptor-mediated gene regulatory network has broad implications not only in drug metabolism, but also in establishing nuclear receptors as therapeutic targets for human diseases, including obesity and diabetes. To better understand the *in vivo* function of these receptors, we have created a wide array of

genetically engineered mice that include transgenic and knockout mice. Various disease models are incorporated into the animal models in order to study the role of these receptors in diseases. This presentation will focus on the role of xenobiotic receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR) in energy metabolism and potential of using xenobiotic receptors for the therapeutic intervention of obesity and diabetes.

Wen Xie

Dr. Xie's research has provided the foundation for understanding the biology of nuclear receptors and their roles in regulating drug metabolism, liver diseases, hormone actions, and metabolic disease. His lab created a wide array of nuclear receptor transgenic, knockout, and "humanized" mice.

He has authored 130 papers and book chapters and has been an invited speaker at over 150 conferences and seminars. He is the sole editor for the book "Nuclear Receptors in Drug Metabolism" published by Wiley in 2008. Among his other achievements, Dr. Xie received 2008 University of Pittsburgh Chancellor's Distinguished Research Award, 2008 James R. Gillette International Society for the Study of Xenobiotics (ISSX) North American New Investigator Award, and 2009 American Society for Pharmacology and Experimental Therapeutics (ASPET) Division for Drug Metabolism Early Career Achievement Award. Dr. Xie was named the Joseph Koslow Endowed Chair in Pharmaceutical Sciences in 2012. Dr. Xie is an editorial member for the journals Molecular Endocrinology, Drug Metabolism and Disposition, and Drug Metabolism Reviews.

Dr. Xie received the MD degree from Peking University Health Science Center and PhD from University of Alabama at Birmingham. After a postdoctoral fellowship at the Salk Institute, Dr. Xie joined University of Pittsburgh in 2002.

A Zebrafish Based Platform for Nuclear Receptor and Cofactor Drug Discovery

Henry Krause, Professor, The Donnelly Center, University of Toronto, Toronto, ON

We are developing transgenic fish lines that respond to small molecules that modulate the activities of transcriptional activators and repressors, focusing primarily on nuclear receptors and their epigenetic regulating cofactors. The fish lines that we have developed indicate the presence of active hormones and drugs via localized GFP production. We can then co-purify the active ligands from responding tissues using triple affinity purification of the activated NR protein, and then identify bound ligands and proteins via mass spectrometry. Several screens have already been carried out, resulting in the isolation of dozens of both known and novel small molecule ligands. One of the new hits discovered is an existing drug with excellent repurposing potential for the treatment of fatty liver diseases. Using affinity chromatography, we have also identified an endogenous ligand for this orphan receptor. The ~50 transgenic fish lines expected should have enormous potential for the understanding and prevention of metabolic disorders and related diseases such as cancer, depression, memory, inflammatory and immune disorders.

Henry Krause

Dr. Krause obtained his B.Sc. at McGill University, Ph. D. at the University of Alabama at Birmingham and PDF at the Biocentre in Basel Switzerland. His research career has focused on the functions of transcription factors in embryonic patterning, developmental timing and disease. He has spent the past 15 years focused on Nuclear receptors, using Drosophila and zebrafish as models. This work has resulted in the discovery of several new endogenous ligands, new functions and potential drugs. He is also a co-founder and President of the drug discovery company InDanio Bioscience Inc.

Selected Abstract for Oral Presentation

LXR Antagonism Attenuates Glucocorticoid-induced Osteoclast Activation in a Mouse Model of Cushing's Syndrome (Abstract # 6)

Jasmine Williams-Dautovich, University of Toronto

Thursday, May 28

SESSION 9:

IV-IVC Modeling and Simulation as a Tool to Facilitate Drug Development and Marketing

SPONSORED BY: COLORCON

Modeling and Simulation in Development and Life-Cycle Management of a Generic Drug Products

Jasmina Novakovic, Apotex Inc., Toronto, Ontario, Canada

For a generic drug product, an ultimate goal is to achieve performance equivalent to that of the reference (innovative) product. Physiologically based pharmacokinetic (PBPK) modeling and simulation is a useful tool to characterize performance of the reference product, facilitate development and support life-cycle management of a generic drug product.

At the early development stage, the PBPK modeling and simulation based on the API physico-chemical (M.Wt., pKa, log P, solubility, particle size distribution etc.) and pharmacokinetic (PK) properties (Vd, CL, plasma protein binding, first-pass effect etc.), reported in the literature or derived from in house PK studies, are used iteratively to assess the API attributes critical for the product performance and to facilitate development of a generic formulation. The applicability of the PBPK modeling and simulation at development stage is illustrated using a BCS 4 drug formulated as an immediate release solid oral dosage form as an example.

During the life-cycle management of a generic drug product, the PBPK modeling and simulation can serve multiple roles, as illustrated using an example of a BCS 1 drug formulated as an extended-release, matrix based formulation. For this type of formulaton, the PKPB modeling and simulation is utilized to: (a) identify bio-relevant dissolution test conditions and establish clinically meaningful specification limits; (b) justify bio-study waiver for the additional strengths, and (c) define boundaries for critical material attributes of a rate–controlling excipient.

In summary, the PBPK modeling and simulation is found suitable for the tasks specific to various stages of a generic drug product development and/or life cycle management.

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Jasmina Novakovic

A pharmacist by training, Jasmina Novakovic holds PhD degrees in Analytical Chemistry (Charles University, Prague, 1992) and in Pharmaceutical Chemistry (University of Belgrade, Serbia, 1994). Jasmina conducted her post-doctoral training in the area of pre-clinical testing of novel drug molecules at the University of Toronto, Faculty of Pharmacy (2000-2004). She was employed as a Research Associate at the Faculty of Pharmacy (2004-2007) prior to joining Apotex Inc in 2007 as a Scientific Leader. In her current role, Jasmina covers diverse and multidisciplinary areas of research, with a particular focus dissolution methodology and specifications, bio-pharmaceutical modeling and simulations to support product development. In

addition to her industrial employment, Jasmina teaches pharmaceutical subjects, such as Introduction to Clinical Pharmacology (Faculty of Continuing Education, Seneca College) and Drug Interactions with Herbs and Dietary Supplements (Canadian Academy of Natural Health). Jasmina is an author or coauthor of more than twenty scientific publications including four recent (2012-2013) comprehensive review articles in “Profiles of Drug Substances” (Academic Press), as well as an invited speaker at scientific events, such as Canadian Society for Pharmaceutical Sciences (CSPS) Conference (Toronto, 2012), the EuroAnalysis Meeting (Belgrade, 2011), and departmental seminars (Department of Analytical Chemistry, Charles University, Prague; Faculty of Pharmacy, University of Belgrade, Belgrade). On a volunteer basis, Jasmina serves as an abstract screener for the AAPS Annual Meetings.

Methods for Bioequivalence of Topical Products for Local Action and IVIVC

Isadore Kanfer, BSc(Pharm), Bsc(Hons), PhD, Emeritus Professor, Rhodes University, Grahamstown, South Africa, and Adjunct Professor, University of Toronto, Toronto, Canada

Unlike drug products which are intended to be absorbed into the systemic circulation and apart from transdermal dosage forms, topical dosage forms are generally not intended to be absorbed, thus the determination of BE of such products presents a formidable challenge. The methodology for assessing BE of systemically absorbed drugs and the statistical assessment of data are well-established procedures. In the latter instance, the regulatory BE acceptance criteria are based on the maximum blood (plasma) concentrations (C_{max}) and the area under the plasma drug concentration versus time profiles (AUC) falling within prescribed limits of the confidence interval (CI) of 90 % and the relative means of test to reference product ratios being within 80–125 % on the log transformed basis. However, in the case of topical drug products not intended to be absorbed into the systemic circulation, the methodology for the assessment of BE is still a “work in progress.” Currently, apart from the Human Skin Blanching Assay (also known as the vasoconstrictor assay or VCA) for the assessment of bioequivalence of topical corticosteroid products, the only means whereby a generic company can demonstrate bioequivalence of a topical dosage form intended for local and/or regional activity is through comparative clinical trials with a clinical endpoint using a randomized, double blind, parallel, placebo-controlled study design comparing the generic product versus an acceptable reference product. This has resulted in a dearth of generic topical products reaching the market since conducting clinical endpoint trials are lengthy and expensive. Several surrogate methods will be discussed including data to show IVIVC.

Isadore (Izzy) Kanfer

Dr. Kanfer was appointed to the Chair as the first Professor of Pharmaceutics at Rhodes University in 1980 and served as Head of Pharmacy at Rhodes University and Dean of the Faculty from 1987-1989 and from 1999-2007. He obtained his BSc (Pharmacy), BSc(Honours) and PhD in Pharmaceutics at Rhodes University and was Visiting Professor in Pharmaceutics at the University of California, San Francisco in 1980/81 and then in 1990 spent a year as Visiting Professor in the Division of Pharmaceutics at the University of North Carolina’s School of Pharmacy in Chapel Hill, in the USA. Prof Kanfer spent several years in the Pharmaceutical Industry in Canada where he was Vice-President: Scientific Affairs. He was appointed by the South African Minister of Health as a member of the South African Medicines Control Council (MCC) and served as Vice-Chairperson of the MCC’s Pharmaceutical and Analytical Committee which includes Bioavailability and Bioequivalence. In 2012, he was appointed as a Member of Health Canada’s Scientific Advisory Committee on Pharmaceutical Sciences & Clinical Pharmacology (SAC-PSCP). He is a Founder Member and Past Chairman of the South African Academy of Pharmaceutical Sciences is currently an Associate Editor of the Journal of Pharmacy & Pharmaceutical Sciences and a member of the Editorial Board of Encyclopedia of Pharmaceutical Science and Technology. Dr. Kanfer has supervised 36 postgraduate students (MSc & PhD) and post-doctoral fellows in Pharmaceutical Sciences and has contributed to over 200 research publications and conference presentations. He is co-editor of 4 books in the series**, Generic Drug Product Development, viz:** Solid Oral Dosage Forms, Bioequivalence Issues, International Regulatory Requirements for Bioequivalence and Specialty Dosage Forms. Professor Kanfer was the recipient of the Rhodes University Vice Chancellor’s Distinguished Senior

Research award for 2007. He is an honorary life member of the South African Academy of Pharmaceutical Sciences and a Fellow of the South African Pharmaceutical Society. He was elected as a Fellow of the American Association of Pharmaceutical Scientists (AAPS) in 2010 and in 2013 as Fellow of the Canadian Society for Pharmaceutical Sciences and as Chair of the AAPS Bioequivalence Focus Group. In 2008, he was appointed as Dean/Professor Emeritus (Pharmacy), Rhodes University, Honorary Professor, KLE University, Belgaum, Karnataka, India in 2010 and Adjunct Professor, University of Toronto in 2014. More latterly, he has been focusing on the development of methods for the bioequivalence assessment of topical dermatological dosage forms where the drug is not intended to be absorbed into the systemic circulation. His research team and collaborators were awarded a substantial grant by the US FDA in 2014 for a project entitled: Novel Methodologies and IVIVC Approaches to Assess Bioequivalence of Topical Drug Products.

How to Develop Clinically Relevant Dissolution Methods

Raimar Löbenberg, Professor, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB

The talk will review how in vitro / in vivo correlations can be developed and their importance in pharmaceutical product lifecycle management. The concept of physiological based pharmacokinetic modeling will be discussed and dextromethorphan data will be shown on how to develop such a model for this drug. A new aspect in oral drug absorption will be introduced. Lyophilic drugs with a high pKa value can get trapped in the lysosomes of the enterocytes. This will cause a delayed appearance of the drug in the systemic circulation. However, the drug is fast absorbed. Setting clinically relevant dissolution specifications for such drugs requires a detailed mechanistic knowledge of the absorption process or an in vitro / in vivo correlation might be based on wrong assumptions.

Raimar Löbenberg

Dr. Löbenberg holds a BS in pharmacy from the Johannes Gutenberg-University in Mainz, Germany. He received his PhD in pharmaceutics from the Johann Wolfgang Goethe-University in Frankfurt in 1996 for his work in drug delivery using nanoparticles. He then joined Dr. Dressman’s lab and investigated the dissolution behavior of drugs in Biorelevant dissolution media. In 1999 he joined Dr. Amidon’s lab in Ann Arbor where he investigated different aspects of oral drug administration, including computer simulations. He joined the University of Alberta in 2000.

Dr. Löbenberg's research interests are in Biopharmaceutics to predict the oral performance of drugs and botanicals and inhalable nanoparticles to treat lung cancer. He is founder and director of the Drug Development and Innovation Centre at the University of Alberta.

He is president of the Canadian Society for Pharmaceutical Sciences. He is a member of the United States Pharmacopeia Dietary Supplement Expert Committee and chair of the subcommittee for performance testing. He is Vice Chair of the Specialty Committee of Traditional Chinese Medicine in Pharmaceutics of the World Foundation of Chinese Medicine Science. He is a member of the Health Canada Scientific Advisory Committee on Pharmaceutical Sciences and Clinical Pharmacology and the Opioid Advisory Committee.

Selected Abstract for Oral Presentation

Appropriateness of Traditional Bioequivalence Metrics to Infer Therapeutic Equivalence for Follow-on Long Acting Injectables with Complex Pharmacokinetic Profiles (Abstract # 113)

Jodi Dickstein, Janssen Pharmaceuticals

Thursday, May 28

CSPS Lifetime Achievement Award Lecture

0.5 X 10^2: Looking Back and Forward

Roger Lea Williams, M.D.

Scientific advances over the last 50 years, coupled with revolutions in many industries, have changed the way we think about health and disease, both diagnostically and therapeutically. These advances have also affected the discovery, development, registration and utilization of medicines, with more changes coming every day. Yet it is hard to argue that patients are more comfortable now with their healthcare than they were 50 years ago and that societies are prepared for what is to come. The presentation will provide a focused view of changes in a specific discipline, pharmaceutical sciences, in the context the past 50 years, looking to the future.

Roger Williams
Dr. Williams received his Bachelor of Arts degree at Oberlin College and his medical doctor degree at the University of Chicago School of Medicine. He received further training in internal medicine at the University of Chicago and also completed a three year fellowship in clinical pharmacology at the University of California, San Francisco. Dr. Williams joined the faculty at the University of California, San Francisco (1977-89), worked briefly in industry (1989-90), then directed FDA's Office of Generic Drugs (1990-3). Thereafter he took on responsibility for a new 'super office', the Office of Pharmaceutical Science (OPS) with oversight for approximately 500 employees. At OPS, Dr. Williams led the creation and direction of the new Offices of New Drug Chemistry and the Office of Clinical Pharmacology and Biopharmaceutics. He also continued his oversight responsibilities of the Office of Generic Drugs and managed as well the Center's research unit, the Office of Testing and Research. At FDA, Dr. Williams championed an extensive series of coordinating committees that developed guidances in the areas of safety, efficacy, and quality. He also took on leadership roles nationally and internationally for FDA at the World Health Organization, the Pan American Health Organization, and the International Conference on Harmonization. In 2000, Dr. Williams departed FDA to join the United States Pharmacopeial Convention (USP) as Chief Executive Officer (CEO) and Chair of the Council of Experts. Dr. Williams' tenure was marked by substantial growth of the organization in terms of volunteers and staff, global linkages, science focus, and financial stability. Dr. Williams is a member of Phi Beta Kappa and Alpha Omega Alpha. He received the U.S. Army Award of Merit from the U.S. Army and many awards while at FDA. He is a fellow of the American Association of Pharmaceutical Scientists and a former expert member of the International Pharmacy Federation Board of Pharmaceutical Sciences. He has received a Doctor of Science degree, *honoris causa*, from Long Island University. He has authored or co-authored over 200 publications on many topics related to applied regulatory and compendial science as well as national and international health.

Thursday, May 28

SESSION 10:

Pharmacogenomics in Drug Development

Application of Pharmacogenomics in Drug Development, Regulatory Review and Clinical Practice

Shiew-Mei Huang, Ph.D., Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD

There are recognized ethnic factors that can affect a patient's response (ICH E5). A recent survey showed that more than 50% of the clinical trials in the submissions that we receive have been conducted in regions outside the United States. In several cases, such as orphan drugs for rare diseases, clinical data have been completely from outside the US. Facing today's globalization in drug development, some factors may be more critical than others depending on a particular drug, the disease under consideration and other factors in different regions of the world. This presentation will focus on the genetic factor and the application of pharmacogenomics in drug development, regulatory review and clinical practice. The evolution of the genomics at the FDA will be reviewed with recent development of relevant regulatory guidance documents and case examples of lessons learned in the incorporation of pharmacogenomics information in the drug product labeling.

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Shiew-Mei Huang

Dr. Huang is currently Deputy Director, Office of Clinical Pharmacology, Center for Drug Evaluation and Research (CDER), FDA. She received her B.S. in Pharmacy from National Taiwan University, School of Pharmacy in 1975 and her Ph.D. from University of Illinois, Medical Center in Pharmacokinetics and Biopharmaceutics in 1981. She has 15+ year drug development experience (Ortho pharmaceutical Corp. and Dupont-Merck Pharmaceutical Company) before joining the FDA in 1996. She chairs CDER working groups that published a draft guidance on drug interactions in 2012 and a draft guidance on pharmacokinetics in renal impairment in 2010. She is a member of the FDA Pharmacogenomics Working Group and CDER Hepatic impairment working group. She is an alternate member of the FDA Drug Safety Oversight Board.

She has published over 150 peer-reviewed articles and book chapters focusing on topics in clinical pharmacology, drug metabolism / transport interactions, physiologically based pharmacokinetic modeling and pharmacogenomics areas and has been invited to present more than 60 presentations in the

past 5 years at national and international meetings and workshops. Dr. Huang is an associate editor for a Nature journal “Clinical Pharmacology and Therapeutics” and on the editorial boards of several other journals including Expert Review in Clinical Pharmacology, Biomarkers in Medicine, Expert Opinion- Pharmacotherapy; Pharmacogenomics. She has received many awards, including an FDA Outstanding Achievement Award, FDA Clear Communication Award, and FDA Distinguished Service Award. Dr. Huang is an AAPS Fellow (American Association of Pharmaceutical Scientists), a JSSX Fellow (Japanese Society of the Study of Xenobiotics) and a diplomate of the American Board of Clinical Pharmacology. She is an Adjunct Professor at the School of Pharmacy, University of Maryland and has been a faculty member of an elective course on pharmacogenomics since 2008. She was the President (2009-2010) of the American Society for Clinical Pharmacology and Therapeutics (ASCPT). She has received a prestigious ASCPT Award “Gary Neil Prize for Innovation in Drug Development” in March 2014.

Short Tandems Repeats [STRs] are more Informative for Disease Diagnosis and Translation than SNP Associations

Allen Roses, MD. Zinfandel Pharmaceuticals, Inc. and Duke University

Short structural variants and in particular short tandem repeats [STRs] are among the most polymorphic loci in the human genome. Expanded repeats have been implicated in several Mendelian diseases, including neurodegenerative conditions. However, studies about their possible role in complex human disorders, including relationship to longevity, are underrepresented. We described a highly variable single sequence repeat [SSR] polymorphism (rs10524523) that is associated with age of onset distributions for mild cognitive impairment due to Alzheimer’s Disease [MCI-AD] in intron 6 of the TOMM40 gene [The Outer Mitochondrial Membrane 40 kB channel]. This highly polymorphic polyT length genetic marker is in linkage disequilibrium with the APOE gene. The APOE4 allele has been verified and validated for more than twenty years for its association with age of onset of AD, and the APOE2 allele with delayed onset of AD and longevity. However, the APOE4 allele is only informative in 29% of Caucasians and the APOE2 allele, only 7%. Cis-haplotypes of APOE-TOMM40’523 provide >99% informative haplotyping, with 97% of individuals mapped to defined age of onset distributions. The TOMMORROW Phase 3 clinical trial [Zinfanel-Takeda Alliance], a biomarker risk assignment algorithm (BRAA) is used identify individuals at high risk for developing MCI-AD to: 1] validate the BRAA for age of onset risk, and 2] determine whether low dose pioglitazone treatment delays the onset of MCI-AD during the duration of the trial (5 years). [Crenshaw et al., Clin.Pharm.Therapeutics, 2013] The BRAA incorporates a normal individual’s age (from 65 to 83 years), and APOE/TOMM40’523 haplotypes to determine subjects at high risk who then receive either drug or placebo. The status of TOMMORROW trial will be updated.

Additional studies are currently underway to determine the specific roles of STR polymorphisms in phenotypic expression that may cross several other complex neuropsychiatric diseases. One example is the predictive differentiation of genetic haplotypes between AD patients with or without Lewy bodies [LB]. Specific haplotypes derived from brain tissue of dementia patients found to have Alzheimer’s disease at autopsy may be useful as a prognostic test to predict the complex neuropathology of AD, AD with LB, and LB dementia associated with Parkinson’s Disease. These genetic haplotypes may be predictive of clinical phenotypes specifically relating to Parkinson’s symptoms to AD, and possibly allow clinical predictions during the course of complex dementing diseases, similar to the use of age of onset data relating to TOMM40-APOE haplotypes in TOMMORROW trial. “Complexity” of many common genetic diseases may be defined by haplotypes of STRs. SV haplotypes can improve the accuracy of disease diagnosis and to discover the mechanisms of action of relevant drugs targeted for prevention or delay.

Allen D. Roses

Dr. Roses was one of the first clinical neurologists to apply molecular genetic strategies to neurological diseases. His laboratory at Duke reported the chromosomal location for more than 15 diseases, including several muscular dystrophies and Lou Gehrig’s disease**.** He led the team that identified apolipoprotein E4 [APOE4] as the major susceptibility gene for common late-onset Alzheimer’s disease in 1992. In 1997, when Dr. Roses left Duke University Medical Center, he was

the Jefferson Pilot Professor of Neurobiology and Neurology, Founding Director of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Chief of the Division of Neurology, and Director of the Center for Human Genetics. Dr. Roses became Senior VP for Genetic Research at GlaxoSmithKline where he supervised the translation of the APOE4 association, finding new metabolic pathways for Alzheimer's disease, as well as new lead molecules for drug development. His laboratory teams then completed an efficacy pharmacogenetic clinical trial - identifying responsive and non-responsive patients to rosiglitazone in a large Phase IIB clinical trial. A Phase 3 trial identified a potential clinical effect for a low dose of 2 mg [p <0.07, but no therapeutic effect of the standard daily dose for diabetes mellitus of 8 mg.

Subsequently, in 2008 Dr. Roses registered a virtual company, Zinfandel Pharmaceuticals, Inc, to commercialize new genetic findings relevant to the prediction of age of onset of late-onset Alzheimer's Disease. The genetic variant, TOMM40-523, is a variable single sequence length polymorphism, and informative in all humans. Age of onset distributions for AD are informative for >97% of Caucasians. There is great variability of the proportion of specific APOE /TOMM40 haplotypes in different ethnic groups, but each haplotype may provide age of onset predictions in each ethnicity. An algorithm for predicting risk for onset of AD was developed in the design of a Phase III delay of onset clinical trial (TOMMORROW Study). This clinical trial will: a] validate the diagnostic algorithm, and 2] test whether low dose pioglitazone can delay the age of onset; using 5800 normal cognitively tested subjects who are followed prospectively with neuropsychological testing every six months.

During the course of these ongoing studies Dr. Roses and collaborators developed a structural variant [SV] database of more than 850,000 SVs across the genome. The initial use of this SV database has been to test candidate genes in other complex diseases, including results from GWAS candidate genes. This new method uses the highly informative SV markers to determine linkage with a candidate GWAS SNP within a region of linkage disequilibrium, as an independent post-GWAS confirmation strategy.

Selected Abstract for Oral Presentation

Preliminary Results on the Impact of Genetic Factors on Gastrointestinal Bleeding in a Prospective Cohort of New Warfarin Users (Abstract # 20)

Stephanie Dumas, Université de Montréal

The Post-Genomic Era: Two Steps Forward and One Step Back?

Edward M. Sellers, MD, PhD, FRCPC, FACP, Professor Emeritus, University of Toronto and Principal, DL Global Partners Inc. Toronto

The use of pharmacogenomics to diagnose and optimize treatment offers great promise. However, from a public health perspective reaping even a portion of the benefits will be difficult.

Current drug development is expensive and prolonged and works best for common diseases when treatment cost is spread widely. Segmenting patients by age, sex, race or disease severity increases costs and hurdles for approval and marketing. Orphan or disease targeted drugs are expensive and personally unaffordable for a variety of reasons (e.g., drugs for hepatitis C; ivacaftor (Kalydeco®) for cystic fibrosis). Pharmacogenomic individualization creates many subsets of "diseases" and an expensive fragmented drug development and therapeutic world. Payment for this segmented market using the current financial models will not be affordable.

A framework of diagnosis and treatment based on genetics does not exist. For example, "schizophrenia" is associated with several hundred genes in 8 clusters. Many of these genes are implicated in bipolar illness and depression. Does this mean we need 8 new "schizophrenia" treatment approaches or that some treatments could be used for treating several disorders?

My recent personal hospital experience reminded me that training of health care professionals in current therapeutics is inadequate - not one mention of side effects from drugs I was to receive nor did any physician know about CYP2D6 deficiency, a known individualized genetic risk factor, occurring in 6% of the population.

Critical reviews indicate best practices for common diseases are rarely followed (e.g., osteoarthritis of knee, pain, anti-psychotic use in the

elderly). If we can't optimize treatment for these how will we provide specialized point of care testing and treatment for sophisticated pharmacogenomic driven treatments? Current knowledge about pharmacogenomic individualization of treatment is barely used, except perhaps in highly specialized centers. For example, few patients benefit from existing pharmacogenetics knowledge in the use of psychotropic drugs.

The promise of pharmacogenomics exceeds what is presently achievable and what is affordable. The risk, without major changes in the financial assumptions underlying drug development, is that the gap will get even wider between patients who can afford and access the best treatments and those who can't. In contrast, precision medicine based on what we already know could benefit many quickly.

Edward M. Sellers

Dr. Sellers is a graduate of the University of Toronto and Harvard University and an internationally recognized clinical neuropsychopharmacologist. He is Professor Emeritus of Pharmacology and Toxicology, Medicine and Psychiatry, University of Toronto and has published more than 600 papers with citations of his work exceeding 26,000. His expertise includes a wide range of basic and clinical neurosciences and drug development including tobacco, drug and alcohol dependence, pharmacogenetics, drug metabolism and kinetics.

He has been President of the American Society for Clinical Pharmacology and Therapeutics, the Canadian Society for Clinical Pharmacology and the College on the Problems of Drug Dependence. In 2014 he received the Oscar B. Hunter Award of the American Society of Clinical Pharmacology and Therapeutics. This most prestigious award given in clinical pharmacology recognizes his achievements in research, teaching and clinical care.

Dr. Sellers is currently a Principal in DL Global Partners Inc.

Clinical Pharmacogenetics: A Case Study in Personalized Medicine

Jami Elliott, Thermo Fisher Scientific

This talk will chart the path of diagnostic Pharmacogenetic testing in the U.S. over the last three years (2012-2015); highlighting both the opportunities as well as the challenges in the deployment of panel-based Pharmacogenetic testing from a policy, reimbursement and utilization perspective.

Jami Elliott

Jami Elliott is founder and CEO of Quantigen Genomics, a specialty contract research organization focused on molecular assay development, exploratory clinical trials support and screening services. Quantigen is a CLIA certified laboratory and is currently pursuing ISO 15189 certification. In addition to his work with Quantigen, Mr. Elliott actively consults in global clinical segment development with Thermo Fisher Scientific's Life Sciences Group where he advises on new platform & test segment development for clinical customers as well as targeted services and manufacturing offerings in support of companion diagnostics enablement.

Thursday, May 28

SESSION 11:

Bioavailability of Novel Dosage Forms

Advances in Peptide Delivery Formulation to Impart Bone Specificity

Michael R. Doschak, MSc, PhD, Associate Professor, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; Past-President, Canadian Chapter of the Controlled Release Society http://www.cc-crs.com

Several naturally occurring peptide hormones in humans, such as Calcitonin and Parathyroid Hormone, play key roles in the regulation of bone turnover. Both Calcitonin and Parathyroid Hormone have been used as therapeutic agents in diseases of low bone volume and fragility fracture, such as Osteoporosis. However, current drug formulations of those hormones are quickly diluted systemically and readily excreted by the body after administration, resulting in the all too common lack of potency and efficacy associated by physicians and patients alike with those biologic drug medications, with the risk of unwanted side-effects with increasing dosages and administration frequency.

As an approach to challenge those shortcomings, we developed and synthesized bone-seeking variants of Calcitonin and Parathyroid Hormone, by chemically coupling them to bone-seeking bisphosphonate drug moieties under highly controlled reaction conditions. As a bone-targeting delivery formulation, the novel drug compounds directly target and coat mineralized bone surfaces after administration by virtue of the specific binding of the bisphosphonate moieties to bone hydroxyapatite mineral, eliminating the problem of systemic drug dilution and side effects when native hormone is administered at very high doses. In particular, novel BP-conjugated Calcitonin drugs have been proven to outperform current commercial formulations of Calcitonin in preclinical rat models of Osteoporosis, indicating these compounds hold promise as a novel platform of bone drugs, namely, bone-targeting biologics.

Michael Doschak

Dr. Michael Doschak is an Associate Professor with the Faculty of Pharmacy & Pharmaceutical Sciences at the University of Alberta in Edmonton, Canada. He received his Ph.D. (2004) in the Medical Sciences with the Faculty of Medicine at the University of Calgary, where his research centered on the mineral binding effects of the bisphosphonate drug family. He completed an Industrial Fellowship with the Canadian biotech company Millenium Biologix Corporation Biologics Division labs in Mississauga, where we worked on developing conjugative strategies to effectively dope biomaterial surfaces with bioactive peptide biologics, before his academic recruitment to the University of Alberta. His academic research program involves advanced drug delivery strategies, to effect the targeting and controlled release of drugs and peptide biologics with bone tissues, with orthopaedic, orthodontic (dental) and biomaterials applications.

Dr. Doschak's translational efforts in bone drug delivery have helped establish a novel platform of bone-targeting drugs, notably for the peptide hormones calcitonin, parathyroid hormone – and recently for MRI-based SPION contrast agents capable of detecting dynamic bone turnover without the use of radionuclides. He has served with the Controlled Release Society as the Canadian Chapter President (2011-13), the 2014 Nominating Committee, and with the newly formed International Committee (2014-15). Additionally, he is President and founder of Osteo-Metabolix Pharmaceuticals Inc., a University of Alberta spin-off company, who are developing a novel suite of proprietary bone-targeting therapeutics capable of reducing the pain and suffering attributed to Bone Diseases such as Osteoporosis, Arthritis and bone pain secondary to cancer metastases. http://www.osteometabolix.com/

Study Samples Re-Analysis Following High Variability of Internal Standard

Adrien Musuku, Ph.D., Director, Biopharmaceutics, Pharmascience, Montreal, Canada

Recently, there is considerable interest in the effect that internal standard variability can have on the reliability of pharmacokinetic data. Bioanalytical laboratories are required to ensure that reported analyte concentration is an accurate reflection of the sample concentration at the time of collection and can be reproduced upon repeat analysis using the validated assay.

Adequate monitoring of internal standard response across a bioanalytical run helps to identify anomalies and to ascertain if the IS compensated for endogenous and exogenous differences between samples as well as samples handling reproducibility during samples extraction and clean up procedure.

To avoid unnecessary findings from Regulatory Authority Inspectors, a guideline for internal standard acceptance criteria was implemented at our laboratory and two case study will be presented to highlight the importance of IS response monitoring and the potential impact of IS variability on the reliability of reported bioanalytical data.

Adrien Musuku

Dr. Musuku has over 19 years of experience in management of Analytical and Bioanalytical Laboratory operations as well as clinical research including phase 1 development studies, drug-drug interaction and bioequivalence. He joined Pharmascience in 2010 and is a Director in charge of the bioanalytical development as well as the pharmacokinetic and clinical operations including bioequivalence studies outsourcing to CROs.

Prior to joining Pharmascience, he served as a Bioanalytical Director, Innovative Sector at Anapharm/Pharmanet in Quebec, Canada. Earlier, he was a Director, Laboratory Operations and Research and Development Director at Cantest in Vancouver, British Columbia, Canada.

He has a Doctor of Science degree in chemistry from the University of Antwerp in Belgium.

Selected Abstract for Oral Presentation

Targeted Delivery of siRNA by Polymeric Nanomicelles (Abstract # 46)

Petro Czupiel, University of Toronto

Role of Excipients in Design of Solid Amorphous Drug Dispersions Using Hot Melt Extrusion and Spray Drying for the Delivery of Poorly Water-soluble Drugs

Krishna Bhandari, BPharm, MSc, PhD, Sr. Staff Scientist, Global Pharmaceutical R&D, Ashland Specialty Ingredients, Ashland Inc., Wilmington, Delaware

Most new drug candidates suffer from poor water-solubility leading to rate-limiting dissolution, slow absorption and limited bioavailability. Solid amorphous drug dispersions are most commonly used to improve bioavailability by enhancing drug solubility. The selection of the right solid dispersion formulation not only requires a thorough understanding of the API itself, but also an extensive knowledge of excipients and solid dispersion technologies.

Controlled release of poorly water-soluble drugs is yet another challenge for formulators. Solid dispersions generally tend to be immediate-release forms with the inherent drawbacks of high peak drug concentrations in the blood as well as relatively short durations of effective concentration levels in the blood. The combined and synergistic approaches of controlled release and solid dispersions containing poorly water-soluble drugs have become a valuable technique to achieve optimal drug bioavailability in a controlled manner and thereby providing the predictability and reproducibility of the drug release kinetics.

Krishna Bhandari

Dr. Bhandari currently works as a Senior Staff Scientist in Ashland Speciality Ingredients, USA.

He holds a Bachelor’s degree in Microbiology and Pharmacy, Masters degree in Pharmaceutical science from South Korea and PhD from the University of Alberta. Canada.

He has approximately 7 years industrial experience in pharmaceutical formulation development and scale up of small molecule oral solid dosage forms including abuse deterrent

platform technologies.

He also has extensive experience in receptor targeting injectable PEGylated drug conjugates which is being further developed by Osteometabolix Pharmaceuticals Inc (OMX) in Edmonton.

His research is primarily focused on improving bioavailability through drug dissolution enhancement using micro- emulsions, inclusion complexation and amorphous solid dispersion for poorly water soluble small molecules; and through receptor targeting for macromolecules.

Dr Bhandari has published 16 peer reviewed papers (~400 citations) and 4 US/worldwide patents on platform technologies for abuse deterrent oral dosage forms and bioavailability enhancement.

Bioequivalence and Pharmaceutical Equivalence Criteria Recommended by US-FDA for Drugs Acting Locally Within GI Tract

Barbara M Davit, PhD, JD, Executive Director, Biopharmaceutics, Merck Research Laboratories, Rahway, NJ, USA

When a new generic drug product is approved by the US-FDA, the Agency makes a determination that it is therapeutically equivalent or switchable with its corresponding reference product. To be deemed therapeutically equivalent to the corresponding reference, the generic product must be a pharmaceutical equivalent to the reference and also be bioequivalent to the reference. Bioequivalence is defined as the lack of a significant difference in the rate and extent to which the active ingredient or active moiety becomes available at the site of action when two drug products are administered at the same molar dose under the same conditions in an appropriate designed study. Determining the rate and extent of availability of a drug at the site of action is challenging for drugs which act locally within the gastrointestinal (GI) tract and which are not systemically absorbed. The US-FDA proposes a variety of in vitro and in vivo bioequivalence approaches for locally-acting GI drugs. These include in vitro binding assays, in vitro release tests, pharmacodynamic endpoint studies, and clinical endpoint studies. Traditional bioequivalence studies based on pharmacokinetic endpoints may be included if the GI drug shows substantial systemic absorption. The choice of bioequivalence approach depends on the reference drug formulation, indication, and mechanism of action, as well as the feasibility and sensitivity of the approach. This presentation will present several case studies to illustrate the scientific reasoning supporting the selection of an optimal bioequivalence approach for a given locally-acting GI drug.

Barbara Davit

Barbara M. Davit is an Executive Director in the Biopharmaceutics Group at Merck Research Laboratories (MRL), responsible for managing clinical bioavailability/bioequivalence (BA/BE) studies in early- and late-stage new drug development. Dr. Davit holds a B.S. in Chemistry from Georgian Court University, a Ph.D. in Nutrition Science from University of California, Davis, and a J.D. from George Mason University School of Law. Her graduate work focused on ADME of water-soluble vitamins, and she trained in Pharmacokinetics as a Fellow at the California Primate Research Center. After several years in the CRO industry, Dr. Davit joined the US-FDA in 1991 as a Pharmacology Reviewer, and, throughout her US-FDA tenure, ascended to positions of increasing responsibility in the Office of Clinical Pharmacology and Office of Generic Drugs. During her FDA career, Dr. Davit actively participated in developing regulatory guidance for industry. She supervised the writing and posting of the Dissolution Methods Database and the BE Recommendations for Specific Products. She also contributed to writing the recently-posted FDA draft guidances on bioanalytical method validation and on general BA/BE requirements for both new and generic drugs. Dr. Davit has written numerous articles and book chapters on various clinical pharmacology and biopharmaceutics-related topics, and is regarded as a national and international expert on BE issues. She is currently the Chair of the MRL Document Review Committee for evaluation of Phase 1 study proposals as well as the Chair of the AAPS Bioequivalence Focus Group.

Poster Session 1

CSPS and CC-CRS Posters

Wednesday, May 27, 2015

Poster Session 1

Wednesday, May 27

Biomedical Sciences

1. Liver X Receptors (LXR) Modulate the Negative Effects on Endothelial Progenitor Cells after Feeding a High Cholesterol Diet

Adil Rasheed and Carolyn L. Cummins
Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Purpose: The liver X receptors (LXRα/β) belong to the nuclear receptor superfamily of ligand activated transcription factors. LXRs are endogenously activated by oxysterols; and one of their primary functions is to facilitate cholesterol efflux out of cells, such as macrophages, via the ABCA1 & ABCG1 transporters. Bone marrow (BM) transplant studies have shown that LXRs are important for preventing the formation of atherosclerotic plaques. Loss of LXRs in the monocyte/macrophage population of the BM was thought to be the primary mediator of the increased atherosclerosis in these studies. However, the BM is comprised of a variety of cell types that are differentiated from hematopoietic stem cells (HSCs). Like numerous other vascular complications, endothelial defects are central to the pathogenesis of the early stages of atherosclerosis. Endothelial progenitor cells (EPCs), derived from HSCs in the BM, contribute to vascular health primarily through the secretion of factors that maintain and repair the endothelium of the damaged vasculature. We hypothesized that LXRs also modulate the negative effects of cholesterol on EPCs, in addition to its effects in macrophages.
Methods: To study the effects of dietary cholesterol on EPCs and the roles of LXRs in modulating these effects, WT and LXRα/β-/- mice were fed a high-fat/high-cholesterol (HF/HC) diet starting at 8-9 weeks of age for a total of 12 weeks. Complete blood counts were used to determine the levels of mature circulating hematopoietic cells. Bone marrow from each of the mice were differentiated 7 days in culture to produce EPCs. Gene expression was performed from these day 7 EOCs.
Results: Feeding a HF/HC diet increased the circulating levels of select myeloid populations, such as monocytes and neutrophils, in only the LXRα/β-/- mice. Differentiated EOC populations from LXRα/β-/- mice fed a HF/HC diet showed significant increase in the gene expression of endothelial markers, CD144 and VEGFR2, suggesting enhanced differentiation to a more endothelial-like cell in the absence of LXRs. Notably, the paracrine reparative actions of EPCs have been shown in populations of cells with low expression of endothelial markers.
Conclusion: Taken together, these results suggest that LXRs modulate the effects of HF/HC diet on the differentiation of EPCs to endothelial cells and thus, may provide another cell type within the BM that would benefit from LXR activation in modulating vascular disease.

2. An *In Vivo* Rat Model of HIV-Induced Neurocognitive Deficits

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Background: The prevalence of neurological complications in Human Immunodeficiency Virus -1 (HIV-1) infected patients is dramatically increasing with about 50% of patients developing HIV-associated neurocognitive disorders (HAND). Clinically, these patients appear to be aging prematurely; perhaps due to persistent low-level viral replication and associated inflammation in the brain leading to neuronal loss.
Purpose: The goal of this project is to implement an *in vivo* rat model of HIV-1 brain inflammation and neurocognitive dysfunction by intracerebro-ventricular (icv) administration of HIV-1 viral coat protein, gp120, in adult male Wistar rats and to investigate the effect of anti-inflammatory

compounds on both the inflammatory/oxidative stress responses and prevention/improvement of neurocognitive deficits.

Material/Methods: Anesthetized rats were injected icv with a single dose of R5-tropic $gp120_{ADA}$ in both lateral ventricles (4μg/ventricle). Lipopolysaccharide (LPS), a bacterial endotoxin that has been reported to induce inflammation and behavioural abnormalities, was administered icv as a positive control. Real-time qPCR was used to assess gene expression of inflammatory/oxidative stress markers in different regions of the brain (i.e. hippocampus, frontal cortex, striatum). Cognitive deficits in spatial learning and memory were characterised using the Morris Water Maze test and immunoblotting was used to quantify expression of activated caspase-3. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) will be used to examine the extent of neuronal apoptosis.

Results: Our data demonstrate high levels of cytokines (IL-1b and TNFa) and oxidative stress marker (iNOS) transcripts in the brain 6h and 24h post injection of gp120/LPS compared to control saline; following behavioural testing, six days after initial injection, the inflammatory response subsided. Rats injected with a single dose of 50μg LPS were significantly impaired in spatial learning/memory compared to saline controls ($P < 0.001$; n = 8-12). However, 8μg gp120 injection did not result in cognitive deficits. Consistent with our behavioural observations, significant activated caspase-3 wasobserved in rats treated with LPS, but not in gp120 treated animals, in both frontal cortex ($p < 0.01$; n = 6) and hippocampus ($p < 0.001$; n = 6). Additional fluorescence imaging analyses are in-progress to quantify DNA fragmentation in neurons.

Conclusion: Our data illustrate HIV-1 $gp120_{ADA}$ and LPS are able to induce a robust inflammatory response in the brain that could initiate caspase-dependent apoptosis leading to observable learning and memory dysfunctions. Our gp120 and LPS-induced neuroinflammatory rat model could constitute a useful tool to examine the potential effect of anti-inflammatory compounds in reversing HAND as adjuvant therapies.

3. Cardioprotective Effects of CB-13, a CB_1/CB_2 Cannabinoid Receptor with Limited Brain Penetration

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Purpose: Cardiac hypertrophy is increased myocardial mass provoked by hemodynamic stress or myocardial injury, and is a convergence point for risk factors of heart failure. Prolonged hypertrophy leads to functional decompensation of the heart, in part by disrupting healthy energy production. We queried whether the endocannabinoid system might be manipulated to attenuate hypertrophy and improve mitochondrial bioenergetics. Endocannabinoids are amides, esters and ethers of long chain polyunsaturated fatty acids that reportedly protect against myocardial ischemia, arrhythmias, and endothelial dysfunction of coronary arteries. However, clinical utility of endocannabinoids is precluded by undesirable psychoactive side effects mediated by central cannabinoid CB_1 receptors. Therefore, we determined the effects of CB13, a peripherally-restricted dual CB1/CB2 agonist, on cardiac myocyte hypertrophy and mitochondrial dysfunction.

Methods: Our experimental paradigm of hypertrophy was rat ventricular myocytes treated with endothelin-1 (ET1; 0.1 μM). The effect of CB13 (1 μM) was assessed by measuring myocyte size, protein synthesis, and hypertrophic gene expression. Changes in mitochondrial membrane potential ($\Delta\Psi_m$) were assessed by fluorescence microscopy using the potential sensitive dye, JC-1. Modulators of mitochondrial function (i.e. PPAR-γ coactivator-1α [PGC-1α - a driver of mitochondrial biogenesis], carnitine palmitoyl transferase 1β [CPT-1β - facilitator of fatty acid uptake], and AMP-activated protein kinase [AMPK - energy sensor]) were assessed by real-time PCR and western blotting. The Seahorse Bioscience XF24 Analyzer was used to measure fatty acid oxidation-related bioenergetics.

Results: ET1 induced myocyte enlargement (122±4%), protein synthesis ([3H]-leucine incorporation; 147±13%), and hypertrophic gene expression (brain natriuretic peptide mRNA levels;

426±88%) ($p<0.05$ vs. controls). CB-13 attenuated all hypertrophic indicators. ET1 also caused mitochondrial aberrations which included membrane depolarization ($\Delta\Psi_m$ 80±5% vs. control; $p<0.05$), reduced PGC-1α (59±7% vs. control; $p<0.01$) and CPT-1β (81±5% vs. control; $p<0.05$) expression, as well as depressed respiration (basal/maximal/reserved respiration respectively: 81±5%, 78±4%, 74±5% vs. control; $p<0.05$), coupling efficiency (83±6% vs. control; $p<0.05$), and respiratory control ratio (79±5% vs. control; $p<0.01$). CB13 improved all mitochondrial parameters. The effects of CB13 on hypertrophy, mitochondrial membrane potential, and PGC-1α were abolished by shRNA knockdown of AMPK. These data suggest that AMPK contributes to the effects of CB13.
Conclusion: CB13 attenuated hypertrophy and mitochondrial aberrations. Therefore, a cannabinoid-based treatment for cardiac disease remains a potential therapeutic strategy that warrants further study.

4. Waddles Mice Experience Altered Calcium Signaling within Cerebellar Granule Cells in Response to Various Glutamatergic and GABAergic System Manipulations

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Purpose: Motor coordination and learning are the primary responsibilities of a brain region known as the cerebellum. The waddles mouse (*wdl*) is characterized by a namesake waddling side-to-side gait, which is the result of a mutation in a gene encoding carbonic anhydrase type 8 (CAR8). CAR8 has previously been shown to reduce the release probability of calcium from intracellular stores of neurons by inhibiting the binding of inositol 1,4,5-trisphosphate to its receptor. However, the cellular mechanism causing impaired motor output from the *wdl* mice is currently unknown.
Methods: Behavioral and calcium imaging experiments were conducted to investigate the *wdl* pathology. Initially, an accelerating rota-rod paradigm was used to characterize the degree of impairment in *wdl* mice motor output and learning. Calcium imaging experiments were then undertaken with acute sagittal slices from the cerebellar vermes. Slices were loaded with Oregon Green BAPTA-1AM (a fluorescent calcium indicator) and imaged either at baseline activity, with exposure to various pharmacological agonists/antagonists, or while undergoing indirect electrical stimulation.
Results: Younger homozygotes outperformed their older cohorts on the rota-rod apparatus; and heterozygotes, which were thought to be free of motor impairment, displayed motor learning deficiencies. Calcium imaging revealed significant alterations in cerebellar granule cell somatic calcium responses when exposed to glutamate, with controls showing a much larger response than homozygotes. A contribution of inhibitory GABAergic signaling to these alterations was verified using bath application of bicuculline which reversed the trend seen with glutamate application alone. Use of a group 1 metabotropic glutamate receptor (mGluR1) agonist, dihydroxyphenylglycine, caused a significantly larger response in mutants versus control animals. Changes in somatic calcium signals were found to be applicable to an *in-vivo* scenario by using electrical stimulation of afferent mossy fiber projections onto granule cells. A portion of the granule cell response to electrical stimulation was found to be mGluR1-dependant as it was reversibly inhibited by the selective antagonist 1-aminoindan-1,5-dicarboxylic acid. Finally, intracellular calcium store function was also found to be significantly altered by the *wdl* mutation when slices were treated with thapsigargin.
Conclusion: Understanding the pathological calcium signaling that underlies the *wdl* ataxia furthers our understanding of other ataxias involving aberrant calcium signaling, as well as our understanding of standard cerebellar calcium signaling as it relates to functional motor output. Findings from this study suggest potential therapeutic targets to help treat and alleviate symptoms in cerebellar based ataxias in the future.

5. Hepatic Ablation of Arginine and Glutamate Rich 1 Impairs Glucocorticoid-induced Gluconeogenesis

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Purpose: The glucocorticoid receptor (GR) has essential roles in development, maintaining carbohydrate and fat metabolism, immune responses, and in the central nervous system. Using a high-throughput expression-cloning screen, we

have identified, Arglu1 (arginine and glutamate rich 1) as a novel protein that strongly potentiates GR transcriptional activity, and is highly conserved across species. Our objective was to uncover the in vivo role of Arglu1 in the context of glucocorticoid signalling.

Methods: To study the cell-autonomous role of Arglu1 in hepatic gluconeogenesis, primary mouse hepatocytes were isolated and transfected with siControl or siArglu1. Ligands were added 48 hrs post-transfection. QPCR analyses and glucose production assays were carried out 4 and 24 hrs later, respectively. Primary macrophages were used to investigate the effects of Arglu1 knockdown on the immunosuppressive action of glucocorticoids (GCs). To investigate the role of Arglu1 in vivo, adeno-associated virus (AAV) was used to knockdown Arglu1 in livers of Arglu1$^{fl/fl}$ mice. AAV8-Cre-Ttr and AAV-GFP (control) were obtained from Vector Biolabs. 10^{12} viral particles were delivered by tail-vein injection to 12-16 week-old Arglu1$^{fl/fl}$ mice (n=10/group). Dexamethasone (Dex) treatment (5 mg/kg, bid) was initiated 8 days later (n=5/group), and animals were sacrificed for analysis after 5 days.

Results: Arglu1 knockdown in primary hepatocytes impaired Dex-mediated induction of gluconeogenic genes (PEPCK and G6Pc) and glucose production, but had no effect on Dex-mediated suppression of IL-1β and TNFα expression in primary macrophages. In vivo, both, Arglu1$^{fl/fl}$ and Arglu1$^{Liv-/-}$ animals were equally responsive to Dex-induced hepatomegaly and spleen atrophy. Plasma glucose levels tended to be increased with Dex in the AAV-GFP group, whereas, at the gene expression level, Dex significantly up-regulated Pepck expression in GFP injected animals, but not in animals lacking Arglu1. Moreover, Dex-treated Arglu1$^{Liv-/-}$ animals had significantly lower plasma cholesterol and triglyceride levels than Arglu1$^{fl/fl}$ mice. Lower plasma and liver triglyceride levels could be explained, in part, by the inability of Dex to induce a number of genes involved in lipid homeostasis, Hmg-CoA synthase 1 (HmgCS1), Fgf21 and Dgat1 in the knockout animals.

Conclusion: It was found that Arglu1 is important for the induction of the gluconeogenic program in primary hepatocytes and in vivo but is not required for immunosuppressive actions of GCs. Moreover, animals lacking hepatic Arglu1 were more resistant to Dex-induced hypertriglyceridemia. Overall, we have identified Arglu1 as a novel GR co-activator that plays an important role in glucocorticoid-induced gluconeogenesis but is dispensable for GC immunosuppressive actions.

6. LXR Antagonism Attenuates Glucocorticoid-induced Osteoclast Activation in a Mouse Model of Cushing's Syndrome

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Purpose: Synthetic glucocorticoids (GCs) have unparalleled anti-inflammatory and immunosuppressive properties that are crucial for the treatment of many inflammatory diseases. Unfortunately, a remaining limitation for the long-term therapeutic use of GCs is the development of major metabolic side effects, including Cushing's syndrome, a disease characterized by metabolic dysregulations such as hyperglycemia, muscle wasting and osteoporosis. The glucocorticoid receptor (GR) and liver X receptor (LXRs) are members of the nuclear receptor superfamily that regulate distinct but overlapping transcriptional programs. Previous work in our lab found that one of the detrimental side effects of long-term GC therapy hyperglycemia was prevented when mice, exposed to a high level of GCs, were treated with a LXR antagonist. Importantly, in this context GCs retain their therapeutic anti-inflammatory effect. The aim of this study was to determine whether LXRs are also involved in modulating GC-induced bone loss and muscle wasting.

Methods: A mouse model of Cushing's syndrome (CRH-tg), a disease characterized by high endogenous glucocorticoids, was used to study GC excess. Wildtype and CRH-tg mice were treated with LXR antagonist (GSK2033, 40mg/kg) or vehicle (control) twice daily for 6 weeks. The gastrocnemius and humeri were flash frozen and gene expression was measured by qPCR for muscle atrophy genes (Murf1, Mafbx and Myostatin), for osteoclast-related bone turnover genes (Oscar Trap and Ctsk) and for osteoblast-related bone forming genes (Col1a1, Osteocalcin). Serum osteocalcin and TRAP-5b were measured by ELISA.

Results: The CRH-tg mice showed a significant increase in osteoclast-related genes responsible for bone turnover (Oscar, Trap and Ctsk) compared to the WT mice. This increase was attenuated in the

CRH-tg mice treated with GSK2033. This pattern was also seen at the protein level when the osteoclast marker, TRAP-5b, was measured by ELISA. Interestingly, the CRH-tg mice showed a decrease in osteoblast-related bone forming genes (as expected) compared to WT, however this decrease was not rescued in the GSK2033 treated CRH-tg mice. Muscle atrophy markers (Murf1, Mafbx) and Myostatin show a trend to increase in the CRH-tg mice compared to WT, which is only slightly attenuated in the GSK2033 treated CRH-tg mice, however the effect did not reach statistical significance.

Conclusion: Together, these data suggest that LXRs significantly modulate the effects of GCs in osteoclasts, marginally in muscle cells, and not in osteoblasts suggesting a cell type specific effect. These data support a beneficial role for LXR antagonism in the treatment of glucocorticoid-induced osteoporosis.

7. Epigenetic Regulation of Brain Derived Neurotrophic Factor (BDNF) by Methyl CpG Binding Protein 2 (MeCP2) Isoforms in an Experimental Autoimmune Encephalomyelitis (EAE) Model of Multiple Sclerosis (MS)

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Rationale: Multiple sclerosis (MS) is a chronic neurological disease of central nervous system (CNS) myelin. At present, there is no cure for MS due to the inability to repair damaged myelin. Although the neurotrophin, brain derived neurotrophic factor (BDNF) has a beneficial role in re-myelination and/or myelin repair, these effects can be hampered by over-expression of its transcriptional repressor methyl CpG binding protein 2 (MeCP2). MeCP2 has been shown to disrupt the homeostatic signaling equilibrium network between cytokines, chemokines and neurotrophins that govern re-myelination and/or myelin repair. We identified three novel biological targets that govern re-myelination and/or myelin repair including BDNF and the two biologically active isoforms of MeCP2:MeCP2E1 and MeCP2E2. However, their exact interactions are currently unknown.

Hypothesis: We hypothesize that following EAE-induced myelin damage, MeCP2 hampers the ability of BDNF to re-myelinate and repair damaged myelin by repressing BDNF expression.

Aims and Methods: Identify and compare the temporal gene and protein expression changes of the two known isoforms of MeCP2 (MeCP2E1, MeCP2E2) and BDNF in EAE mice during the acute and chronic progressive phases of EAE. These changes will be correlated with the changes in the neurological disability scores (NDS). The expression changes in the biological targets selected will be analyzed at the transcript (*via* quantitative real time PCR (qRT-PCR). Fresh DRG and SC tissue will be removed for the purpose of molecular gene (qRT-PCR) and protein [western blotting (WB), enzyme-linked immunosorbent assay (ELISA)] analysis.

Preliminary Findings: Our research findings confirm that the immune system-induction of MeCP2 disrupts the normal homeostatic signaling equilibrium network between cytokines, neurotrophins and chemokines by functioning as a transcriptional repressor of BDNF. Our findings also indicate that the immune system-induced increase of MeCP2 expression inversely correlates with BDNF.

Conclusion: Our results indicate that the elevations in immune system-induced spinal cord (SC) MeCP2 are involved in the localized repression of SC BDNF. As such our findings confirm that the MeCP2-induced repression of BDNF disrupts the homeostatic signaling equilibrium between cytokines, chemokines and neurotrophins which subsequently interferes with the re-myelination and/or myelin repair process.

8. Development of a Mouse Smooth Muscle Cell Model for Assessing the Role of Chemerin/ Chemokine-like Receptor 1 Signalling in Atherosclerosis

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Purpose: Chemerin is a fat tissue-secreted protein with cell proliferative, migratory and pro-inflammatory properties. Its presence in human serum correlates with atherosclerosis severity however, its role is unknown. This study aims to develop a protocol to isolate aortic vascular smooth

muscle cells VSMCs from wild-type (WT) and chemerin receptor (CMKLR1) knockout (KO) mice, in order to assess the role of chemerin/CMKLR1 signalling in SMC migration and proliferation (important stages of atherosclerotic plaque formation).

Methods: Aortic VSMCs were harvested from adult WT and CMKLR1 KO mice and were cultured and characterized after 0, 7 and 14 days. BrdU and MTT assays were used to assess proliferation and viability, respectively, of VSMCs upon treatment with chemerin (0- 30 nM) alone and in combination with the inflammatory cytokine TNFα (0-10nM), in the presence and absence of fetal bovine serum (FBS).

Results: Endothelial-specific gene expression decreased while VSMC-specific gene expression increased with increased WT VSMC confluence. WT VSMC proliferation increased by 100- and 200-fold in the presence of 1% and 5% FBS (positive control), respectively. Chemerin did not affect the proliferation of WT VSMCs in the presence or absence of 1% FBS. Co-treatment with chemerin and 1 or 10 nM TNFα reduced WT SMC proliferation by 50%; however, MTT analysis showed no reduction in cell viability. In preliminary studies, chemerin did not affect the proliferation of CMKLR1 KO SMCs.

Conclusions: Mouse aortic VSMCs were successfully isolated, cultured and characterized. Chemerin does not independently affect aortic VSMC proliferation, but may attenuate proliferation in the presence of additional inflammatory mediators.

9. Fractal Circuit Sensors Enable Rapid Genetic Profiling of Donor Organs for a Personalized Medicine Approach to Lung Transplantation

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Purpose: Biomarker profiling is being rapidly incorporated in many areas of modern medical practice to improve the precision of clinical decision-making. This potential improvement however, has not been transferred to the practice of organ assessment and transplantation due to the fact that previously developed gene-profiling techniques require an extended period of time to perform, making them useless in the time-sensitive organ assessment process. Herein, we sought to develop novel electrochemical sensors that would rapidly quantify the expression of genetic biomarkers known to be predictive of primary graft dysfunction (PGD) in donor lungs.

Methods: Electrochemical sensors, fractal circuit sensors (FraCS), were fabricated using conventional photolithography and standard glass substrates. FraCS were electrodeposited in exposed linear apertures in microchips using DC potential amperometry and functionalized with peptide nucleic acid (PNA) probes that specifically reported on the mRNA levels of IL-6, IL-10, and ATP11B. Electrochemical measurements were made using a redox-active Ru^{3+}/Fe^{3+} reporter system and differential pulse voltammetry (DPV). Lung tissue biopsies were collected during the cold ischemic period of the transplantation process, and a small portion (5 mm^3) was lysed then used for genetic analysis with FraCS. A proof-of-concept study using (n = 52) donor lungs was performed and used to develop the FraCS prediction model (FPM).

Results: We developed a novel class of three-dimensional electrochemical biosensors (FraCS) with large surface areas. These sensors displayed signals with maximal concentration dependence and were able to rapidly (< 20 minutes) and reproducibly quantify small differences in the expression of IL-6, IL-10, and ATP11B mRNA. The FraCS platform was validated using a logical buildup of analyte complexity and ultimately validated using crude, unpurified tissue biopsies. Using FraCS, the predictability of each biomarker was confirmed using receiver-operator characteristic curves with a significant AUC for each biomarker. In a clinical study, we demonstrated that a FraCS-model could be used to predict (AUC = 0.82, P<0.05), with excellent sensitivity (74%) and specificity (91%), the incidence of PGD for a donor lung, thus delivering a key prognostic test that could be applied to enhance

patient outcomes.

Conclusion: This work provides an important step towards bringing rapid mRNA diagnostic profiling to clinical application in lung transplantation. This study demonstrates the value of integrating rapid, personalized diagnostics into the donor lung assessment process.

10. Role of *Hoxa2* in Regulating Osteoblast Differentiation during Palate Development

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Background: Cleft palate is one of the most common birth defects in humans with an incidence of 1 in 400 to 1 in 1000. *Hoxa2* plays a direct role in secondary palate development and *Hoxa2*$^{-/-}$ mice exhibit cleft palate. The molecular mechanism governing the cleft palate phenotype of *Hoxa2* null mice is unknown. *Hoxa2* is a known inhibitor of osteoblast differentiation in the second pharyngeal arch. However, the role of *Hoxa2* in the osteoblast differentiation of the developing palate has not been investigated.

Purpose: Our objective was to investigate the role of *Hoxa2* in osteoblast differentiation during palate development.

Methods: Osteoblast differentiation in the developing palate *in vivo* was studied using *Hoxa2* null mice by quantitative real-time PCR (qRT-PCR), Western blot analysis and immunohistochemistry. Primary mouse embryonic palatal mesenchyme (MEPM) cells isolated from wild type and *Hoxa2*$^{-/-}$ mice were subjected to *in vitro* osteoblast differentiation. Alkaline phosphatase and Alizarin red staining were used to study the bone matrix deposition and mineralization *in vitro.*

Results: Data collected indicate increased expression of osteoblast markers such as alkaline phosphatase (Alp), runt-related transcription factor 2 (Runx2) and *Sp7* transcription factor in the *Hoxa2*$^{-/-}$ mouse palatal mesenchyme at embryonic day (E) E16.5. In addition, mRNA expressions of osteoblast markers (*Runx2, Alp, Sp7* and *Bglap*) were significantly increased at E13.5 and E15.5 in the developing palatal shelves. *Hoxa2*$^{-/-}$ MEPM cell cultures also exhibited increased bone matrix deposition and mineralization *in vitro*. Consistent with the upregulation of osteoblast markers, canonical Bmp signaling mediated by phosphorylated Smad 1/5/8 was increased in the *Hoxa2*$^{-/-}$ palatal shelves.

Conclusion: Our findings demonstrate that *Hoxa2* plays an important role in osteoblast differentiation in the palate and has a regulatory role in maintaining the temporal and spatial expression of osteoblast markers in the palatal mesenchyme. In conclusion, we show for the first time that *Hoxa2* regulates palate development by controlling osteoblast differentiation of the palatal mesenchyme.

Acknowledgement: Supported by a grant from the NSERC to AJN.

11. The Effect of Diet, Chronic Inflammation, and Hypertension in MCA Function and Hemorrhagic Stroke Development

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Introduction*:* Rheumatoid arthritis (RA) patients have a higher mortality rate associated with stroke and develop hypertension, a known risk for hemorrhagic stroke (HS), than the general population. HS is associated with loss of cerebrovascular function; the normal protective functions of the middle cerebral artery (MCA) is likely lost, predisposing the brain to cerebral hemorrhage. We believe chronic inflammation, similar to that seen in RA patients, causes alterations to MCA's function, leading to HS. Our aims were to create and define an animal model which has both chronic inflammation and hypertension, and study the changes to the MCA function.

Methods: We established a hypertensive-arthritis animal model using stroke-resistant spontaneously hypertensive rats (SHR). They were divided into four groups; one group was fed a high salt diet while a second group was fed Purina diet. Arthritis was induced by intradermal injection of Complete Freund's adjuvant (CFA) into the left paw. Control SHRs were injected with saline (n=6-14/group). The rats were monitored for 21 days. At study endpoint, some were infused with Evans Blue (EB) dye to identify signs of cerebral hemorrhage. Upon sacrifice, the MCAs were isolated and their ability to undergo pressure dependent constriction (PDC) and to react to vasoactive peptides was evaluated.

Results and Discussion: All groups exhibited significant increases in blood pressure. The CFA

groups had a significant increase in paw inflammation. EB infusion identified signs of intracerebral hemorrhage in CFA groups but not in saline controls, irrespective of diet. The MCAs' ability to undergo PDC was significantly diminished in the high salt diet group injected with CFA vs. saline. However, they had comparable response to vasoactive peptides. On the other hand, PDC response in Purina diet cohort did not differ between the CFA and the saline groups, while the response to certain vasoactive peptides was significantly less in the CFA group.

Conclusion: We were successful in inducing HS in our hypertensive-arthritis model regardless of type of diet. Chronic inflammation impairs aspects of the MCA function in the endothelial and vascular smooth muscle cells, resulting in cerebral hemorrhage. It is likely that high salt diet plays a role in cerebral vessel impairment and the extent to its involvement requires further research.

12. Knockdown of Scavenger Receptor Class B type I is Correlated with Reduced Prostate Specific Antigen Secretion and Cell Cycle Arrest in Castration-resistant Prostate Cancer Cells

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Purpose: Existing therapies for castration-resistant prostate cancer (CRPC) extend life and provide clinical benefit; however, patients still develop therapeutic resistance. Persistent androgen signalling in CRPC has been proposed to be maintained by *de novo* intratumoral steroidogenesis from the precursor cholesterol. The high density lipoprotein-cholesterol receptor, scavenger receptor class B type I (SR-BI), is upregulated in CRPC models *in vitro* and *in vivo*. The purpose of this study was to test the potential of SR-BI as a target in CRPC as a novel approach to overcome therapeutic resistance.

Method: The effects of SR-BI knockdown were studied using the CRPC C4-2 cell line. Cells were transfected with either stealth RNAi duplexes targeting SR-BI (SRBI-KD) or Lo GC Negative Control (NC) duplexes. Cells were grown for several days post-transfection and samples were collected at various time points; media samples were collected for prostate specific antigen (PSA) quantification (chemiluminescence), while cell lysates were collected for cholesterol (fluorometric), steroid (LC-MS), and protein (immunoblot) analysis. Additionally, cells were subjected to propidium iodide staining for cell cycle analysis by flow cytometry and MTS cell proliferation assay.

Results: SR-BI knockdown was correlated with a 39% reduction in PSA secretion compared to control (20.9 ± 1.4 ng/mL/μg protein SRBI-KD vs. 34.5 ± 2.4 ng/mL/μg protein NC; n=4, $p < 0.05$). Testosterone concentrations were also reduced in SRBI-KD (0.20 ng/mL/mg cell pellet) compared to NC (0.41 ng/mL/mg cell pellet; n=1). These changes were accompanied with reduced proliferation in SRBI-KD at 6 days post-transfection and G_1/S cell cycle arrest (70.9 ± 7.9% G_0-G_1 phase fraction SRBI-KD vs. 58.3 ± 4.1% NC; n=4, $p < 0.05$) in the absence of apoptosis.

Conclusion: Knockdown of SR-BI resulted in reduced PSA secretion as well as reduced androgen concentrations suggesting decreased androgen signalling ultimately leading to G_1/S arrest and decreased proliferation in the absence of an apoptotic induction. These data indicate SR-BI may be required to provide cholesterol for *de novo* steroidogenesis for continued androgen signalling.

Funding acknowledgements: CIHR and Prostate Cancer Canada

13. Propofol Mediated Cardioprotective Signal Transduction

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Purpose: Propofol protects against myocardial ischaemia-reperfusion injury[1]. However, the precise molecular mechanism remains to be elucidated. The purpose of this study is to explore the role of the lipid membrane in mediating propofol signaling. Two implicated components are the endothelin receptors, and the organelle in which they reside—caveolae (a subtype of lipid raft). We hypothesize

that propofol mediated signal transduction is dependent on the Endothelin A receptor (ETAR) and caveolae.

Methods: H9c2 (rat cardiomyocyte) cells were treated with propofol. To investigate the role of ETAR, we pretreated the cells with a specific ETAR inhibitor, and measured phosphorylated STAT3 tyrosine protein expression via Western Blot as a downstream functional outcome[1]. To determine if propofol was affecting the cellular localization of either ETAR or Caveolin-1, Immunocytochemistry techniques were used to observe both the membrane (non-detergent permeabilised) and cytosolic (detergent permeabilised) localizations. To determine caveolar-dependance of propofol signaling, all of the cellular lipid rafts were disrupted by the use of methyl beta cyclodextrin, and the levels of phosphorylated STAT3 tyrosine determined via Western Blot.

Results: Specific inhibition of ETAR significantly reduced the amount of propofol induced STAT3 tyrosine phosphorylation. Immunofluorescence suggested that while caveolin-1 levels stayed relatively constant in both the cytoplasm and membranes, the levels of ETAR increased in the cytosol. Disrupting lipid rafts, did not decrease the amounts of propofol-induced STAT3 tyrosine phosphorylation.

Conclusion: The results suggest that propofol is acting in part through ETAR. Propofol-mediated activation and subsequent vesicular internalization of ETAR may explain the increase in immunofluorescence cytosolic expression of ETAR. Disruption of lipid rafts and thus the association between Caveolin-1 and ETAR does not inhibit propofol-mediated signaling.

References: JAKSTAT 2014(3), e29554

14. Identification of Novel Smo Ligands Using Structure-Based Docking

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Purpose: The cell surface transmembrane protein Smoothened (Smo) mediates hedgehog (Hh) signalling, a critical pathway required for embryonic development and adult tissue homeostasis. Hh proteins control cell growth and migration, as well as stem cell maintenance. Inappropriate activation of the pathway is directly linked to cancer, including basal cell carcinoma and medulloblastoma. The recent determination of the Smo crystal structure offers an opportunity for structure-based ligand discovery. This structure-based virtual docking campaign is the first reported for the Frizzled G-protein coupled receptor (GPCR) family.

Methods: We first performed a retrospective docking of 308 known Smo ligands to the available atomic structure, comparing the docking scores to those of a library of decoy molecules. For the prospective virtual screen, we used DOCK to screen a small molecules library containing 2.4 millions commercially available compounds with drug-like properties: MW<350, logP<3.5 and a maximum of 7 rotatable bonds. After visually inspecting the top 1000, 21 compounds were selected for experimental testing. Preliminary testing was performed at 30□M in $Ptch1^{-/-}$ MEFs expressing a Gli-Luciferase reporter gene, where the pathway is constitutively active due to deletion of the Hh receptor Ptch. We used an analog-by-catalogue approach with the ZINC database to improve the affinity of chemical families identified. We then validated the best candidates using qPCR to determine their ability to inhibit the Hh target gene Gli1. We also confirmed specificity to Smo by testing for their ability to displace a fluorophore-tagged Smo antagonist in HEK293 cells overexpressing Smo-Cherry. Since treatment with available Smo inhibitors rapidly leads to the emergence of Smo mutations providing resistance, we asked if the new candidates could inhibit the activity of these Smo mutants.

Results: In the initial screen, we successfully identified four antagonists of three novel families of ligands (19% hit rate). We further explored the chemical spaces and identified a total of 11 novel Smo antagonists that can specifically inhibit the pathway and displace a known Smo antagonist, both in a dose-dependent manner with IC50 in the low micromolar range. Interestingly, the best candidate can also inhibit Smo mutants that confer resistance to the clinically available Vismodegib drug.

Conclusions: We are presenting the first structure-based virtual screen for Smoothened, and for the class F of GPCRs. We identified 11 new antagonists with three novel scaffolds. One lead candidate still

exhibit efficacy at Smo mutants previously identified to provide resistance to other Smo inhibitors.

15. Effect of Oral Egg Yolk Antibody on Inhibition of Gliadin Induced Intestinal Inflammatory Response

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Purpose: In Celiac disease (CD) individuals, ingestion of gliadin promotes intestinal tissue inflammatory response, owing to the predisposing genetic factor and presence of pepsin-trypsin resistant gliadin (PT-gliadin) peptides. This study aims to prevent PT-gliadin absorption using egg yolk antibody (IgY) in order to inhibit gliadin-induced celiac pathogenesis. To prove this, specific anti-gliadin IgY antibody was produced and evaluated for its efficacy on PT-gliadin induced proinflammatory effects on intestinal epithelial (Caco-2) cell culture model for CD.
Methods: Caco-2 (passages 20-24) monolayers were subjected to 7 experimental conditions (n=3 each): phosphate-buffered saline (PBS; control), pancreatic digested-casein (PD-casein; negative control), PT-gliadin (positive control), non-specific IgY with PT-gliadin, and anti-wheat gliadin IgY with PT-gliadin at a ratio of 1:6,000, 1:3,000 and 1:1,500. Caco-2 monolayers were then evaluated for effects of gliadin and/or anti-wheat gliadin IgY after 24 h exposure. Enzyme-linked immunosorbent assay (ELISA) was used to quantify anti-inflammatory markers (TNF-α and IL-1β) 5 days after cells were exposed to PT-gliadin and/or anti-wheat gliadin IgY.
Results: PT-gliadin has shown to stimulate significant release of pro-inflammatory cytokines (IL-1β and TNF-α) in Caco-2 cell culture system after 24 h incubation ($P < 0.05$), as compared to cultures exposed to control PBS, PD-casein, and non-specific IgY. Upon PT-gliadin stimulation, a 6.77 fold higher of TNF-α content than that of IL-1β was detected in the cell culture supernatant. When anti-gliadin IgY and PT-gliadin were co-incubated at a ratio of 1:6,000, TNF-α concentration was significantly decreased ($P < 0.05$), whereas IL-1β levels were undetectable. However, with higher content of anti-gliadin IgY co-incubation with PT-gliadin at a ratio of 1:3,000, there were undetectable levels of both TNF-α and IL-1β.
Conclusion: The anti-wheat gliadin IgY antibody produced in this study, used at a ratio of 1:3,000 (anti-gliadin IgY: PT-gliadin), has proved to prevent intestinal inflammatory response at cell culture level, thus resulting in undisturbed epithelial cells upon gliadin stimulation.

16. Maternal Bacterial Infections Impact Expression of Drug Transporters in Human Placenta

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Purpose: Several drug transporters are highly expressed in the human placenta. The ABC efflux transporters, ABCG2 (BCRP) and ABCB1 (PGP/MDR1), and SLC uptake transporter, SLCO2B1 (OATP2B1), are amongst key proteins that are involved in the transmembrane transport of endogenous substrates and xenobiotics in placenta. In turn, they are crucial in impacting fetal drug exposure during pregnancy. It has previously been shown that placental expression and activity of these transporters are decreased in rodents exposed to bacterial endotoxin. Our objective was to examine the expression in placental tissue isolated from human subjects diagnosed with chorioamnionitis, a bacterial infection of the placental chorion and amnion.
Methods: Human placental samples were collected from preterm and term pregnancies diagnosed with chorioamnionitis and were gestational age-matched with samples from pregnancies with no obstetric complications using predefined exclusion criteria. Protein expression was quantified on Western blots using antibodies specific for ABCG2 (BXP-21), ABCB1 (F4) and SLCO2B1 while transporter and cytokine mRNA was measured via real-time qPCR.
Results: mRNA levels of pro-inflammatory cytokines IL-6, IL-1β and TNF-α were profoundly induced by 2.5- to 3-fold in preterm placentas of women with chorioamnionitis, relative to preterm controls ($p < 0.05$). Gene expression of ABCG2 and SLCO2B1 was downregulated by 48 to 57 % ($p < 0.05$) in placentas obtained from infected preterm pregnancies, as compared to preterm healthy controls. Protein expression changes were generally consistent with mRNA data. At term, ABCG2 mRNA and SLCO2B1 protein expression levels were significantly downregulated, relative to

controls. Changes in ABCB1 and SLCO4A1 expression did not reach statistical significance, however ABCB1 gene levels strongly correlated with IL-6, IL-1β and TNF-α expression ($p < 0.001$), possibly suggesting involvement of cytokine-mediated regulation.
Conclusion: Maternal infections impose significant changes in the expression of key placental drug transporters. The data suggests that materno-fetal drug transport may be altered by changes in the expression of placental ABC and OATP transporters. Further studies are needed to determine the underlying mechanisms involved in the regulation of placental transporters during infection.

17. Analysis of Common Polymorphisms in the hKCNE4 Potassium Channel β-Subunit Gene

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Purpose: Voltage-gated potassium channels (Kv) are macromolecular complexes composed of the pore-forming (β -subunits) and ancillary subunits (β-subunits). Ubiquitously expressed in excitable tissues, the *KCNE* genes (*KCNE1-5*) encode promiscuous β-subunits which exert tissue-specific effects on Kv channel function. Two genes encoding Kv β- subunits, *KCNA1* and *KCNQ1*, are known channelopathy genes where genetic mutations can cause a variety of excitability disorders such as cardiac arrhythmia, epilepsy, hypothyroidism and diabetes, at times in combination in the same patient. These comorbidities coupled with the known complexity in personal "channotypes" implicate functionally-associated genes as disease modifiers. *KCNE4* is an inhibitory subunit of Kv channels when co-expressed *in vitro*. Therefore, single mutations in *KCNE4* alone, or in combination with other Kv mutations, present an intriguing molecular candidate for phenotype modulation.
Methods: Population genome databases were queried to identify potentially pathogenic common polymorphic (MAF>0.1) gene variants in *KCNE4*. The full length human KCNE4 gene was subcloned into a pcDNA3.1-GFP expression plasmid. Missense mutations were introduced into hKCNE4 via site directed mutagenesis. Positive ampicillin resistant clones were isolated, sequence validated and propagated for plasmid purification. The resulting mutant plasmid combinations were coexpressed in HEK293 cells along with wild-type and mutant *KCNA1* and *KCNQ1* subunits. Channel biogenesis was monitored with fluorescent microscopy followed by semi-quantitative Western blot analysis. Patch-clamp electrophysiology will be used to functionally characterize biophysical properties.
Results: Population analysis revealed 30 known missense mutations of which three were selected for mutagenesis: L70Q, E141A, and D145E in human *KCNE4*. These mutations are located in highly conserved regions located at the protein:protein interface shared by α and β subunits. Characterization experiments have revealed mutation-specific effects and unanticipated interactions within Kv channels containing >1 mutation.
Conclusions: These studies provide mechanistic insight into how mutations within the *KCNE4* gene differentially modulate channel function, depending on the tissue-specific β -subunit partner. Ultimately this will ultimately address the question of whether compound polymorphisms in the same macromolecular complex can fully explain the intermittent manifestation of clinical comorbidities. These studies will be used to elucidate the genetic basis of pathophysiology in excitability disorders and to identify novel drug targets for therapeutic intervention.

Clinical Sciences & Pharmacy Practice

18. Systematic Review of Pharmacist-led Interventions for the Identification and Prevention of Medication Misuse, Divergence, and Overdose

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Purpose: Medication misuse is a common problem in the community pharmacy setting and a formidable obstacle in providing safe, effective drug therapy. Despite the prevalence of this issue, there are few high quality studies available in the literature. We

aim to systematically review the literature to identify studies that have examined interventions executed by a pharmacist for identifying and intervening on patients at risk of medication misuse, divergence, and overdose.

Methods: PUBMED, MEDLINE, EMBASE, Psych Info, and IPA were searched for English language studies published from inception to June 2014 that investigated interventions carried out by a pharmacist for identifying and intervening on patients at risk of medication misuse. Studies of any design were eligible for inclusion. Two reviewers assessed abstracts and full articles to identify relevant literature.

Results: Of 308 unique articles identified, 31 studies were eligible for inclusion. Prescription drug monitoring programs have been investigated as tools to reduce medication misuse in four studies. Three of the four PDMP focused studies that were reviewed showed that while they may be effective in identifying patients at risk of misusing medications, they have not been shown to significantly reduce medication misuse or overdose mortality. Drug package size reductions were recommended to reduce overdose efficacy and deaths in three articles. Harm reduction services and pharmacy staff-patient relationships were identified in six of the studies as critical to preventing medication misuse. Five studies evaluated the community pharmacist's impact on prevention and identified their role as a positive influence on patient care and patient education. While public respect for pharmacists is high, pharmacists were identified as having negative opinions and views of the pharmacist-physician relationship. Pharmacists also have a negative perception of their ability to intervene on patients suspected of abusing or misusing medications, citing their lack of education or training as a barrier.

Conclusions: Data support the role for community pharmacists to help reduce gaps in care for patients at risk for medication misuse, divergence, and overdose. However, pharmacists themselves have identified a lack of education in dealing with patients that misuse drugs as a hindrance to patient communication and their interventions. In order to effectively reduce the scale of this issue, more research is needed to develop effective and feasible strategies for pharmacists to perform these interventions and relations between members of the health care team and their perceptions of each other must be improved for the benefit of the patient.

19. Olanzapine for Treatment and Prevention of Acute Chemotherapy-induced Vomiting in Children: A Retrospective, Multi-center Review

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Purpose: This retrospective review provides preliminary data regarding the safety and efficacy of olanzapine for chemotherapy-induced vomiting (CIV) control in children.

Methods: Children <18 years old who received olanzapine for acute chemotherapy-induced nausea and vomiting (CINV) control from December 2010 to August 2013 at 4 institutions were identified. Patient characteristics, chemotherapy, antiemetic prophylaxis, olanzapine dosing, CIV control, liver function test results and adverse events were abstracted from the health record. Toxicity was graded using CTCAEv4.03.

Results: Sixty children (median age 13.2 years; range: 3.10-17.96) received olanzapine during 158 chemotherapy blocks. Olanzapine was most often (59%) initiated due to a history of poorly controlled CINV. The mean initial olanzapine dose was 0.1mg/kg/dose (range: 0.026-0.256). Most children who received olanzapine beginning on the first day of the chemotherapy block experienced complete CIV control throughout the acute phase (83/128; 65%). There was no association between the olanzapine dose/kg and complete CIV control (OR 1.01; 95% CI: 0.999 to 1.020; p=0.091). Sedation was reported in 7% of chemotherapy blocks and was significantly associated with increasing olanzapine dose (OR: 1.17; 95% CI: 1.08 to 1.27; p=0.0001). Of the 25 chemotherapy blocks where ALT and/or AST were reported more than once, grade 1-3 elevations were observed in 5. The mean weight change in 31 children who received olanzapine during more than one chemotherapy block was 0% (range: -22 to +18).

Conclusion: Olanzapine may be an important option to improve CIV control in children. Prospective controlled evaluation of olanzapine for CINV prophylaxis in children is warranted.

20. Preliminary Results on the Impact of Genetic Factors on Gastrointestinal Bleeding in a Prospective Cohort of New Warfarin Users

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Purpose: Warfarin, a commonly prescribed oral anticoagulant, is well known for its narrow therapeutic index. Warfarin causes a third of emergency hospitalization due to adverse events in the elderly. Most of these complications are major gastrointestinal (GI) bleeds. Our goal was to investigate the potential risk factors of warfarin-related minor and major GI bleeding events and to examine their potential differential impact according to patient's genetic profile.
Methods: This study was based on a prospective cohort of new warfarin-users the objectives were to assess the genetic, clinical and environmental risks associated with the effectiveness and safety of warfarin. Data was collected on 1069 patients who began the treatment between May 1st, 2010 and July 31st, 2013. Patients were followed-up each three months for a year. The primary outcomes were the occurrence of a first minor or major GI bleed. We used a Cox regression analysis.
Results: Mean age was 70.8, 61.8% of patients were men, 68.4% had a history of hypertension and 60.8% of dyslipidemia and 76.4% had atrial fibrillation as a primary indication for warfarin. Overall, 4.8% of patients reported ≥1 minor GI bleed and 1.6% reported ≥1 major GI bleed. Patients with ≥1 polymorphism on both the CYP2C9 and the VKORC1 were significantly more at risk of having a major GI bleed (HR 10.72; p=0.023). Patients with a history of MI or angina were at risk of having both minor and major GI bleeds (HR=1.73 and HR=2.63; $p<0.05$). The impact of MI and angina disappeared for patients with no SNP on the CYP2C9 gene but was higher for patients with ≥1 SNP (HR=2.44 and HR=5.26, $p<0.05$).
Conclusions: Our preliminary results suggest an interaction between MI or angina history and , the occurrence of major and minor GI bleeds, especially in patients with ≥1 polymorphism on the CYP2C9 gene. Further analysis including concomitant use would help clarify clinical guidelines for this population and underlie the potential benefit of genetic testings.

21. Comparative Efficacy of Esomeprazole and Omeprazole: A Case of Racemate to Single Enantiomer Switching

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Purpose: The stereochemical differences in drug action and disposition have long been used to justify the development of single enantiomers of already available racemates as new drugs, often, without meaningful therapeutic advantage. One such example is esomeprazole, the S-isomer of racemic omeprazole. At cellular level, both S and R-isomers convert to the same inhibitor of H+/K+/ATPase producing the same level of acid suppression, hence they are equipotent. The results of the randomised controlled clinical trials comparing efficacy of these two drugs are contradictory, hence, they have been criticized particularly for comparing unequal therapeutic doses. Now that omeprazole and esomeprazole have been used for more than a decade, a logical comparison of these drugs is warranted to justify the rationale of switching from an inexpensive generic racemate to a costly branded single enantiomer. The aim of the present systematic review is to present the result of our test of the hypothesis that esomeprazole is superior to racemic omeprazole if used in equal therapeutic doses.
Methods: A web search was carried out for randomized controlled trials with head to head comparisons of omeprazole and esomeprazole in equivalent daily doses in an adult population (>18 years age). The data was abstracted and odd ratios were calculated for outcomes categorised as therapeutic cure, symptomatic relief and pH control. Then individual OR were combined together to assess the overall effect (combined odd ratio, OR') employing the random effect inverse variance method. Omeprazole was assigned as the reference (OR'=1). The Review Manager software as recommended by Cochrane was used for analysis.
Results: Out of 1171 studies, 14 were deemed

eligible. In terms of therapeutic cure outcomes [OR', 1.06; 95% CI, 0.83, 1.36; p = 0.63] and intragastric pH control [OR', 1.13; 95% CI, 0.85, 1.51; p = 0.40] there were no significant differences between the two products. However, for the relief of gastroesophageal reflux disease, S-omeprazole was found significantly but marginally more effective than omeprazole [OR', 1.14; 95% CI, 1.01, 1.29; p = 0.03].
Conclusion: The therapeutic benefit of the single enantiomer of omeprazole (esomeprazole) over racemic omeprazole is questionable.

22. Risk of Adverse Event and Survival Following Generic Valsartan Substitution

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Purpose: Once the patent has expired, brand name drugs such as Diovan® (valsartan) can be substituted with generic analogs in pharmacies. Few studies have assessed the clinical impact of this substitution on outcomes. We aimed to evaluate the impact of generic substitution on adverse events (hospitalization, emergency consultation and death of any causes) among Diovan® consumers.
Methods: Between February 2009 and 2011, a cohort of Diovan® users among newly diagnosed coronary heart disease or heart failure patients aged > 65 years was constituted with data from the Quebec Integrated Chronic Disease Surveillance System. Exposition to substitution was determined by the date when Diovan® was substituted to any of its generic versions. A fictive date of exposition was randomly assigned to Diovan® users without substitution (unexposed) by matching. The outcome of adverse event 60 and 365 days following the exposition date (or fictive date) was compared between groups using a logistic regression and Cox regression models, both adjusted for age, sex, antipsychotics concomitant treatment and disease severity.
Results: This cohort (n=1,467) was constituted of 895 women and 572 men mostly aged between 75-84 years at exposition. Among them, 96/791 exposed (12.1%) and 96/676 unexposed (14.2%) had an adverse event within 60 days following substitution. There was no significant association for the combination of adverse events 60 days following exposition for exposed subjects as seen by global odds ratio (OR: 0.82; p=0.20), hospitalization (OR: 0.75; p=0.28), emergency consultation (OR: 0.85; p=0.31) and death (OR: 0.48; p=0.32). A total of 1209 subjects were evaluated 365 days post-exposition and OR were also not significant, globally (OR: 0.90; p=0.37), hospitalization (OR: 0.95; p=0.76), emergency consultation (OR: 0.92; p=0.49) and death (OR: 0.62; p=0.11). Survival analysis 365 days following exposition revealed a global hazard ratio (HR) of 0.91 for exposed subjects (p=0.31), itemized as follows: hospitalization (HR: 0.95; p=0.73), emergency consultation (HR: 0.93; p=0.42) and death (HR: 0.63; p=0.11).
Conclusion: Generic substitutions of Diovan® do not increase the risk and delay before an adverse event among elderly newly diagnosed with heart failure or coronary heart disease.

Drug Delivery & Pharmaceutical Technology

23. Assessing the Role of HIF1alpha as a Target for Overcoming Drug Resistance in Breast Cancer

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Purpose: The long-term objective of this study is to overcome hypoxia induced drug resistance in breast cancer. For this purpose, we have developed a drug resistant model of MDA-MB-231 cells (a triple negative metastatic breast cancer cell line) through its culture under hypoxic condition. The potential role of hypoxic inducible factor alpha 1 expression as a target for overcoming hypoxia-induced resistance to cisplatin (an anticancer drug) in this cell line was then investigated.

Methods: MDA-MB-231 cells were maintained in RPMI medium (supplemented with 10% FCS, 100 IU/mL penicillin and 100 mg/mL streptomycin) either under normoxia (20% oxygen) or hypoxia (1% oxygen) at 37 °C. Viability of cells were measured by MTT assay and Flow Cytometric analysis using propidium iodide and FITC Annexin V staining for cells which were treated with different concentration of cisplatin under normoxia and hypoxia for different length of incubation. Expression level of HIF-1α protein was analyzed by transcription factor assay kit and immunoblotting under normoxic and hypoxic condition for different incubation times. Small interfering RNA (siRNA) techniques were used to knockdown HIF-1alpha expression in response to hypoxia. The effect of this treatment on the cytotoxicity and apoptosis induction by cisplatin under normoxic and hypoxic condition was assessed.
Results: Significantly higher level of HIF-1α expression was measured under hypoxia comparing to normoxia in MDA-MB-231 cells. Treatment of MDA-MB-231 cells with cisplatin (1-100 μg/mL) under hypoxia led to a higher cellular viability comparing cells treated with cisplatin at similar concentrations under normoxia. Successful knockdown of HIF-1alpha expression by lipofectamine 2000 or PEO-(PCL-SP) HIF1a-siRNA complexes (the latter is developed in our lab) didn't translate to lower cellular viability or induction of apoptosis after drug exposure under hypoxic or normoxic condition.
Conclusion: Treatment of cells with cisplatin under hypoxia induced drug resistance and resulted in lower level of cellular toxicity comparing to the cells which were treated under normoxia. Higher expression level of HIF-1α may play a role in hypoxia induced drug resistance.

24. Targeted Brain Delivery of siRNA using Dual-antibody Modified Chitosan Nanoparticles

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Purpose: Human immunodeficiency virus (HIV) is commonly known for its devastating effects on the immune system, leading to acquired immunodeficiency syndrome (AIDS) and the increased danger of opportunistic infections. The virus can also cause the development of several HIV-associated neurocognitive disorders (HAND) and it is estimated that nearly half of all adults with AIDS suffer from neurological complications related to HIV. Unfortunately, the blood brain barrier (BBB) is a major obstacle impeding effective treatment of HIV in the brain. The goal of this study was to develop and characterize a nanomedicine that can improve delivery of siRNA across the BBB and inhibit HIV-1 replication.
Methods: Nanoparticles were fabricated from chitosan and functionalized with antibodies against the transferrin receptor-1 on brain endothelia and insulin like growth factor receptor on astrocytic glial cell for the delivery of siRNA specific for SART3, a host gene important for HIV replication. Dynamic light scattering was used to determine particle size and zeta potential. Transmission electron microscopy (TEM) was performed to characterize the morphology of the nanoparticles with and without siRNA. In vitro cytotoxicity studies were evaluated in the human glioma cell line U138-MG and cell viability was determined using the MTS assay. Cellular uptake studies of the nanoparticles were performed in U138-MG cells over a 4 h period.
Results: Antibody-conjugated nanoparticles encapsulated with siRNA (siRNA-NP-Ab) had an average diameter of 215±13.6 nm and an siRNA encapsulation efficiency of 98.2±1.6%. TEM images indicate that the nanoparticles maintained a spherical structure even after siRNA encapsulation. After 24 h of incubation of our nanoparticle formulation with U138-MG cells, there was no significant decrease in cell viability when compared to controls. Cellular uptake studies indicate a time-dependent increase in the amount of Cy3-siRNA-NP-Ab taken up by U138-MG cells over a 4 h period. Our nanoparticle formulation significantly enhanced Cy3-siRNA uptake after 2 h and 4 h in comparison to Cy3-siRNA delivered by the commercially available transfection reagent Lipofectamine2000.
Conclusions: Overall, we have described a nanomedicine that is non-cytotoxic and can efficiently deliver siRNA into brain cells in vitro. This platform demonstrates potential utility for the prevention of HIV replication in the brain and for the treatment of other CNS disorders.
Acknowledgement: Jessica Adria is the University of Alberta recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

25. Biodegradable Film for the Targeted Delivery of siRNA-loaded Nanoparticles to Vaginal Immune Cells

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Purpose: Development of small interfering RNA (siRNA)-based vaginal microbicides is a promising approach for the treatment and prevention of sexually transmitted human immunodeficiency virus (HIV) infections. Synaptosome-associated 23-kDa protein (SNAP-23) is a SNARE protein located on the plasma membrane. Studies have shown that the absence of SNAP-23 expression on host cells results in defects with HIV-1 particle production. The goal of this study was to develop and characterize a novel intravaginal film platform for the delivery of SNAP-23 siRNA-loaded nanoparticles as a potential gene therapy for the prevention of sexually transmitted HIV infection.

Method: Poly (ethylene glycol) (PEG)-functionalized poly (D, L-lactic-co-glycolic acid) (PLGA)/polyethylenimine (PEI)/siRNA nanoparticles (siRNA-NP) were fabricated using a modified emulsion-solvent evaporation method. Nanoparticles were decorated with anti-HLA-DR antibody (siRNA-NP-Ab) for targeting delivery to HLA-DR+ dendritic cells (DCs) and homogeneously dispersed in a biodegradable film consisting of poly vinyl alcohol (PVA) and λ-carrageenan. We investigated the physicochemical properties of the siRNA-NP-Ab and siRNA-NP-Ab-loaded films (siRNA-NP-Ab-film) and determined its impact on the viability of epithelial and immune cells. Most importantly, we evaluated the ability of siRNA-NP-Ab released from film to penetrate epithelial cells and specifically target delivery into HLA-DR+ immune cells (HIV target cells) using a co-culture vaginal mucosa model. SNAP-23 knockdown efficiency was also determined at both mRNA and protein levels.

Results: The fabricated siRNA-NP-film was homogeneous, transparent, displayed suitable physico-mechanical properties, and was non-cytotoxic. Targeting activity was evaluated in a vaginal mucosal co-culture model consisting of a vaginal epithelial monolayer (VK2/E6E7 cells) and differentiated KG-1 cells (HLA-DR+ DCs). siRNA-NP-Ab was rapidly released from the film and was able to penetrate the epithelial layer to be taken up by KG-1 cells. siRNA-NP-Ab demonstrated higher targeting activity and significantly higher knockdown of SNAP-23 mRNA and protein when compared to siRNA-NP (without antibody conjugation). siSNAP-23-NP-film and siSNAP-23-NP-Ab-film achieved approximately 28.3±14.17% and 59.3±7.45% down-regulation of SNAP-23 mRNA with 200 nM siRNA after 48 h treatment. KG-1 cells treated with siSNAP-23-NP-film significantly knocked-down SNAP-23 protein expression by 21.6± 6.61% compared to non-treated controls, whereas mKG-1 cells treated with siSNAP-23-NP-Ab-film knocked down expression by 51.9 ± 6.82%.

Conclusion: These data suggest that our novel siRNA-NP-Ab-film may be a promising platform for preventing HIV infection within the female genital tract.

26. Preparation and Characterization of Solid Lipid Nanoparticles for Bioavailability Enhancement of Agomelatine

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Purpose: To enhance the bioavailability of Agomelatine (AGM) which is an antidepressant drug with an agonist action at the melatonergic MT1 and MT2 receptors combined with a 5HT2c antagonist effect. It has low absolute bioavailability caused by a high level of first-pass metabolism. Its elimination half-life is 2.3 hours. It is metabolized to a major extent (90%) by CYP1A2 and the rest by CYP2C9. It has no active metabolites and is excreted in urine. Solid lipid nanoparticles are lipid based colloidal carriers that have the potential to allow controlled drug release, drug targeting, increased drug stability and protecting labile drugs from degradation. Therefore SLNs can be used as drug carrier to enhance AGM bioavailability.

Methods: Solid lipid nanoparticles were prepared by ultrasonication technique using lipids (gelucire 01/43 and glyceryltripalmitate) and stabilizer (poly vinyl alcohol). Different formulations were prepared by changing the type of lipid, the concentration of lipid and the drug to lipid ratio. The prepared nanoparticles were characterized and evaluated. The in-vitro release was performed in phosphate buffer at

pH 6.8.

Results: The particles size, polydispersity index, zeta potential and entrapment efficiency of the prepared formulations were found to be 238.4 to 380 nm, 0.166 to 0.9, -12 to -19.7 mV and 64 % to 91 % respectively. Cumulative amount of drug released was found to be 38.5 % to 81.9 % after 8 hrs.

Conclusion: Solid lipid nanoparticles formulations were able to entrap a high amount of AGM with small particle size constituting a promising carrier to enhance bioavailability.

27. Polymeric Micelles for siRNA Delivery to Breast Cancer following Systemic Administration: The Effect of Lipid Modification on siRNA Delivery

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Purpose: Our objective was to study the effect of lipid modification in micellar nano-carriers on the delivery of siRNA to breast cancer.

Methods: Poly(ethylene oxide)-*block*-poly(ε-caprolactone) with grafted spermine (PEO-*b*-P(CL-g-SP)) were synthesized. The spermine group was substituted with cholesterol (PEO-*b*-P(CL-g-SP-Chol)). Block copolymers self-assembled to polymeric micelles. The effect of cholesterol substitution on the micellar size, siRNA binding, release, cellular uptake and cytotoxicity was investigated. MCL-1 siRNA was complexed in PEO-*b*-P(CL-g-SP) (MCL-SP) and PEO-*b*-P(CL-g-SP-Chol) (MCL-SP-Chol) micelles. The siRNA formulations were assessed for their cytotoxicity and MCL-1 mRNA down-regulation in MDA-MB-435 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and qPCR, respectively. Female nude mice bearing MDA-MB-435 xenografts were treated intratumorally with MCL-SP and MCL-SP-Chol and their counterparts containing scrambled siRNA. Tumor growth was followed and MCL-1 mRNA expression in tumor was measured 24h after the last injection. Tumor bearing mice were also treated intravenously with MCL-SP and MCL-SP-Chol with or without RGD4C peptide modification and assessed for tumor growth, MCL-1 mRNA expression, and major organ pathology.

Results: Cholesterol modification of PEO-*b*-P(CL-*g*-SP) enhanced the siRNA uptake and the transfection efficiency of MCL-1 siRNA complexes in MDA-MB-435 cells leading to 2.5-fold reduction in cell viability. Following intratumoral administration, MCL-SP and MCL-SP-Chol treated animals showed 34% and 39% down-regulation in relative MCL-1 mRNA expression in tumor compared to scrambled siRNA (no down-regulation). Both MCL-SP and MCL-SP-Chol, showed delayed tumor growth in mice compared to the vehicle or scrambled siRNA formulations. On day 10, MCL-SP and MCL-SP-Chol treated mice showed 3.5- and 5.4-fold decrease in tumor volume compared to animals receiving vehicle. Following intravenous administration, MCL-SP and MCL-SP-Chol with RGD4C on their surface showed 30 and 38% down-regulation in relative mRNA expression as compared to 15 and 4% for micelles without RGD4C, respectively. Despite better silencing of MCL-1 for RGD4C modified micelles, similar inhibition in tumor growth was observed for both siRNA complexes with or without RGD4C modification. On day 13, MCL-SP and MCL-SP-Chol with RGD4C modification showed a 1.8- and 1.7-fold decrease in tumor volume, as compared to 1.7- and 1.5-fold decrease for groups without RGD4C modification, respectively. Histology studies showed no signs of toxicity in liver, spleen, and kidney for all treatments.

Conclusion: Cholesteryl-modified nano-carriers showed better transfection efficiency of complexed siRNA *in vitro* and following intratumoral administration *in vivo*. Cholesteryl modification, however, did not affect the transfection efficiency of complexed siRNA by plain or RGD4C targeted nano-carriers in MDA-MB-435 tumors following systemic administration.

28. Traceable Nanocarriers for Targeted Therapy of Primary and Metastatic Breast Cancer

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Purpose: The objective of this study was to: i) develop traceable polymeric micelles using chemical conjugation of a near infra-red (NIR) dye to the block copolymers; and ii) investigate the effect of core as well as shell structure on breast cancer targeting of polymeric micelles using NIR imaging.
Methods: Block copolymers of poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) and PEO-*b*-poly(α-benzyl-carboxylate-ε-caprolactone) (PEO-*b*-PBCL) were synthesized. The polymers were modified at their hydrophobic end with a linker, i.e., α-propargyl carboxylate-ε-caprolactone (PCC). Click chemistry was used to react PCC with cy5.5-azide leading to the production of cy5.5-conjugated PEO-*b*-PCL and PEO-*b*-PBCL block copolymers. Acetal-PEO-*b*-PCL and acetal-PEO-*b*-PBCL were also synthesized and used to attach breast cancer targeting P18-4 peptide on the micellar shell at different densities (0.1 and 0.2 mole/mole of peptide/polymer). Cy5.5-labeled PEO-*b*-PCL or cy5.5-labeled PEO-*b*-PBCL were mixed with P18-4-modified PEO-*b*-PCL or P18-4-modified PEO-*b*-PBCL at molar ratios of 0.084 and 0.053, respectively. Mixed micelles were formed using a co-solvent evaporation method and analyzed for their size, CMC, and uptake into human MDA-MB-231 breast cancer cells and MCF10A breast epithelial cells at 4 and 24h incubation using flow cytometry. Confocal microscopy was used to characterize micellar internalization into MDA-MB-231 cells.
Results: ^{1}H NMR spectroscopy confirmed successful synthesis of PEO_{114}-*b*-PCL_{7}-PCC and PEO_{114}-*b*-$PBCL_{7}$-PCC, respectively. Cy5.5 was successfully conjugated to the PCC end group of PEO-*b*-PCL and PEO-*b*-PBCL block copolymers with an efficiency of 34.75% and 52.56%, respectively. P18-4 was conjugated to acetal-PEO-*b*-PCL and acetal-PEO-*b*-PBCL polymers with a conjugation efficiency of 100% at both densities. PEO-*b*-PBCL micelles exhibited smaller size and a 7-fold decrease in CMC compared to PEO-*b*-PCL indicating greater thermodynamic stability. Results from flow cytometry showed higher uptake of PEO-*b*-PCL micelles as compared to PEO-*b*-PBCL micelles in both MDA-MB-231 and MCF10A cells. Peptide conjugation increased cell uptake into MDA-MB-231 cancer cells for both peptide densities with 0.2 mole/mole density showing significantly higher cell uptake. However, this density also showed higher non-specific uptake in MCF10A cells as compared to 0.1 mole/mole density which showed decreased uptake in MCF10A cells. Confocal microscopy confirmed similar pattern of cell internalization to the cell association data obtained by flow cytometry.
Conclusion: These findings show the potential of NIR labeled polymeric micelles in providing further information towards development of polymeric micelles with optimized properties for targeted delivery to breast cancer. The *in vivo* imaging of the developed polymeric micelles in MDA-MB-231 xenograft mice models is currently underway.

29. Taguchi Design for the Preparation and Optimization of Poly (lactide-co-glycolide) (PLGA) Nanoparticles for Intravenous Delivery of Docetaxel

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Purpose: Cancer is the leading cause of death in Canada. Today, common and conventional methods to treat cancer offer good means to fight off the disease, but they are not without drawbacks. An example is the issues accompanied with cancer chemotherapy. Docetaxel is a potent anticancer agent used in various cancer types. There are concerns regarding its pharmacokinetics after administration of conventional docetaxel formulation. To date, nano-particulate drug carriers as a category of novel drug delivery systems (NDDs) have been invented to address such issues in chemotherapy. On the other hand, Poly (lactide-co-glycolide) (PLGA), a biocompatible/biodegradable polymer with FDA-approved application in human gives polymeric nanoparticles with unique characteristics that can potentially modify pharmacokinetics/pharmacodynamics of docetaxel in cancer patients. Accordingly, there are different nanoparticle characteristics that can affect the fate of nanoparticle/drug in the systemic circulation. These characteristics are further controlled by factors attributed to nanoparticle preparation technique and factors attributed to the PLGA polymer itself. Here in, the purpose of the study is to evaluate the effect of different factors on particle-important characteristics as a mean to determine factor(s) that significantly affect nanoparticles and optimize nanoparticle preparation to get nanoparticles with properties that best match their future application in the body.
Method: In this study, Taguchi robust fractional

factorial design was used to evaluate the effect of different variables on particle-important characteristics. A modified method of emulsification-solvent-evaporation was used to fabricate nanoparticles. Six relevant factors were selected as the main determinants of particle-important characteristics (table 1). The first two factors were evaluated at 4 levels and the rest at 2 levels. Different combinations of factors/levels were considered to prepare nanoparticles based on L16 Taguchi design and particle-important characteristics including size, poly dispersity index (PDI), surface electric charge (zeta potential), and loading efficiency of the method were further determined. Analysis of the data was done by running different statistical tests on obtained data.

Results: The simple and efficient approach used throughout the study allowed us to rank factors based on their level of effectiveness on particle-important characteristics. Also, the factors that could affect nanoparticle properties with statistical significance were determined. Attempts to fit models to explain different nanoparticle characteristics resulted in models with good robustness and acceptable significance.

Conclusion: it is now possible to predict particle characteristics even from combinations of factors/levels that have not been experimentally studied. Optimized preparation condition(s) for an intravenous sustained-release nanoparticle formulation is now achievable.

		Level			
Code	Factor	1	2	3	4
A	Drug Concentration (mg/ml)	0.25	0.5	1	1.5
B	Organic/Aqueous phase ratio	1:2	1:3	1:4	1:5
C	Polymer Molecular weight (dL/g)	0.15 - 0.25	0.55 - 0.75		
D	Polymer Terminus	Acid	Ester		
E	Lactide:Glycolide Ratio	50:50	75:25		
F	PVA Concentration	2.2%	9%		

Table 1. Variables (Factors) along with their corresponding values and trial levels in the Taguchi experimental design used in fabrication of docetaxel-loaded PLGA nanoparticles through emulsification solvent evaporation technique.

30. Polymeric Nano-micelles for Delivery of STAT3 Inhibitors to Multiple Myeloma

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Purpose: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is constitutively activated in many types of human cancer including multiple myeloma (MM), for which there is currently no cure. Despite the universal acceptance of STAT3 inhibition as a promising strategy in cancer treatment, STAT3 inhibition has yet to be successfully translated to clinical settings. This is mostly due to toxicity and inefficient delivery of STAT3 inhibitors to tumor cells. S3I-201 and its derivative S3I-1757 are effective inhibitors of STAT3 dimerization that have shown activity against MM, but their further development to drug candidates has been hampered due to their low water solubility and poor tumor selectivity. The aim of this research was to design and develop polymeric micellar nano-formulations for delivery of these STAT3 inhibitors to MM tumors. Polymeric micelles have shown promise in solubilisation and tumor targeted delivery of poorly water soluble drugs.

Methods: Diblock copolymers of poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL_X) or PEO-*b*-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-*b*-$PBCL_Y$) having similar degree of polymerization in PEO segment (114 ethylene oxide units) and different degrees of polymerization in the PCL (X= 22, 44, 66) and PBCL (Y = 15, 20, 30) were synthesized by ring-opening polymerization. S3I-201 and its derivative, i.e., S3I-1757 were encapsulated via co-solvent evaporation in the PEO-*b*-PCL or PEO-*b*-PBCL block copolymer micelles. Drug-loaded micelles were characterized for their size, encapsulation efficiency, drug release profile, and cytotoxicity against human U266 MM cell line.

Results: PEO-*b*-PCL and PEO-*b*-PBCL block copolymers were successfully synthesized as evidenced by ^{1}H NMR. Block copolymers self-associated to form micelles in aqueous solution. Successful encapsulation of S3I-201 and particularly S3I-1757 in all micellar structures under study was evidenced by HPLC (~25 and 75% encapsulation

efficiency, respectively). Dynamic light scattering revealed micelle diameters of 30-70 nm. The release of S3I-1757 from polymeric micelles was slower than that of S3I-201 (~35% in 24 h versus ~100% in ≤ 8 hours, respectively) irrespective of polymer structure. Interestingly, despite slow drug release, significantly better cytotoxicity (lower IC_{50}) for S3I-1757-loaded in PEO_{114}-*b*-$PBCL_{15}$ (10.2 ± 0.7 mM) and PEO_{114}-*b*-$PBCL_{20}$ (11.0 ± 1.3 mM) than free S3I-1757 (13.1 ± 0.9 mM) was observed against human U266 cells, *in vitro*.

Conclusion: PEO-*b*-PCL and PEO-*b*-PBCL micelles are promising nano-delivery systems for STAT3 inhibitors S3I-201 and S3I-1757 delivery against MM.

31. Panitumumab Modified with Metal Chelating Polymers (MCPs) for Dual Labeling with ^{111}In and ^{177}Lu as a Potential Theranostic for Pancreatic Cancer

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Purpose: Our objective was to develop a novel "theranostic" agent that combines imaging of EGFR-positive pancreatic cancer (PnCa) with radio-immunotherapy (RIT). Anti-EGFR panitumumab (PmAb), was modified with hydrazino nicotinamide metal chelating polymers (HyNic-MCP) that harbor 13 DOTA chelators for high specific activity (SA) labeling with ^{111}In and ^{177}Lu and 10 PEG groups to minimize liver and spleen uptake. ^{111}In is a γ-emitter [$t_{1/2}$ = 2.8 days; $E\gamma_1$=171, 245 keV] useful for SPECT imaging and emits Auger electrons for RIT of *micrometastases*. ^{177}Lu is a β-emitter [Eβ (max) =498, 385 and 176 keV] useful for RIT of millimeter-sized tumours, and emits imageable γ-photons [$E\gamma_1$=113, 208 keV].

Methods: PmAb was reacted with *N-succinimidyl-4-formylbenzamide (S-4FB) to install aldehyde groups* for derivatization with HyNic-MCP which forms a UV-measureable (354 nm) bis-arylhydrazone bond. The reaction was terminated when two MCPs were conjugated to PmAb. Purity and MCP conjugation was confirmed by SDS-PAGE. PmAb-HyNic-MCP was labeled with $^{111}InCl_3$ or/and $^{177}LuCl_3$ in 0.1M HEPES pH 5.5. For comparison, PmAb was directly modified with two DOTA and labeled with ^{111}In and ^{177}Lu. Labeling efficiency was measured by ITLC-SG in 0.1 M sodium citrate. Stability was studied in the presence of a 500-fold excess of EDTA for 1 h at 25 °C and in plasma at 37°C for 1 week by size-exclusion HPLC and ultrafiltration. EGFR binding affinity was assessed in a saturation binding assay using EGFR-overexpressing MDA-MB-468 human breast cancer cells.

Results: The purity of PmAb-HyNic-MCP was >96%. Labeling yields with ^{111}In or ^{177}Lu were >96%. PmAb-HyNic-MCP and PmAb-DOTA were with both ^{111}In and ^{177}Lu in high yield (>96% and >89%, respectively). PmAb-HyNic-MCP was labeled with ^{111}In and ^{177}Lu to higher SA than PmAb-DOTA: 72.4 ± 2.0 and 48.0 ± 2.0 MBq/µg, vs. 6.6 ± 0.6 and 5.5 ± 0.6 MBq/µg, respectively. Radioimmunoconjugates were stable in plasma and to EDTA challenge. Binding to MDA-MB-468 cells revealed a dissociation constant (K_d) of 2.2 ± 0.6 nM and B_{max} of 0.6 ± 0.07 x 10^6 receptors/cell for ^{177}Lu-PmAb-HyNic-MCP vs. K_d of 1.0 ± 0.4 x 10^{-9} mol/L and B_{max} of 0.5 ± 0.07 x 10^6 sites/cell for ^{177}Lu-PmAb-DOTA.

Conclusions: Novel PmAb-HyNic-MCP immune-conjugates were constructed and labeled to high SA with ^{111}In and/or ^{177}Lu. ^{177}Lu-PmAb-HyNic-MCP exhibited preserved EGFR binding. Studies are planned to evaluate the biodistribution and imaging properties of these dual-labeled theranostic agents.

Research Support: This study was supported by Cancer Research Society

32. 2-In-1 Polymeric Micelles of Reparaxin and Paclitaxel for Targeting Cancer Stem Cells and Bulk Tumor Cells

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Purpose: Cancer stem cells (CSCs) play an important role in the development of drug resistance, metastasis and recurrence of cancers. Conventional therapies do not normally target CSCs, sometimes may even increase CSCs proliferation. We developed a 2-in-1 polymeric micelle delivery system (PMs) of reparaxin (RPX) and paclitaxel (PTX) to target both CSCs and non-CSCs, respectively.

Methods: Poly(ethylene glycol)-block-poly(D,L-lactic acid) PMs were prepared by lyophilization, and optimized for particle size, drug loading, and formulation stability. *In vitro* drug release was performed in PBS (pH 7.4) using a dialysis method. 3D-Mammosphere culture was developed for breast cancer cell line MCF-7 to enhance CSC population and characterized using flow cytometry. *In vitro* cytotoxicity of PMs was evaluated in both mammosphere cells and adherent cells using MTS assay. Effect of PMs on stemness of CSCs was also assessed using mammosphere formation assay.

Results: All PMs formulations were easily reconstitutable (≤1 min) in H_2O/PBS, and showed similar particle size of 23.2±1.2 nm with narrow PDI of 0.119±0.009. 2-in-1 micelles dissolved PTX and RPX in water at 6.40 mg/ml, and remained stable for 24 h at 25° C. *In vitro* drug release from 2-in-1 PMs represented first-order release kinetics, with drug release of PTX and RPX at 85.11±2.49% and 94.36±5.5% in 24 h, respectively. In adherent cell culture PMs demonstrated IC50 of 204±28 nM (PTX micelles) and 152±38 nM (2-in-1 micelles), indicating synergistic anticancer effect for the drug combination. In mammosphere cells 2-in-1 PMs demonstrated lower IC50 value (124±33 nM) than PTX micelles (223±41 nM), confirming improved efficacy of 2-in-1 PMs in decreasing CSCs viability *in vitro*. Treatment with 2-in-1 micelles also suppressed mammosphere formation by approximately 24-30% in comparison to PBS control.

Conclusion: 2-in-1 PMs significantly enhanced the aqueous solubility of PTX and RPX, and demonstrated formulation stability. They also possessed synergistic cytotoxic effect in both adherent and mammosphere cell cultures. This confirmed their anticancer efficacy in eliminating both CSCs and bulk tumor cells *in vitro*.

33. RNAi-Based Nanomicrobicide for the Efficient Intravaginal Gene Knockdown of CCR5 and Nef in CD4+ Immune Cells

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Purpose: The goal of this study is to develop and characterize a RNA interference (RNAi)-based nanomicrobicide for the delivery of small interfering RNAs (siRNAs) knocking down the host factor gene CCR5 and viral gene nef simultaneously as a pre-exposure prophylaxis to prevent the intravaginal transimission of HIV-1. This technology platform consists of siRNA-encapsulated nanoparticles (siRNA-NPs) loaded into a vaginal gel.

Methods: siRNAs targeting CCR5 and nef were first condensed by polyethyleneimine (PEI) and then co-encapsulated into NPs by double-emulsion evaporation method using the biodegradable polymer, poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG). Resulting NPs were then formulated into a 0.5% hydroxyethyl cellulose (HEC) vaginal gel.

Results: NPs showed a particle size of 256.6±8.3 nm and a zeta-potential (net surface charge) of -9.78±1.03 mV in pH 5.0 buffer. In pH 7.4 buffer, the NPs had a particle size of 246.3±10.2 nm and a zeta-potetial of -24.95±5.55 mV. The encapsulation efficiency of siRNA was 86.76±0.14%. NPs showed a pH-dependent release profile, with sustained release of siRNA under pH 7.4 (approximately 40% over 13 days) and minimal release of siRNA under pH 5.0 (less than 5% over 2 days). The siRNA-NPs were rapidly taken up by Sup-T1 cells (CD4+ immune cells). In 2 hr, approximately 40% of cells were siRNA+ and in 24 hr, 100% of cells were siRNA+. The siRNA-NPs were successfully formulated into a vaginal gel with approximately 17% of siRNA-NPs released within 24 hr. In a vaginal mucosal co-culture cell model (upper chamber comprising a vaginal epithelial cell layer and a lower chamber comprpising CD4+ T cells stably expressing the viral gene nef), the vaginal gel loaded with siRNA-NPs could efficiently knock down CCR5 (?% gene knockdown) and nef (>70% gene knockdown) in CD4+ T cells over 3 days after a 24 hr treatment.

Conclusions: We have developed a novel RNAi-based nanomicrobicide that can efficiently knock down CCR5 and nef simultaneously in CD4+ immune cells. The NPs have desirable particle size and zeta potential for intravaginal delivery and a pH-dependent release profile that can preserve siRNA under acidic environments. NPs are formulated into a gel dosage-form to provide ease in vaginal administration and retention within the female genital tract.

34. Development of a Reservoir-Type Intravaginal Ring for the Sustained Delivery of Hydroxchloroquine as a Potential Strategy for HIV Prevention

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Purpose: The goal of this study was to develop a novel intravaginal ring (IVR) drug delivery system for the controlled, sustained (>14 days) release of hydroxychloroquine (HCQ) as a novel strategy for the prevention of HIV-1 infection within the female genital tract.

Methods: Polyether urethane reservoir IVRs were fabricated by hot-melt injection molding. The IVR was loaded with HCQ for release studies at 37°C in pH 4 release medium for 2 weeks. Released HCQ was quantitated using reversed-phase high performance liquid chromatography. Accelerated stability studies were conducted at room temperature (RT) or at 40°C/75% relative humidity (RH) in an environmental chamber. *In vitro* biocompatibility was evaluated using the MTS cell viability assay, colony formation assay, and analysis of pro-inflammatory cytokine production in vaginal and ectocervical epithelial cells incubated with IVR elution medium up to 30 days.

Results: Reservoir IVR segments loaded with HCQ exhibited a near zero-order release profile with no burst release. The average release rate observed was 195.59 ± 24.96 µg (4.67 ± 0.59%) per day for 14 days. HCQ within the IVR segments were stable when incubated at RT or at 40°C/75% RH. The IVR segments had no significant impact on cell viability, pro-inflammatory cytokine production, or colony formation of vaginal and ectocervical epithelial cells.

Conclusion: This is the first study to fabricate and characterize a reservoir-type IVR for the controlled, sustained release of HCQ over 14 days. The IVR was non-cytotoxic in vitro and in vivo and may be a suitable platform for the prevention of HIV-1 transmission.

35. ^{177}Lu-labeled and Dual-receptor Targeted Radiation Nanomedicine for Simultaneous Targeting of HER2 and EGFR on Breast Cancer Cells

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Purpose: Our aim was to develop a dual-receptor-targeted radiation nanomedicine (^{177}Lu-DRT-AuNPs) for personalized treatment of breast cancer (BC). Since heterodimerization of EGFR with HER2 promotes cell growth and co-expression of EGFR and HER2 in BC cells is a negative prognostic marker, gold nanoparticles (AuNPs) were modified with trastuzumab or panitumumab to target both HER2 and EGFR, respectively. AuNPs were further modified with polymers to complex ^{177}Lu, which emits β-particles ($E\beta_{max}$=0.5 MeV (100%); half-life=6.7 d) suitable for radiation treatment of tumors.

Methods: Bispecific ^{177}Lu-DRT-AuNPs were constructed by linking polyethyleneglycol (PEG) polymers conjugated to trastuzumab and panitumumab or to ^{177}Lu-DOTA chelators to 15 nm AuNPs. Monospecific ^{177}Lu-panitumumab-AuNPs, ^{177}Lu-trastuzumab-AuNPs and non-targeted ^{177}Lu-NT-AuNPs (without antibody modification) were constructed for comparison. The terminus of the PEG incorporated lipoic-acid presenting two-thiols for strong bivalent linking via a gold-thiol bond. Dynamic light scattering (DLS) was used to determine the size of the AuNPs. Competition receptor binding and cell fractionation studies were used to study the binding and internalization of bispecific and monospecific AuNPs into MDA-MB-231-H2N (HER2+/EGFR+), MDA-MB-468 (HER2-/EGFR+) or BT-474 (HER2+/EGFR-) cells.

Results: The size of ^{177}Lu-DRT-AuNPs was 33.6±0.1 nm whereas unmodified AuNPs were 14.5±0.5 nm. ^{177}Lu-DRT-AuNPs bound specifically to both EGFR and/or HER2 on MDA-MB-231-H2N

cells, with 1.5-, 1.3- and 4.6-fold displacement by excess panitumumab, trastuzumab or both, respectively. ^{177}Lu-DRT-AuNPs bound specifically to EGFR and HER2 on MDA-MB-468 and BT-474 cells, respectively, with 12.4- and 4.5-fold displacement by panitumumab and trastuzumab. ^{177}Lu-panitumumab-AuNPs bound specifically to EGFR on MDA-MB-231-H2N and MDA-MB-468 cells with 5.2- and 7.7-fold decreased binding with excess panitumumab. There was no displacementt of binding to BT-474 cells by panitumumab. Similarly, ^{177}Lu-trastuzumab-AuNPs specifically bound HER2 on MDA-MB-231-H2N and BT-474 cells with 5.9- and 4.2-fold decreased binding caused by trastuzumab. There was no displacement of the binding to MDA-MB-468 cells by trastuzumab. No specific binding was observed to any cells treated with ^{177}Lu-NT-AuNPs. Cell fractionation revealed receptor-mediated internalization of ^{177}Lu-DRT-AuNPs in cells expressing HER2 and EGFR. There was 5.4- and 4.8-fold decreased internalization in the presence of excess panitumumab and trastuzumab, respectively in MDA-MB-231-H2N cells.

Conclusion: Bispecific ^{177}Lu-DRT-AuNPs specifically bind to both HER2 and EGFR in BC cells providing an opportunity to target radiation to tumors that co-express these receptors.

Acknowledgement: Supported by a grant from the Canadian Breast Cancer Foundation and a scholarship to S.Y. from the Terry Fox Foundation Strategic Initiative for Excellence in Radiation Research for the 21st Century at the CIHR.

36. Evaluating the Drug Release and In Vivo Biocompatibility of an Intravaginal Implantable Device in a Rabbit Model

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Purpose: Previously, we developed and characterized *in vitro,* a reservoir-type intravaginal ring for the sustained and controlled release of hydroxychloroquine (HCQ), an immunomodulatory and anti-HIV agent, as a strategy for preventing male-to-female transmission of HIV. The aim of the current study is to translate this knowledge towards the development of a novel implantable device for the delivery of HCQ using a rabbit model and assess its *in vivo* drug release profile and biocompatibility.

Methods: The reservoir-based polyurethane implantable devices were designed and fabricated via hot-melt injection molding. Each device was embedded with a RFID micro-transponder. The RFID micro-transponder was X-ray detectable and can be tracked real-time by a handheld RFID reader. HCQ was either pre-formulated in a semi-solid with a rate-controlling excipient at various weight ratios (5:1 or 50:1) or directly loaded in a buffered aqueous solution without the excipient. Each device was loaded with 60 mg of HCQ and non-invasively implanted at a distance of 8-10 cm in the vaginal tract of New Zealand White rabbit. Rabbit cervicovaginal lavage (CVL) was collected at pre-determined intervals. HCQ levels CVL was quantitated using reverse-phase high performance liquid chromatography (RP-HPLC). Pro-inflammatory cytokine production (IL-1β and IL-8) was evaluated in CVL using sandwich ELISA. The histological morphology of rabbit vaginal tissue was assessed via hematoxylin and eosin staining.

Results: X-ray analysis revealed that the implant device was well retained within the rabbit female genital tract for over 40 days. HCQ exhibited a near zero-order release profile in CVL from the medical device. HCQ release was modulated by varying the weight ratios of rate-controlling excipient to HCQ. Highest HCQ release was observed without the presence of the excipient. The release rate was approximately 10.67 μg/mL per day in CVL for 7 days. The CVL HCQ level was within reported concentrations required to induce T-cell immune quiescence and inhibit HIV replication. No significant differences were observed in rabbit vaginal tissue morphology or in the CVL levels of IL-1β and IL-8 among the naive (no implant), placebo (implant without HCQ), and the HCQ implant group over 30 days.

Conclusion: We have described a novel non-invasive implantable device for evaluating the intravaginal delivery of HCQ in rabbits. This system was non-cytotoxic and may be a suitable platform for the *in vivo* evaluation of other drug candidates for the prevention of sexually transmitted diseases.

37. ^{64}Cu-NOTA-pertuzumab F(ab')$_2$ Fragments, a Second-Generation Probe for PET Imaging of the Response of HER2-positive Breast Cancer to Trastuzumab (Herceptin)

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Purpose: SPECT/CT imaging with ^{111}In-BzDTPA-pertuzumab detected trastuzumab (Herceptin)-mediated HER2 downregulation in human breast cancer (BC) xenografts in mice and was correlated with a good response to treatment. This agent is now being studied in a Phase I/II clinical trial (PETRA; ClinicalTrials.gov identifier: NCT01805908) sponsored by the Ontario Institute for Cancer Research (OICR). Our objective was to develop a second-generation positron-emitting analogue for PET/CT imaging to provide greater sensitivity, more accurate radiotracer quantitation and a lower radiation absorbed dose. This agent is composed of F(ab')$_2$ fragments of pertuzumab modified with NOTA chelators that complex the positron-emitter, ^{64}Cu ($t_{1/2}$ = 12.7 h; $E\beta^+$ = 278 keV, 19%).
Methods: Pertuzumab F(ab')$_2$ were produced by proteolytic cleavage of pertuzumab IgG, then derivatized with S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (*p*-SCN-Bn-NOTA). The purity of NOTA-pertuzumab F(ab')$_2$ was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion high-performance liquid chromatography (SE-HPLC). NOTA-pertuzumab F(ab')$_2$ was radiolabeled with $^{64}CuCl_2$ in 0.1 M sodium acetate pH 5.5 (specific activity = 185 MBq/mg). Labeling efficiency was determined by instant thin layer chromatography-silica gel (ITLC-SG) in 0.1 M sodium citrate pH 5. An impure sample of the conjugation reaction was labeled with ^{64}Cu and analyzed by ITLC-SG to determine the number of NOTA per pertuzumab F(ab')$_2$. The binding affinity of ^{64}Cu-NOTA-pertuzumab F(ab')$_2$ was assessed in a saturation radioligand binding assay using HER2-overexpressing SK-BR-3 BC cells.
Results: The purity of NOTA-F(ab')$_2$ was >95%. There were 4.8 ± 2.1 NOTA per F(ab')$_2$. Labeling efficiency was 84.8 ± 8.7% and following purification by ultracentrifugation, the radiochemical purity was 94.2 ± 2.0%. HER2 binding assays revealed a dissociation constant (K_d) of 2.6 ± 0.3 nM and B_{max} of $0.95 \pm 0.28 \times 10^6$ receptors/cell.
Conclusion: ^{64}Cu-NOTA-pertuzumab F(ab')$_2$ fragments exhibited strong HER2 binding. Evaluation of the ability of ^{64}Cu-NOTA-pertuzumab F(ab')$_2$ to image HER2-positive tumours and detect trastuzumab-mediated HER2 downregulation is in progress.
Acknowledgement: Supported by grants from the OICR Smarter Imaging and High Impact Clinical Trials (HICT) Programs.

38. Dermal Delivery of Terbinafine Hydrochloride Nano Vesicular Chitosan Gel: *in vitro* Characterization, *ex vivo* Permeation and Clinical Investigation

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Purpose: The aim of the current study is to evaluate the potential of Terbinafine hydrochloride containing vesicles in topical delivery.
Methods: Terbinafine hydrochloride loaded vesicles were prepared with different penetration enhancers and studied for their effect on physical properties as well as skin delivery of Terbinafine HCl. Penetration enhancer-containing vesicles (PEVs) were formulated with labrasol, transcutol, terpenes like cineole and limonene in addition to ethanol and compared with conventional liposomes. Physical characters as size, surface charge, drug loading and deformation index were investigated. Moreover, vesicles were evaluated for ex-vivo dermal delivery, skin imaging and irritation studies. Furthermore, *in vivo* animal and clinical studies were performed for selected formulae in chitosan gel.
Results: Results showed the formation of stable nearly spherical vesicles with size range from 95-500 nm and zeta potential range (10 to 15) mv. EE% ranged from 30-96%. Prepared vesicles showed no irritation on rat skin. PEVs prepared with 4% limonene showed superior results in *ex-vivo* permeation studies. Moreover, those vesicles in chitosan gel showed best results in term of reduction

of fungal burden in animal study. PEVs prepared with limonene were able to clinically cure subjects with tinea pedis within one week compared to market product which required from two to six weeks for complete cure.

Conclusion: Based on the obtained results it can be concluded that limonene nano vesicular chitosan gel is a potential approach for the topical delivery of Terbinafine hydrochloride.

39. Microgel-Facilitated Drug Delivery to the Brain

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Purpose: Developing treatments for central nervous system (CNS) disorders is challenging as it requires an effective means for crossing the blood-brain barrier (BBB). Nanoparticle-based therapies are of significant interest as they can effectively reach specific targets, control drug release and lower the required drug dosage for therapeutic intervention. Microgels, hydrogel-based nanoparticles, composed of poly(oligoethylene glycol methacrylate) (POEGMA) are promising drug delivery vehicles to the BBB as they can improve drug loading and biodistribution of the hydrophilic drug 3(R)-[(2(S)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide) (PAOPA).

Methods: Precipitation polymerization of diethylene glycol methacrylate, acrylic acid (AA) and 2-hydroxyethyl disulfide dimethacrylate (crosslinker) with various concentrations of sodium dodecyl sulfate (SDS) was used to prepare POEGMA microgels. Carbodiimide chemistry was used to functionalize the AA groups with *Solanum tuberosum* lectin (STL) to enable binding to the nasal epithelium for intranasal delivery. Microgel characterisation was carried out using light scattering (size), transmission electron microscopy (morphology) and conductometric titration (functional group content). Microgels were passively loaded with PAOPA, with excess drug removed using centrifugation. The *in vitro* PAOPA release was assessed using HPLC, and microgel cytotoxicity was examined using an MTT assay with RPMI 2650 nasal septum and SH-SY5Y dopamine 2D receptor-transduced neuronal cells. To assess the *in vivo* biodistribution, rhodamine-labelled microgels were injected into the peritoneum of male Sprague-Dawley rats. Blood and tissues were recovered one hour after injection and analyzed using fluorescence to quantify microgel biodistribution.

Results: Three batches of microgels were prepared by altering the concentration of SDS used: microgels with sizes of 99±2 nm, 152±3 nm, and 249±4 nm were prepared with low polydipsersity (<0.1). PAOPA was loaded with an encapsulation efficiency of 30±2%, independent of microgel size. The diffusion of PAOPA from the two smaller microgels occurs over 4 days while the largest microgel prolongs release to 5 days. The *in vitro* cytotoxicity assays revealed that the microgels had no appreciable toxicity (>85% cell viability) to the neuronal and nasal septum cells. The biodistribution studies with the rhodamine-labelled microgels demonstrated that the smallest microgels were the most effective at crossing the BBB; however, the largest microgels were transported in significant numbers across the BBB and accumulated less in major non-target organs.

Conclusion: POEGMA-based microgels are effective delivery vehicles for hydrophilic drugs to the brain. Due to their elastic nature, larger microgels may be advantageous for the transport of drugs across the BBB as the compressibility may enable the delivery of bigger particles across tight biological barriers.

40. Heat-triggered Release of Cisplatin from Thermosensitive Liposomes Improves Drug Efficacy in Models of Human Triple Negative Breast Cancer

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Purpose: Liposomes are a well-established system for delivery of chemotherapy to solid tumours. They frequently afford improvements in systemic toxicity; however, efficacy is often limited by poor availability of drug owing to slow and/or limited drug release at the tumour site. The research presented herein uses mild hyperthermia to trigger release of cisplatin (CDDP) from thermosensitive

liposomes as they pass through tumour vasculature. The efficacy of this heat-activated thermosensitive liposomal CDDP (HTLC) formulation is compared to free CDDP in mice in two human TNBC orthotopic tumour models that have significantly different sensitivities to CDDP.

Methods: HTLC containing a molar ratio of 57:29:10:4 DPPC:DPPG:MSPC:DSPE-PEG_{2000} was prepared using thin film hydration and high pressure extrusion. The sensitivity of both cell lines to CDDP was determined using the acid phosphatase metabolic assay. Efficacy studies were conducted in female SCID mice that were inoculated in the right inguinal mammary fat pad with $2x10^6$ MDA-MB-231 or $4x10^6$ MDA-MB-436 cells. When tumours reached ~100 mm^3 in volume, mice were administered saline, free CDDP, or HTLC as an intravenous bolus dose at the MTD for each formulation. The tumours of half the mice in each treatment group were heated to 42.5°C for 5 min prior to drug administration and 20 min afterwards using a 763 nm laser illuminated uniformly over the tumour surface. Temperature in the centre of the tumour was measured using a fibre optic probe. Tumour growth was measured using calipers while drug tolerance was determined by changes in body weight and behaviour.

Results: HTLC was prepared at a lipid concentration of 60 mg/mL and contained 1 mg/mL CDDP. MDA-MB-231 cells had an IC50 of 19 ± 5 µM while MDA-MB-436 cells were more sensitive with an IC50 of 0.53 ± 0.12 µM. Thermometry confirmed that the temperature in the centre of the tumour was maintained between 42–43°C over the 25 min heating period. Similar weight loss was observed in both treatment groups and was not affected by tumour heating. In both tumour models, HTLC + heat resulted in statistically significant ($p < 0.05$) differences in tumour volume compared to all other treatments, as well as statistically significant improvements in survival times ($p < 0.05$).

Conclusion: HTLC + heat resulted in significantly improved control of tumour growth in two models of TNBC, in comparison to treatment with free drug administered both with and without heat, while not increasing systemic toxicity. Importantly, this improvement in efficacy was independent of drug sensitivity.

41. Synthesis and Characterization of Functional Block Copolymers obtained by Living Anionic Polymerization for Delivery of Cisplatin

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Purpose: Block copolymer micelles (BCMs) have proven to be a viable delivery technology with several drugs relying on formulation in BCMs in clinical development. Well-designed copolymers with the appropriate physico-chemical characteristics can improve the properties of the encapsulated drug, including solubility, stability, and circulation lifetime *in vivo*. Cisplatin (CDDP) is a well-established platinum-based chemotherapeutic agent used for the treatment of several cancers. The current study aimed to prepare a series of block copolymers that enable stable encapsulation and delivery of cisplatin for treatment of breast cancer.

Method: A series of amphiphilic copolymers that include a hydrophilic block, composed of poly(ethylene glycol) (PEG), and a core-forming block, with functional groups for complexation of CDDP, were synthesized. In brief, methoxyPEG (MePEG) was used as a macroinitiator for living anionic polymerization of allyl glycidyl ether (AGE) monomer with subsequent chemical modification to introduce pendant carboxyl groups, separated by variable length alkyl chains ($3<C_n<11$). The block copolymer precursors (mPEG-5K-*b*-PAGE) showed a narrow molecular weight distribution (polydispersity index < 1.04) and ^{1}H NMR spectra confirmed the addition of thiol−ene couplings of the carboxyl groups (mPEG-5K-*b*-(AGE-C_nCOOH$)_{20}$) and the disappearance of pendant allyl groups.

Results: Inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis confirmed a drug loading capacity of up to 65 wt% of CDDP for the MePEG-5K-*b*-(AGE-C_nCOOH$)_{20}$ copolymers. Dynamic light scattering revealed the formation of nanostructures between $25 < D_h < 220$ nm in aqueous solution. *In vitro* cytotoxicity studies in the MDA-MB-468 human breast cancer cell line demonstrated that the IC_{50} of the copolymer complexed drug was approximately 10 fold higher than that of the free-drug following a 72 hour incubation period.

Conclusion: The results obtained to date demonstrate high drug loading of CDDP in the

newly synthesized copolymers. As well, the preliminary studies, confirm the stability of the nano-sized aggregates *in vitro* and maintenance of drug activity. Based on this data, further optimization of the formulation and *in vivo* evaluation are warranted.

42. The Impact of Tumor Microenvironment on Delivery of a Heat-activated Thermosensitive Liposome Formulation of Cisplatin for Treatment of Cervical Cancer

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Purpose: Cisplatin (CDDP) plays an important role in the treatment of cervical cancer; however, clinical application of this drug has been limited by its dose-limiting toxicities. Liposome formulations have been developed for delivery of CDDP as a means to increase tumor accumulation and reduce systemic toxicity. Yet, clinical evaluation of these formulations has revealed modest activity owing to limited drug release from the carrier at the tumor site. We have developed a heat-activated thermosensitive liposome formulation of CDDP (HTLC) that was found to result in a significant improvement in efficacy, relative to free drug when administered at chemically equivalent doses of CDDP. In order to fully explore the therapeutic potential of this formulation, we evaluated the efficacy of HTLC at its maximum tolerated dose (MTD), in combination with heating of the tumor volume to 42°C, in different xenograft models of human cervical cancer that vary in terms of sensitivity to CDDP and tumor microenvironment.

Methods: The therapeutic effect of HTLC (-/+ heat) was evaluated in subcutaneous ME-180, SiHa, and four patient-derived xenograft (PDX) models of human cervical cancer in comparison to free drug (-/+ heat) with each administered at their MTD. *In vitro* cell sensitivity of CDDP was determined using the MTS assay. *Ex vivo* characterization of tumor microenvironment within each model was performed through H&E immunohistochemistry and immunofluorescent staining of hypoxia, blood vessels, proliferating cells and extracellular matrix.

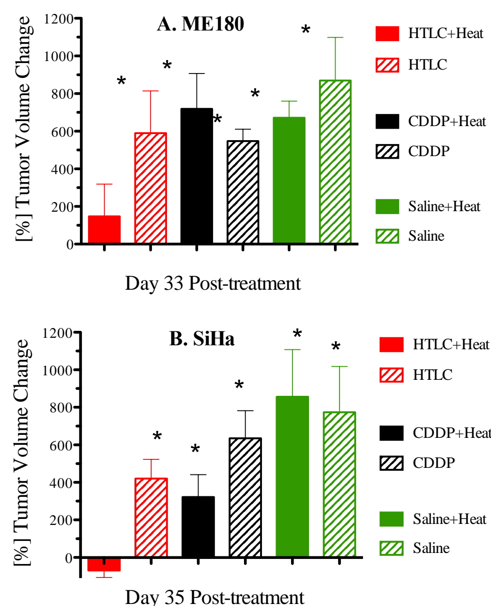

Figure 1. Tumor volume change [%] for the various treatment and control groups (*i.e.* HTLC + heat, HTLC, CDDP + heat, CDDP, saline + heat, and saline) of female SCID mice bearing subcutaneous A. ME-180 and B. SiHa cervical tumors implanted on the left hind limb. Data represent mean ± SD (n = 3-5). * indicates p < 0.01 when comparing heated HTLC group to other treatment groups.

Results: The heated HTLC group resulted in a significant improvement in tumor growth delay in the ME-180, SiHa tumor models, and PDX models, in comparison to treatment with free drug (-/+ heat) (Figure 1). Treatment with HTLC + heat resulted in a greater decrease in tumor burden in the SiHa model relative to the ME-180 model, despite *in vitro* cell sensitivity data showing that ME-180 cells are more sensitive to CDDP. Immunohistological characterization of both tumor models revealed significantly higher stromal content and larger areas of hypoxia in the ME-180 tumors, in comparison to SiHa tumors, both of which are well-known barriers to drug delivery.

Conclusion: This HTLC formulation in combination with mild heating resulted in a significant therapeutic advantage in comparison to free drug (-/+ heat) in different xenograft models of human cervical cancer. Interestingly, *in vitro* cell sensitivity alone was not predictive of *in vivo* efficacy. Rather, tumor microenvironment parameters, in particular stromal content and hypoxia, appear to serve as overriding factors that limit drug delivery and efficacy.

43. Evaluation of Ovomucin Nanoparticles as a Potent Mucosal Drug Delivery System

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Purpose: Mucus is a viscoelastic hydrogel layer covering all epithelial surfaces in the body. Regulating the uptake of nutrients and drugs and also protecting the underlying cells from pathogens are some functions for the mucus. As mucus acts as a barrier in drug and nutraceutical delivery, the barrier should be overcome to have better medical treatments or bioactive compounds absorption. We hypothesized that ovomucin, an egg albumin glycoprotein, due to ability to form gel at room temperature, similar structure to the body mucus and presence of both negatively charged and sulfhydryl groups, is an appropriate mucosal carrier for heat-sensitive compounds.
Methods: To evaluate this potential, the mucoadhesive property of ovomucin was compared with that of chitosan, poly acrylic acid (PAA) and alginate. Maximum detachment force and total work of adhesion were measured using tensile strength method. Nanoparticles (nanogels) were prepared from ovomucin suspensions in water at room temperature, and their size and morphology were studied using dynamic light scattering and TEM. Three model compounds of brilliant blue (negatively charged), riboflavin (non-ionic) and ciprofloxacin (positively charged) were encapsulated in the particles. The release profile of the encapsulated ciprofloxacin was evaluated through incubation of the loaded particles in phosphate-buffered saline (PBS) and simulated intestinal fluid (SIF), and the kinetic of the releases were studied using different mathematical models.
Results: Our results showed that the mucoadhesive property of ovomucin was stronger than that of poly acrylic acid, close to alginate, but slightly weaker than chitosan. According to the particles characterization studies, ovomucin particles were basically spherical in shape ranging from 120 to 160 nm in size. Drug loading efficiency of ovomucin particles for brilliant blue, riboflavin and ciprofloxacin were 87.7%, 25.4%, and 89.1%, respectively. Approximately 61% and 67% of loaded ciprofloxacin were sustainably released from the particles over 7 h incubations in PBS and SIF, respectively. The kinetic of the drug releases in PBS and SIF followed Fickian diffusion mechanism (Korsmeyer-Peppas model).
Conclusion: The appropriate mucoadhesive property, drug loading, release, shape and size of the particles suggested that ovomucin nanoparticles are promising carriers for mucosal delivery of pharmaceutical and bioactive compounds.

44. Polysorbate 80 and Cremophor RH 40 as a Unique, Non-ionic Surfactant Pair for Complex, Multi-API Microemulsion Drug Delivery

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Purpose: Of recent, the scientific community has gravitated toward the use of microemulsions as a drug delivery tool. In addition to consisting of both an organic and aqueous phase in which hydrophobic and hydrophilic components may be solubilized, microemulsions possess unique drug delivery properties such as small particle sizes, increased thermodynamic stability and high bioavailability potential. A number of publications have discussed surfactant use in single- active pharmaceutical ingredient (API) microemulsion formulations. Unfortunately, this is not the case for complex, multi-API microemulsion (CMM) formulations. Given that microemulsion formation is heavily dependent on surfactant choice, the aim of this study was to identify non-ionic surfactants with monophasic, microemulsion formation potential for future use in CMM formulations.
Methods: Literature support for microemulsion formation dictated the selection of surfactant and oil components. APIs were also selected in this manner, using a pre-natal supplement as a model for both hydrophilic and hydrophobic APIs. Ternary Phase Diagram (TPD) analysis was performed for each surfactant by combining various ratios of surfactant, oil and water. Samples were then left for 24 hours and phase behaviour was observed. Microemulsion formation was confirmed visually with a laser, making use of the Tyndall effect. Preliminary temperature and salinity scan studies were conducted to identify possible differences between Miglyol 812 and the multi-API oil component.
Results: The selected surfactant components were Cremophor RH 40, Gelucire 50/13, Poloxamer 188, Polysorbate 80 and Vitamin E TPGS, while the

selected oil component was Miglyol 812- a medium-chain triglyceride. TPD analysis on all surfactants revealed that Polysorbate 80 was ideal for monophasic (Type IV) microemulsion formation; this microemulsion type promotes content uniformity in drug delivery. Due to literature support for the hydrophilicity and bioavailability potential of Cremophor RH 40, Polysorbate 80 was combined with Cremophor RH 40, in an attempt to induce monophasic microemulsion formation. This proved successful, resulting in the selection of Polysorbate 80 and Cremophor RH 40 as a surfactant pair. Preliminary temperature and salinity scans reveal similarities between Miglyol 812 and the multi-API hydrophobic component, lending credibility to the use of this surfactant pair in CMM formation.
Conclusion: TPD analysis indicated favourable monophasic, microemulsion formation potential arising from the combination of Polysorbate 80 and Cremophor RH 40 as a non-ionic surfactant pair. This, along with preliminary oil-comparison data, qualifies the pair for potential use in CMM formation.

45. Smart Liposomal Chitosan–based Autogel for Intrapocket Delivery of Ofloxacin in the Treatment of Aggressive Periodontitis in Human Volunteers

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Purpose: An appreciated treatment modality for periodontitis is scaling and root planning followed by a controlled-release antimicrobial intrapocket delivery system. The aim of the present work was to develop a smart controlled-release liposomal autogel system of ofloxacin and evaluate it for the management of aggressive periodontitis in adult patients.
Methods: Autogels based on chitosan neutralized by β-glycerophosphate were prepared after setting a high degree of deacetylation for chitosan. The systems were characterized for mucoadhesion, syringibility and gelation onset and in-vitro drug release. The microbiological and clinical evaluations were performed in human subjects.
Results: *In vitro* ofloxacin release in pH 6.8 revealed about 83% release after 3 days. Further release modulation was done by encapsulation of ofloxacin in liposomes. Upon inclusion into the gel, liposomes afforded 80% of drug release in 7 days. When tested microbiologically in adult volunteers the ofloxacin liposomal autogel demonstrated markedly lower anaerobes bioburden in subgingival plaque samples than ofloxacin solution after 7 days. In clinical trials, the liposomal autogel formula showed significant improvement in the different clinical parameters evaluated (plaque index, gingival index, probing depth and clinical attachment level).
Conclusion: It can be concluded that the developed ofloxacin liposomal autogel is promising in the management of aggressive periodontitis in adults.

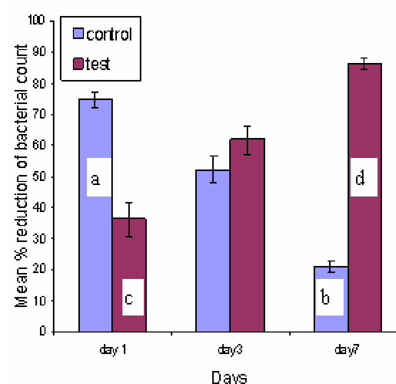

Figure (1): Mean % reduction in bacterial count in control and test human periodontal pockets receiving ofloxacin solution and ofloxacin liposomal autogel respectively (n=6).
a and b show significant difference (unpaired t test, $p<0.05$). c and d show significant difference (unpaired t test, $p<0.05$). a and d show significant difference (unpaired t test, $p<0.05$).

46. Targeted Delivery of siRNA by Polymeric Nanomicelles

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Purpose: Ribonucleic acid interference therapy is a promising alternative cancer treatment, which

utilizes small interfering RNAs (siRNAs) to target and degrade messenger RNAs. Unfortunately, due to the body's natural defenses, siRNA is degraded rapidly, resulting in poor cell uptake and specificity. Polymeric micelles are promising for effective delivery of siRNA, yet questions remain in terms of serum stability, cellular specificity and uptake. With this in mind, we designed biodegradable polymeric nanomicelles that enable conjugation of trastuzumab and siRNA and studied their serum stability cellular delivery.

Methods: Micelles were composed of poly(D,L-lactide-co-2-methyl-2-carboxytrimethylene carbonate)-graft-poly(ethylene glycol-azide) and assembled through the dialysis method. Traztuzumab and siRNA were each modified with a dibenzylcyclooctyne (DBCO) for copper-free click conjugation to azide-functional polymers and a dye for quantification. Trastuzumab and siRNA were surface conjugated to the micelles and purified by fast protein liquid chromatography (FPLC). Conjugations were incubated both at room temperature or 37 °C and for varied time points to monitor the conjugation efficiency onto the micelles. The serum stability of the siRNA-modified nanomicelles were monitored when exposed to PBS, 10% FBS, or 50% FBS at 37 °C and purified by FPLC. Four siRNA sequences were compared: the first containing unmodified RNA, and the other three containing partially or fully fluoro-modified RNA. The intracellular delivery of the siRNA- and trastuzumab-conjugated nanomicelles were observed by confocal microscopy on a human epidermal factor receptor 2 (HER2) overexpressing cancer cell line, MDA-MB-231-H2N.

Results: The optimal conjugation parameters were 2 hours at 37 °C. Using these conditions, 47.6% ± 15.8% of the siRNA and 62.5% ± 12.8% of the trastuzumab were conjugated on the nanomicelles after FPLC purification. In the presence of 50% FBS, the unmodified siRNA degraded rapidly with a $t_{1/2}$ of 4 h while the fully modified siRNAs exhibited a $t_{1/2}$ of 17.6 h. Trastuzumab-modified nanomicelles were endocytosed into breast cancer cells as determined by confocal microscopy. In ongoing studies, we are developing strategies to enhance siRNA delivery into the cytosol.

Conclusions: Optimal conjugation parameters were chosen for the preparation of siRNA/trastuzumab-nanomicelles. The serum stability of fully fluoro-modified siRNA was superior to that of the unmodified counterpart. Trastuzumab-nanomicelles were endocytosed, but the delivery of siRNA into the cytosol is still being optimized.

47. Onset of Action and Efficacy of Low Dose Ibuprofen Liquigel Compared to Conventional Solid Oral Dosage Forms of Ibuprofen: A Systemic Review and Meta-analysis

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Purpose: Ibuprofen liquigel, a soft gelatin capsule that contains liquid ibuprofen free acid and potassium salt in a solubilised form, has been suggested to provide higher plasma concentrations leading to faster analgesic effect in acute episode of pain such as those experienced after certain dental procedures or migraine. We evaluated the onset of action and efficacy of the maximum over-the-counter dose of ibuprofen liquigel (Advil, Pfizer Consumer Healthcare) (IBU_{LG}) vs Motrin IB (McNeil Consumer Healthcare, PA) (IBU_{Mot}). To our knowledge, no head-to-head comparison between these products has been published.

Methods: Published reports of randomized control trials on ibuprofen liquigel 400 mg were identified through a systematic search of PubMed, Embase, and the Cochrane library from inception until March, 2015. Key words used in the search included: ibuprofen, onset, human, dental, oral surgery, migraine, and tension type headache. Moreover, the reference lists of the retrieved articles were scanned for relevant studies. Clinical studies were included if they were randomized, double-blind, and placebo-controlled reports that evaluated ibuprofen administration after moderate to severe episodes of pain, made use of the two stopwatch method to report perceptible and meaningful times of pain relief, had a patient population aged at least 12 yr old, and included monitoring the patients for 3 h or longer post-dose. Relevant studies were categorised on the basis of whether the conventional ibuprofen tablets/caplets or the Ibuprofen liquigel were used.

Results: Initially 20 eligible studies were retrieved. However, due to excessive inter-products variability in the studies that used tablets, we limited the analysis to 4 IBU_{LG} and 3 IBU_{Mot} studies. The median time to the first confirmed perceptible pain relief was 13.6 min for IBU_{Mot} (no variance reported, 3 studies; n=251) and 12.0 min (95% CI 10.7 to 15.6, 2 studies, n=119). The time to the meaningful

relief was 48.7 min for IBU_{Mot} (no variance reported, 3 studies; n=251) and 30.5 min for IBU_{LG} (95% CI 27.7 to 34.5, 4 studies, n=260). The odd ratio (against placebo) to achieve a meaningful relief of pain within 2 h was 4.95 (95% CI, 3.30 - 7.40) for IBU_{Mot} and 18.62 (95% CI, 9.6 - 35.9) for IBU_{LG}. The efficacy of the treatments to yield the total pain relief score (TOTPAR) at 6 h post-dose was 12.34 (no variance reported, 3 studies, n=252) for IBU_{Mot} and 17.02 (95% CI, 16.02 - 18.02, 2 studies, n=126) for IBU_{LG}. The odd ratio for achieving at least 50% pain relief (based on TOTPAR) at 6 h was 3.6 (95% CI, 2.12 - 6.11) in favour of IBU_{LG} over IBU_{Mot}.
Conclusion: The available evidence suggest that both products at the OTC dose of 400 mg are effective in controlling moderate to severe episodes of dental pain, migraine, or tension-type headache. When compared to one another, the evidence tends to favour the liquigel form of ibuprofen, but does not clearly show a major advantage to one form over the other. Due to the limited data and the shortcoming of the current evidence, face-to-face studies are needed.

Pharmaceutical & Analytical Chemistry

48. HDAC Inhibitor Induced Changes in Histone Methylation

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Purpose: Epigenetic drug therapies like, histone deacetylase (HDAC) inhibitors are a new class of cancer treatment that inhibit the addition or removal of epigenetic post-translational modifications to histones, resulting in altered gene expression. Such changes in expression may influence other histone post-translational modifications as an auxiliary effect. Methods are therefore needed to monitor the efficacy of these drugs and to screen for effects on other epigenetic modifications. Here we describe a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously quantify acetylation and methylation marks on histones extracted from cultured cells treated with HDAC inhibitors.
Methods: Human embryonic kidney (HEK293) and chronic myeloid leukemia (K562) cells were treated with HDAC inhibitors for 24 hours, harvested and histones isolated. Histones from control and treatment groups were hydrolyzed by exhaustive proteolysis to component amino acids using w/w pronase for 72 hours. Histone hydrolysates were analyzed by LC-MS/MS. Multiple reaction monitoring transitions were developed and optimized using synthetically modified amino acid standards and their separation was achieved using a Primesep200 column.
Results: Treatment with HDAC inhibitors induced 400-600% hyperacetylation relative to control in both cell lines. Vorinostat produced increases in histone acetylation in K562 cells with an IC_{50} of 1.3±0.3 μM which correlated with growth inhibition measured using the MTS assay (1.4±0.2 μM) indicating that HDAC inhibitor induced growth inhibition is correlated with histone hyperacetylation. Entinostat resulted in an IC_{50} of 0.67±0.1 μM for histone acetylation and 0.97±0.1 μM for growth inhibition. Changes in histone lysine and arginine methylation with entinostat and vorinostat were consistent but not dose dependent. Similar results were observed with mocetinostat except a dose dependent increase in lysine methylation was observed. Mocetinostat was the most potent of the HDAC inhibitors tested with an IC_{50} of 0.48±0.06 μM, which agreed with that measured by the MTS assay (0.56±0.01 μM). An overall decrease in asymmetric dimethylarginine was also found for all HDAC inhibitors.
Conclusion: We have demonstrated that changes in histone methylation occur with HDAC inhibitor treatment, possibly due to changes in gene expression induced by histone hyperacetylation. Mocetinostat was found to induce increases of lysine methylation in tandem with increasing lysine acetylation, a newly discovered epigenetic activity that may contribute to its mechanism in cancer treatment.

49. BioID-based Identification of SCF$^{\beta\text{-TrCP1/2}}$ E3 Ligase Substrates

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Purpose: Protein ubiquitination is a conserved post-translational modification implicated in various cellular processes and disrupted in human disease. E3 ubiquitin ligases confer specificity in ubiquitination reactions via substrate recognition. Identification of ubiquitin E3 ligase substrates has been challenging, due to the transient nature of E3-substrate interactions, the subsequent degradation of substrates, and limitations in purification of proteins from poorly soluble cellular compartments. Current approaches to identifying E3 substrates are unable to resolve these obstacles while remaining widely accessible.
Methods: SCFβ-TrCP1 and its paralog SCFβ-TrCP2 target proteins for degradation in numerous signaling pathways including Wnt, Hippo and NFκB signalling. To covalently tag SCFβ-TrCP1/2 substrates, we applied a protein-labeling technique (BioID) that utilizes a pleiotropic mutant of BirA, a biotin ligase found in bacteria. BioID enabled *in vivo* biotinylation of proteins in a proximity-dependent manner and was coupled with treatment of cells with the proteasomal inhibitor MG132 to prevent substrate degradation. Enriched, biotin-tagged SCFβ-TrCP1/2 interactors were affinity purified and identified with semi-quantitative mass spectrometry. Putative substrates were validated by observing an increase their in half-lives in SCFβ-TrCP1/2 depleted cells.
Results: We identified 50 new putative SCFβ-TrCP1/2 substrates enriched with MG132 treatment. We validated 12 novel substrates with established cellular functions, implicating β-TrCP1/2 in the maintenance of nuclear membrane integrity, processing (P)-body turnover, and translational control. We showed that the stability of PPP1R15B, a subunit of protein phosphatase PP1 involved in the ER stress response, is controlled by β-TrCP1/2. β-TrCP1/2 recognizes substrates via a phosphorylated degron motif. Mutation of a potential phosphodegron motif on PPP1R15B (serine 459-466 to alanine) reduced interaction with β-TrCP1/2 and led to increase in half-life of PPP1R15B. We describe that this regulation plays a pivotal role in the ER stress pathway. During cellular stress, the unfolded protein response slows protein translation. The eukaryotic translational initiation factor EIF2 is inhibited by phosphorylation of its EIF2S1/eIF2α subunit. PPP1R15B opposes this phosphorylation, thereby activating EIF2S1/eIF2α and promoting translation. Expression of the PPP1R15B mutant deficient for β-TrCP1/2 binding inhibited eIF2α phosphorylation resulting from stress-inducing drugs and led to sensitization of their growth arrest properties.
Conclusions: We demonstrate E3 substrates may be identified by proximity-based biotin labeling (BioID) followed by semi-quantitative mass spectrometry. This technique is straightforward, scalable, conducted in live cells, and likely to be broadly applicable to identify transient substrates of other E3s. We validated PPP1R15B as an SCFβ-TrCP1/2 substrate, and reveal a novel role for this E3 ligase in the regulation of translation initiation.

50. Biotinylated Double Antibody Sandwich ELISA for the Quantification of Phosvitin

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Purpose: Phosvitin (PV) is a phosphoprotein found in egg yolk and it is used as a mineral chelating agent. However, there is no rapid and reliable immunoassay for PV quantification. Development of an effective immunoassay for PV detection and quantification is in-direct need to study the PV bioactivities and its mode of action. Thus, a reliable and sensitive double antibody sandwich ELISA (DAS-ELISA based on monoclonal (MAb) and chicken egg yolk IgY antibodies was developed to determine phosvitin content.
Methods: Leghorn laying hens were immunized with purified PV to produce anti-PV IgY antibody in the egg yolk. After 4 to 8 weeks of immunization, anti-PV IgY was extracted and analyzed by SDS-

PAGE and western blot. Biotinylated DAS-ELISA was based on MAb sa a capture antibody and biotinylated IgY as a detection antibody. The ELISA was performed to quantify PV found in egg yolks.
The cross-reactivity of IgY between PV and egg proteins was determined by using western blot method.

Results: The activity of anti-PV IgY was undetectable right after immunization (on day 0), then rapidly increased from week 2 to week 4, and thereafter remained relatively constant up to 9th week. The eggs obtained from 4th to 7th weeks were found with high levels of anti-PV IgY activity. The total content of IgY in the yolks from immunized chickens was approximately 1.1 times greater than that of the yolks from non-immunized chickens. The proportion of PV-specific IgY in the total egg yolk IgY was 6.2% in the immunized egg yolks. The modified DAS-ELISA using Mab as capture and biotinylated IgY as detection antibody showed the detection limit ranging from 2.5-40 ng/µL. Specific IgY antibodies against PV did not cross reacted with other proteins in egg white and yolk, examined by ELISA and western-blot assay.

Conclusion: IgY, which can be simply produced in large quantities and high titers may replace other sources of polyclonal and monoclonal antibodies conventionally used in ELISA or other immunological assays without compromising accuracy of the assay. The present study indicates that DAS-ELISA provides a wider working detection range for PV (5.625-90 µg/µL) compared to the reference method of biotin-avidin DAS-ELISA (2.5-40 ng/µL). The IgY based phosvitin detection assay could be a reliable and sensitive method for quantification of phosvitin.

Pharmacokinetics & Pharmacodynamics

51. Effect of Dipyridamole on Red Blood Cell Concentrations of Purine Nucleotides after Acute Myocardial Infarction in a Freely Moving Rat Model

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Purpose: The objective of the research was to study the effect of dipyridamole on the red blood cell (RBC) concentrations of purine nucleotides after acute myocardial infarction induced by isoproterenol.

Methods: Male Sprague Dawley rats (SDR) weighing between 250 and 300 g were used. Each rat received either 10 mg/kg of dipyridamole (n = 8) or vehicle (n=6) twice daily for 4 doses by subcutaneous (sc) injection. Blood samples were collected from 0 to 6 hours for measurement of RBC concentrations of purine nucleotides. Isoproterenol (30mg/kg) was injected (sc) 1hour after dipyridamole to induce cardiovascular injury. Data between the two groups were compared and differences considered significant at $p < 0.05$ (Student's t-test).

Results: Dipyridamole reduced mortality induced by isoproterenol from 50% to 25%. It increased GTP concentrations in RBC before isoproterenol (AUC T0 - T1 0.05 ± 0.01 vs 0.03 ± 0.01 mM*T) and reduced the breakdown of GTP to GDP in RBC after isoproterenol (AUC ratio of GDP/GTP from T1 – Tlast 0.25 ± 0.09 vs 0.44 ± 0.13, $p < 0.05$ for both). It also decreased the Cmax of ADP and GDP after isoproterenol ($p = 0.067$ for ADP and $p = 0.065$ for GDP), and breakdown of ATP to AMP, although the difference did not reach statistical significance (AUC ratio of AMP to ATP from T1 – T last 0.01 ± 0.01 vs 0.05 ± 0.05, $p = 0.076$).

Conclusion: Dipyridamole decreased mortality and breakdown of GTP and ATP induced by isoproterenol.

52. Altered Hepatic Protein Expression of CYP2c and CYP4a in Mouse Models of Type I and Type II Diabetes

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Purpose: Most patients with type I (T1D) and type II (T2D) diabetes are treated with multiple drugs. Evidence suggests that drug disposition is altered in T1D and T2D. It was already shown that in mouse,

hepatic activity of CYP3a is increased in both T1D and T2D. We then hypothesized that hepatic expression of CYP2c and CYP4a, catalyzing biotransformation of xenobiotics and endogenous substrates such as arachidonic acid (AA), is also modulated by T1D and/or T2D.

Methods: Mouse models of T1D (streptozotocin) and T2D (C57BLKS/Jdb/db) were used in this study. After sacrifice, livers were collected, washed in cold PBS and snap frozen. Total proteins were extracted using ice-cold lysis buffer. Western blots were performed to assess CYP2c and CYP4a protein expression.

Results: Our results showed a significant decrease in relative protein expression of hepatic CYP2c in T2D (1.55 ± 0.16, n=19, p<0.05) compared to control (2.21±0.19, n=17). A significant increase was observed in relative protein expression of hepatic CYP4a in both TD1 and TD2 groups (0.92±0.06, n=19, p<0.001;1.06±0.40, n=19, p<0.001, respectively) compared to control (0.18±0.01, n=17).

Conclusion: This study shows that disease conditions such as T1D and T2D alter protein expression of cytochromes P450, likely affecting drug metabolism and disposition and leading to various clinical consequences in patients suffering from diabetes and exposed to CYP2C substrates such as warfarin. Moreover, these alterations could induce disturbances in the endogenous pathway (CYP450) of AA metabolism and increase the risk of cardiovascular disease by disrupting the fine equilibrium between cardioprotective (CYP2C-generated) and cardiotoxic (CYP4A-generated) metabolites of AA and pushing the balance towards the cardiotoxic side.

Acknowledgement: Audrey Blais-Boilard is the recipient from Université Laval of the GSK/CSPS National Undergraduate Student Research Program Award.

This work has been presented at the ASCPT Conference in March 2015. This abstract has been published in Clim Pharm Ther. 2015: 97(sup 1): S44 (P1-082)

53. Enantiospecific Pharmacokinetic Disposition of Isoxanthohumol in the Rat

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Purpose: Isoxanthohumol is a bioactive dietary prenylflavanone found in hops (*Humulus lupulus* L.), beer and dietary supplements. Isoxanthohumol is formed *in vivo* by xanthohumol, a related hops prenylchalcone, and is also a prodrug of 8-prenylnaringenin, a potent phytoestrogen. Both isoxanthohumol and 8-prenylnaringenin contain one chiral carbon center but their chirality has largely been ignored due to lack of enantiospecific bioanalytical methods of analysis. Additionally, no single-dose pharmacokinetic studies of isoxanthohumol currently exist in the literature for any species. The purpose of this study was to develop an enantioselective bioanalytical assay to quantify isoxanthohumol, evaluate the enantiospecific pharmacokinetic disposition of isoxanthohumol in a rodent model and subsequent enantiospecific formation of the 8-prenylnaringenin metabolite using a previously validated and published method of analysis[1].

Methods: An enantiospecific LC-ESI-MS method was developed and utilized to quantify IX in rat serum and urine using a Chiracel® OD-RH column with an isocratic mobile phase consisting of methanol, acetonitrile and 10 mM ammonium formate (pH 8.5) (8.125:24.375:67.5, v/v/v) and a flow rate of 0.8 mL/min. Detection was achieved using SIM negative mode with single plot transition at *m/z* 353.15 for isoxanthohumol. After intravenous (10 mg/kg) or oral (100 mg/kg) administration of racemic isoxanthohumol to rats (n = 3 per route of administration), serum and urine were collected over a period of 120h. Serum and urine samples were analyzed for isoxanthohumol and 8-prenylnaringenin using novel enantiospecific LC-ESI-MS methods. Non-compartmental pharmacokinetic analyses of isoxanthohumol were carried out.

Results: The developed LC-ESI-MS method for isoxanthohumol showed sensitivity, accuracy and reproducibility and was successfully applied to the stereospecific pharmacokinetics of isoxanthohumol in the rat. Both isoxanthohumol and 8-prenylnaringenin were found as aglycones and glucuronide conjugates. Both compounds displayed multiple peaking in serum suggestive of

enterohepatic recycling. Isoxanthohumol is primarily excreted through non-renal routes. *S*-8-Prenylnaringenin was excreted in the urine in greater amounts than *R*-8-prenylnaringenin. Bioavailability was determined to be ~4-5% for isoxanthohumol.

Conclusion: Further enantiospecific and pharmacokinetic disposition of isoxanthohumol and 8-prenylnaringenin are warranted along with continued enantiospecific bioactivity studies, especially in relation to possible gut microbial metabolism of isoxanthohumol and subsequent formation of 8-prenylnaringenin.

References: [1] Martinez SE, Lakowski TM, Davies NM. Enantiospecific analysis of 8-prenylnaringenin in biological fluids by liquid-chromatography-electrospray ionization mass spectrometry: application to preclinical pharmacokinetic investigations. *Chirality*. 2014; 26(8):419-26.

54. Regulation of Hepatic Gene Expression in Mice during Acute Inflammation: The Involvement of NF-κB and Pregnane X Receptor

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Purpose: Endotoxin-induced inflammation is known to alter the expression and activity of several drug transporters and drug-metabolizing enzymes in the liver. Since these pronounced changes in hepatic transporters contribute to altered drug disposition during inflammation, it is important to understand the underlying regulatory mechanisms. Many key transcription factors involved in regulating transporters and metabolic enzymes are modulated during inflammation including the nuclear factor NF-κB and the pregnane X nuclear receptor (PXR). NF-κB activation is known to be a major mediator of endotoxin signaling, and a reciprocal repression or cross talk-between PXR and NF-κB activation has been reported. The role of PXR and NF-kB signaling pathways on the regulation of transporters during inflammation has not been clarified. Hence, our objective was to examine whether NF- κB directly regulates the expression of drug transporters or exert its effect indirectly via PXR.

Method: Ten to twelve weeks old PXR deficient [PXR (-/-)] or wild-type [PXR (+/+)] male mice were treated with endotoxin (LPS, 5 mg/kg i.p) or saline 30 minutes after i.p administration of the NF-κB inhibitor: PHA408 (40 mg/kg) or vehicle (n=4-8/group). Animals were sacrificed and livers collected 6 hr later. Gene expression was measured in liver samples using qRT-PCR and cytokine levels were measured in serum using ELISA.

Results: Following administration of endotoxin, the NF- κB target genes TNF-α, IL-6, IL-1β mRNA and serum levels were induced to a similar extent in PXR (-/-) and PXR (+/+) mice. Likewise, as comparted to saline controls, LPS administration imposed 30-70% significant decreases in the expression of Abcb11, Abcc2, Abcc3, Abcg2, Slc10a1, Slco2b1, Slco1a4, Mdr1a and Mdr1b in PXR (+/+) and (-/-) mice to a similar extent. Pre-administration of the NF- κB inhibitor, PHA408significantly attenuated endotoxin-mediated changes in the expression of proinflammatory cytokines and hepatic transporters in both PXR (+/+) and (-/-) mice.

Conclusion: Our results indicate that NF- κB is the major signaling pathway involved in endotoxin-mediated down regulation of hepatic transporters and acts independent from PXR.

55. Synergistic Effect of Platinum with PARP Inhibitor in BRCA1/2 Deficient Ovarian Cancer Cells

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Purpose: Ovarian cancer remains a lethal gynaecological disease due to ineffective screening, leading to 80% of patients presenting with late stage tumors at diagnosis. Despite initial high responsive rate to current first line chemotherapy with platinum agents, 85% of patients relapse with resistant disease. Therefore, novel treatment strategies are needed. BRCA proteins are involved in homologous recombination (HR) that repair DNA double strand break. This impairment on HR repair machinery has led to the use of poly (ADP-Ribose) polymerase (PARP) inhibitors where by these drugs inhibit repair of single and double strand breaks, thereby increasing the efficacy of cytotoxic chemotherapeutic drugs. Olaparib (OPB) is a selective PARP1 inhibitor which has shown a favourable toxicity profile and promising anticancer activity in patients with BRCA mutations. Optimal dosing with these agents has not yet been

established. Therefore the purpose of this study is to determine an optimal dosing ratio of Carboplatin (CPT) and OPB that can result in synergistic effects.
Methods: Using the method developed by Chou and Talalay, we determined the combination index (CI) for CPT and OPB in solution on a panel of human ovarian cancer cell lines with either intact, mutated or defective BRCA1/2 genes. Briefly, inhibitory concentration (IC_{50}) of CPT and OPB given as single agent and in combination were measured by MTT assay. Dosing ratios of CPT (1) to OPB (10, 5, 2, 1, 0.5, 0.1, 0.05, 0.03, 0.01) were explored. CI was calculated as $CI = \frac{D_1}{(Dm)_1} + \frac{D_2}{(Dm)_2}$, where CI >1 is antagonism, CI = 1 is additive, and CI < 1 is synergism.
Results: Synergism was observed in BRCA1/2 mutated or defective cell lines (PEO1, OVCAR8, and UWB1.289), suggesting that addition of OPB potentiates the cytotoxic effect of CPT in the absence of functional HR repair. Interestingly, synergism was also observed in OV90 cells with intact BRCA1/2. This may be explained by p53 mutation which can also alter DNA repair mechanism.
Conclusion: Combination therapy of CPT with OPB results in synergistic effect at a molar ratio of 1:0.1 and 1:0.05 in BRCA1/2 mutated or defective cell lines. Mutation in DNA damage response protein, such as p53, can also be synthetic lethal with PARP inhibition.

Table1. Combination Index (CI) of carboplatin-olaparib combination treatment. < 0.30, strong synergism; 0.30-0.70, synergism; 0.70-0.85, moderate synergism; 0.85-0.90, slightly synergism; 0.90-1.10, additive; > 1.10, antagonism

	CI-Carboplatin:Olparib-72hr			
	1:0.5	1:0.1	1:0.05	1:0.03
HeyA8	1.42	1.50	1.50	1.43
OV90	0.70	1.00	0.67	1.36
OVCAR8	1.20	0.48	0.59	0.62
PEO1	1.20	0.79	0.73	1.00
UWB1.289	0.66	0.56	0.47	0.54

56. The Impact of Diet-induced Obesity on Proteins Involved in Drug Disposition in Livers of Rats

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Purpose: The prevalence of obesity worldwide has increased in the last few decades. The risk of obesity comes from its association with various clinical diseases especially cardiovascular diseases and diabetes. The purpose of this study was to assess the expression of some drug metabolizing enzymes and transport proteins in livers of rats fed normal rat chow with water or one of three types of high calorie-containing diets.
Methods: Four groups of rats (10/group) were housed for 14 weeks and they had access either to normal rodent food and water (control group), normal rodent chow and high fructose-corn syrup water (HFCS), 45% high fat (HF) diet and water, or 45% HF and HFCS. All biochemical and hematological parameters were assayed using standard diagnostic kits. The mRNA levels and protein contents of cytochrome P450 (CYP) enzymes and membrane transporters in rat liver were determined using real-time PCR and Western blot respectively.
Results: There were significant increases in plasma glucose, insulin, leptin, triglyceride and cholesterol in each of the high calorie fed groups compared to that of the control group. Relative to the control, the mRNA and/or protein levels of CYP 3A1, 3A2, 1A1 and 2C11 were decreased in the FD and HFCS/FD groups. Although mean OCT1 and MATE1 transporters protein levels appeared lower in a HFCS-HF diet group, the differences were not significant.
Conclusion: Our findings suggest that HFCS and/or HF diet induced obesity can reduce the expression of some hepatic microsomal enzymes.

57. The Pharmacokinetics of Dronedarone in Normolipidemic and Hyperlipidemic Rats

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Purpose: Dronedarone is a benzofuran derivative of amiodarone that is used for the treatment of cardiac

arrhythmias. It is a lipophilic drug which might be a candidate for binding by lipoproteins, and hence altered pharmacokinetics in hyperlipidemia. The objective of this study was to compare the pharmacokinetic profile of dronedarone after oral doses in normolipidemic (NL) and hyperlipidemic (HL) rats.

Methods: Sprague Dawley rats were administered poloxamer 407 (P407) 1 g/kg as intraperitoneal (ip) injection to induce HL. The day before the pharmacokinetic assessment, cannulas were implanted into the right jugular veins of NL and HL rats. Dronedarone HCl was dosed orally as methylcellulose suspension (55mg/kg). After dosing, serial blood samples were collected for up to 24 h. Samples were assayed for dronedarone using a validated reverse phase HPLC method.

Results: The geometric mean pharmacokinetic data (CV of geometric mean in parentheses) are summarized below:

Parameter	NL(n=6)	HL(n=6)
$AUC_{0\text{-}24}$, mg·h/L	4.78 (39.7%)*	12.4 (105%)
$AUC_{0\text{-}\infty}$, mg·h/L	5.03 (40.5%)*	12.7 (105%)
$t_{½}$, h	5.85 (22.0%)	5.39 (59.5%)
C_{max}, mg/L	1.19 (156%)	3.27 (340%)
t_{max}, h	2.57 (124%)	1.27 (133%)
CL/F, mL/min/kg	215 (40.5%)*	85.0 (105%)
Vdss/F, L/kg	95.7 (46.5%)*	28.9 (97.6%)
MRT, h	7.41 (39.1%)	5.66 (35.8%)

*Significantly different compared to HL rats (single factor ANOVA)

The pharmacokinetic data showed that the AUC of HL rats was significantly higher than the NL rats. Moreover, the CL/F and Vd/F were significantly lower in the HL rats. There were no significant differences in Cmax, t½ and MRT. In the HL rats, pharmacokinetic variability was generally higher than that seen in the NL rats.

Conclusion: Hyperlipidemia altered the pharmacokinetic parameters of dronedarone in rats. This might be due to factors such as decreases in metabolism, and/or increases in the extent of drug bound to plasma proteins; further study is needed to determine the cause.

58. Systemic Levels of Flaxseed Lignans Following Oral Administration of Flaxseed Lignan-enriched Complex in Young and Elderly Adults

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Purpose: Human clinical trials with flaxseed lignan-enriched complex (FLC) (BeneFlax®) demonstrate therapeutic effects in patients with dyslipidemia. Yet, uncertainty exists regarding the primary lignans available from FLC and which lignan is biologically active. Data on their bioavailability and pharmacokinetics is necessary to better understand the lignan role in the putative health benefits. The purpose of this study is to understand systemic lignan levels of the principal lignan of flaxseed (secoisolariciresinol diglucoside (SDG), and its primary metabolites, secoisolariciresinol (SECO), enterodiol (ED), and enterolactone (EL) and their respective glucuronic acid conjugates) following a single oral dose (SOD) administration in young and elderly participants and following multiple oral dose (MOD) administration in elderly participants.

Methods: FLC was administered to young (age 18-35 yr, n=12) and older (age 60-80 yr, n=10) patients as a SOD (300 mg SDG with 100 mL of water). In a double blind randomized control study (n=32) multiple oral doses equivalent to 600 mg/day of FLC was administered for 6 months to healthy 60-80 year old individuals. Primary flaxseed metabolites were extracted from human serum with and without β-glucuronidase treatment and were analyzed using a validated LC/MS-MS method.

Results: Detectable levels of SECO, ED and EL but not SDG were observed. Following glucuronidase treatment, PK parameters for EL following the SOD in both young and old patients, respectively, were: C_{MAX} = 53.9 ± 39.1 and 105.2 ± 52.59 ng/mL, T_{MAX} = 37.0 ± 7.7 and 32.4 ± 7.5 h, AUC (0-96 h) = 1958 ± 1462 and 4277 ± 2013 ng*h/mL, and half-life = 21.4 ± 12.1 and 19.1 ± 7.6 h. PK parameters for ED with glucuronidase treatment following SOD in both young and old patients, respectively, were: C_{MAX} = 73.7 ± 40.1 and 170.1 ± 150.9 ng/mL, T_{MAX} = 26.8 ±

3.9 and 24.3 ± 7.8 h, AUC (0-96 h) = 1612 ± 1109 and 3530 ± 3231 ng*h/mL, and half- life = 14.8 ± 4.9 ± 9.03 ± 2.1 h. Analysis of serum samples from the MOD study is underway.

Conclusion: The single oral dose administration study showed a trend towards age-dependent differences in the overall exposure to lignans. In this study, the elderly demonstrate higher exposure levels following an oral dose, which, in turn, may suggest this natural product has a propensity to have more important favourable effects for an age group where chronic disease is a common finding.

59. CYP1A2 Binding Studies of Novel Tacrine Derivatives

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Purpose: Tacrine (Cognex®) was the first drug to be approved by the US FDA for the management of the symptoms of Alzheimer's disease. This compound promotes the maintenance of adequate levels of the neurotransmitter acetylcholine by inhibiting acetylcholinesterase, the enzyme responsible for its degradation. However, tacrine was withdrawn from the market due to its hepatotoxicity concerns. This effect was attributed to hydroxytacrine metabolites formed via the hepatic enzyme cytochrome P450 1A2 (CYP1A2). We hypothesize that the substrate behaviour of tacrine towards CYP1A2 can be changed by chemical modification.

Methods: We prepared tacrine derivatives with chemical modifications at the C-6 and C-9 positions. In vitro inhibition assays were carried out using human recombinant CYP1A2 enzymes. The metabolic profile of the tacrine derivatives was further elucidated using rat liver microsomes. A method for the quantitation and identification of metabolites was developed using tandem liquid chromatography-mass spectrometry (LC-MS). In addition, in silico molecular docking experiments were carried out to determine the binding modes of tacrine and its derivatives and corroborate the in vitro findings.

Results: Our results indicate that modification of the chemical structure of tacrine can significantly alter its binding mode within the CYP1A2 catalytic site. In particular, the addition of a picolyl group at the C-9 position results in a greater affinity for CYP1A2 while a substituted benzyl moiety at the same position greatly reduces CYP1A2 affinity.

Conclusions: Appropriate chemical modification of tacrine has the potential to prevent the formation of its hepatotoxic metabolites, while maintaining its ability to inhibit cholinesterase enzymes. These novel tacrine derivatives may represent a new class of anti-Alzheimer's therapies with reduced adverse effects.

Poster Session 2

CSPS and CC-CRS Posters

Thursday, May 28, 2015

Posters - Session 2

Thursday, May 28

Biomedical Sciences

60. Assessment of Acute Toxicity of Methoxy Poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) Polymeric Micelles Following Oral and Intraperitoneal Administration to Rats

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Purpose: Methoxy polyethylene oxide-*block*-polycaprolactone (PEO-*b*-PCL) copolymers are amphiphilic, biodegradable copolymers that have been extensively used for drug delivery. Polymeric micelles prepared from these copolymers have been successfully used to deliver a variety of drugs and diagnostic agents. The aim of this study was to assess the acute toxic effects, following oral or intraperitoneal (ip) injection, of drug-free PEO-*b*-PCL micelles after multiple-dose treatments in rats.
Methods: Methoxy PEO-*b*-PCL block copolymers with different molecular weights of PCL were synthesized. Assembly of block copolymers was achieved by co-solvent evaporation method. The self-assembled structures were characterized by dynamic light scattering. To investigate the toxicity profile of PEO-*b*-PCL micelles, sixty animals were divided into two main groups: The first group received PEO-*b*-PCL micelles (100 mg/kg) or vehicle (control) by oral gavage daily for seven days. The other group of animals received the micelles (100 mg/kg) or vehicle by ip injections daily for seven days. Twenty-four hours following the last dose, half of the animals were sacrificed and blood and organs (lung, liver, kidneys, heart and spleen) were collected. Remaining half of the animals was observed for further 14 days and was sacrificed at the end of third week, and blood and organs were collected. The organ-to-body weight ratios from different treatment groups were calculated. Moreover, leukocytes and % lymphocytes in blood samples were analyzed using flow cytometry. In addition, the influence of different polymeric micelles on biochemical parameters such as LDH, AST, GGT, creatinine, and blood urea were assessed one week- and three-week post-dose. Data obtained from different animal groups were statistically analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparisons ($\alpha = 0.05$).
Results: The diameter size of all micelles was less than 100 nm with a narrow distribution. None of the polymeric micelles administered caused a significant change in organ-to-body weight ratios, leucocytes count or % lymphocytes. Moreover, no significant changes on any of the measured biochemical parameters were observed.
Conclusion: Although the dose of copolymers used in this study is much higher than those used for drug delivery, it did not cause any significant toxic effects. Assessment of the toxicity of PEO-*b*-PCL micelles is a crucial step for future design and clinical application. These findings are in agreement with previous reports suggesting the biocompatibility of PEO-*b*-PCL micelles.

61. Regulation of Folate Permeability at the Blood Brain Barrier

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Purpose: Folates (vitamin B9) are required by humans for the synthesis of DNA, regulation of gene expression, and the production of S-adenosylmethionine (universal methylation agent). The movement of folates across epithelia is largely due to three different transport systems. In the intestine the proton-coupled folate transporter

(PCFT) uses a hydrogen gradient to facilitate folate uptake into enterocytes. Found ubiquitously throughout the body is the reduced folate carrier (RFC) that functions as a low affinity organic anion antiporter. In the CNS, at the choroid plexus, folate receptor alpha (FRα) constitutes a major transcytosis pathway for folates into the cerebrospinal fluid (CSF). The aim of this project was to investigate folate transport at the blood-brain barrier (BBB), an area of the brain hitherto largely unstudied in relation to folate transport but critical to the maintenance of CNS homeostasis.

Methods: The expression of PCFT, RFC, and FRα was studied in human and rat brain microvessel endothelial cell lines (representatives of the BBB), primary rat astrocytes and microglia, and isolated mouse brain capillaries. Western blotting was used to determine protein levels in each model system while qPCR analysis was done to detect gene expression.

Results: Expression of RFC was found in all of the model systems (n=3). PCFT was expressed in both rat brain miscovessel cells and primary rat astrocyte cultures (n=2) while FRα was detected in the human and rat BBB cell lines as well as in rat microglia and astrocytes (n=3).

Conclusion: The detection of FRα by western blot in representative BBB cell lines is a novel finding and indicates it may be the predominant pathway for folate transport at the BBB, as it is in the choroid plexus. RFC detection in endothelial cells as well as brain parenchyma cells suggests it may play an important role in folate permeability at the BBB especially in the case where mutations in FRα impair this mode of folate transport. Characterizing folate transport at the BBB could have great implications for the treatment of neurometabolic disorders caused by folate deficiency. Further work will involve undertaking transport assays with folic acid to characterize the activity of each transporter/receptor at the BBB and investigating the role of nuclear receptors in folate transporters functional expression.

Acknowledgement: Theodora Bruun is the University of Toronto recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

62. Folate Transport at the Blood-brain Barrier

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Purpose: Folates (vitamin B9) play a critical role in DNA synthesis, gene regulation, and the production of the universal methylation agent, S-adenosylmethionine. There are three major transport systems that facilitate gastrointestinal absorption and movement of folates across epithelial barriers. In the small intestine, the proton-coupled folate transporter (PCFT) mediates uptake of folates into enterocytes by using a proton gradient. Expressed ubiquitously in mammalian tissues is the reduced folate carrier (RFC), which functions as a low affinity organic anion antiporter. In the central nervous system (CNS), particularly in the choroid plexus, folate receptor alpha (FRα) constitutes a major transcytosis pathway for folates into the cerebrospinal fluid. Mutations of the FRα can cause severe folate deficiency in the CNS resulting in childhood neurodegeneration characterized by ataxia, dyskinesia, epilepsy, and abnormal brain myelination. The aim of this project was to investigate folate transport at the blood-brain barrier (BBB), an area of the brain largely unstudied in relation to folate transport.

Methods: Applying qPCR and immunoblotting, the expression of PCFT, RFC, and FRα was examined in: i) human and rat brain microvessel endothelial cell lines representative of the BBB, ii) primary cultures of human brain microvessel endothelial cells, and iii) isolated rat and mouse brain capillaries.

Results: The folate transporters demonstrated robust expression (gene and protein) in all BBB model systems with western blots revealing multiple protein bands for PCFT (50-60kDa) and RFC (58-75kDa), indicative of differential glycosylation. Interestingly, FRα mRNA expression was not detected in human brain microvessel endothelial cells but was present in rodent brain capillaries.

Conclusion: The detection of PCFT and RFC in various brain cell culture systems and rodent brain capillaries representative of the BBB suggests a potential role for these transporters in folate permeability at the BBB, especially when FRα mutations impair the predominant mode of brain folate uptake in the choroid plexus. Transport assays

with folic acid (specific PCFT substrate) and methotrexate (PCFT and RFC substrate) to characterize the function of each transporter in the various BBB models are ongoing. Modulating folate transport at the BBB could potentially constitute a novel strategy for the treatment of neurometabolic disorders caused by folate deficiency.

63. Interaction of Chemotherapeutic Drugs with Gold Nanoparticles

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Purpose: Grafting nanotechnology onto existing medical systems is gaining exponential interest in the field of biomedical sciences. Gold nanoparticles (GNPs) in particular have been extensively used in cancer research due to their ability to act as an anti-cancer drug carrier and as a dose enhancer. Therefore, it potentially can be used in conjunction with chemotherapy or radiotherapy. Previous studies that used gold nanoparticles with anti-cancer drugs, focused mostly on optimizing the GNP-drug complex using linkers. This study aims to observe the change in efficacy of the drug by having GNPs present in the system.
Method: The effectiveness of two chemotherapeutic drugs, bleomycin and cisplatin, is observed in presence of spherical GNPs, by calculating the survival fraction of human breast cancer cells, MDA-MB-231 *in vitro*. Bleomycin is conjugated onto the GNPs through the sulfur bond while cisplatin does not attach onto the GNPs.
Results: The sample treated with bleomycin – GNP complex had a decrease in therapeutic effect compared to the sample treated with bleomycin alone. However, the sample treated with cisplatin and GNPs had the same therapeutic result with the sample treated with cisplatin alone.
Conclusion: Having GNPs in the cells do not affect the mechanism of the drug but conjugating chemotherapeutic drugs onto GNPs may change the uptake pathway of the drugs into cells. Having a better understanding of the interaction of GNPs and drugs will establish a more successful NP-based platform for GNP combined chemotherapy studies in the future.

64. Novel Missense Mutations in SCN1B as a Cause of Pediatric Epilepsy

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Purpose: Sodium channel (NaCh) mutations are a principle cause of pediatric epilepsy. Voltage-gated NaChs (Navs), which initiate neuronal action potentials, are composed of a pore-forming α subunit and one or more regulatory β subunits. *SCN1B* encodes the Navβ1 abundant in cardiac, skeletal muscle and neuronal tissues. Navβ1 mutations cause a spectrum of pediatric epilepsies, including GEFS+ and Dravet's Syndrome. While the mechanism by which many *SCN1B* mutations cause hyperexcitability is unclear, Navβ1 mutations can alter cellular response to drug therapy, making this protein an intriguing candidate for the development of epilepsy therapeutics and comorbid excitability disorders.

The study objectives were to use bioinformatics and structural modeling tools to identify potentially deleterious *de novo SCN1B* mutations in the human population; to generate ion channel expression constructs with novel and known *SCN1B* mutations; and to functionally characterize these mutations in a cellular expression system.
Methods: Candidate nonsynonomous single nucleotide polymorphism (nsSNP) mutations were identified via literature search and evaluation of the 1000 Genomes and Exome Variant Server. Mutation pathogenicity was evaluated *in silico* using Polyphen, SIFT, GERP, and Grantham scores. Navβ1 homology models were generated and subjected to *in silico* mutagenesis independently and in complex with α subunits. The full-length isoform 1 of the rat *Scn1b* gene was subcloned into the pcDNA3.1 expression vector and validated by Sanger Sequencing. *Scn1b* Mutations were generated using overlapping extension PCR, cloned into pcDNA3.1 and validated by Sanger sequencing. Positive clones were transfected into HEK 293 cells for trafficking and patch-clamp electrophysiological assays.
Results: Our analysis identified five missense amino acid substitution mutations to be of particular interest: C121W, R125C, R125L, N114S and N135K. These mutations are in close proximity to the extracellular immunoglobulin loop, an established hotspot for epileptic-inducing variants.
Functional characterization has revealed mutation-

dependent effects on channel biogenesis, pharmacology and electrophysiology, even for different mutations at the same protein location.

Conclusions: Navβ1 amino acid substitutions, whether common (population) or novel (personal), impact structure-function relationships, including biogenesis and biophysics, in the functional NaCh. Future experiments will perform combinatorial analysis of Navβ1 mutation effect in complex with a mutated α subunit. More in-depth characterizations of *SCN1B* mutations will allow us to gain a greater understanding of epilepsy pathophysiology and the role of compound mutations in pharmacoresistant epilepsy, which may potentially improve our therapeutic selection.

Acknowledgement: Miao Yan Sun is the UBC recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

65. Discrepancy Between In Vitro and In Vivo Potencies of Vitamin D Analogs on Cholesterol Lowering

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Purpose: Treatment with 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], active ligand of the vitamin D receptor (VDR), led to cholesterol lowering in mice fed a Western diet due to down-regulation of the small heterodimer partner (Shp) and induction of cholesterol 7α-hydroxylase (Cyp7a1). Here, we compared the in vitro and in vivo potencies and cholesterol lowering properties of several vitamin D analogs.

Methods: In vitro activities of the vitamin D analogs were estimated upon incubation of the ligands over a concentration range (0.0001-150 μM), which were cotransfected with the GAL4-hVDR, UAS-luciferase reporter and β-galactosidase in HEK293 cells to determine the EC_{50}. In vivo activities were appraised in male C57BL/6 mice fed a Western diet for 3 weeks and given intraperitoneal doses of vehicle, dietary vitamin D_3 (1625 nmol/kg), 25-hydroxyvitamin D_3 [25$(OH)D_3$, 1248 nmol/kg], 1α-hydroxyvitamin D_3 [1α$(OH)D_3$, 1.75 nmol/kg] or 1α-hydroxyvitamin D_2 [1α$(OH)D_2$, 1.21 nmol/kg] every other day for 8 days during the last week of diet. 1,25$(OH)_2D_3$ and cholesterol concentrations were measured by enzyme-immunoassay and enzymatic method, respectively. mRNA and protein expression were assayed by qPCR and Western blotting, respectively.

Results: In vitro, the vitamin D analogs were less transcriptionally active compared to 1,25$(OH)_2D_3$ (EC_{50} of 2.6 nM): the EC_{50} of 1α$(OH)D_3$ was similar to that for 25$(OH)D_3$ (302 vs. 307 nM), but was higher for 1α$(OH)D_2$ (650 nM) and dietary vitamin D_3 (2130 nM). In vivo, dietary vitamin D_3 failed to produce additional 1,25$(OH)_2D_3$ over control conditions (74 pM), while 25$(OH)D_3$ treatment resulted in similar 1,25$(OH)_2D_3$ levels (76 pM). By contrast, 1α$(OH)D_3$ treatment readily furnished more 1,25$(OH)_2D_3$ in plasma (162 pM). Treatment with 25$(OH)D_3$ increased Cyp7a1 protein (1.4-fold) expression and decreased Shp mRNA (44%) and liver cholesterol (41%) levels, and treatment with 1α$(OH)D_3$ led to induction of Cyp7a1 mRNA (1.7-fold) and protein (1.6-fold) expression and decreased hepatic Shp mRNA (49%) and plasma cholesterol (13%) levels, despite that the 25$(OH)D_3$:1α$(OH)D_3$ dose ratio was greater than 700-fold. Administration of 1α$(OH)D_2$, an analog that forms 1,25$(OH)_2D_2$ and 1,24$(OH)_2D_2$ as active metabolites, failed to elicit Cyp7a1, Shp or cholesterol changes.

Conclusion: While in vitro results suggest that 1α$(OH)D_3$ and 25$(OH)D_3$ are of similar potency, VDR genes in vivo were more responsive to treatment with 1α$(OH)D_3$ than 25$(OH)D_3$. This discrepancy could be attributed to the facile bioactivation that readily converts 1α$(OH)D_3$ to 1,25$(OH)_2D_3$ via 25-hydroxylase, contrasted by the much slower, rate-limiting conversion of 25$(OH)D_3$ to 1,25$(OH)_2D_3$ via 1α-hydroxylase.

66. Immunogenetic Basis of Platelet - Inhibitors Induced Hypersensitivity - Lymphocyte Toxicity Assay

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Purpose: Clopidogrel and prasugrel are commonly prescribed platelet inhibitors for patients diagnosed

with acute coronary syndrome or undergoing stent placement. Hypersensitivity reaction (HSR) after anti-platelet initiation manifesting clinically as rash, serious cutaneous adverse reactions (SCAR), and drug-induced liver injury (DILI) are HSRs affecting up to 3% of treated patients. The immunologic basis of anti-platelets HSR diagnosis remains unconfirmed in many patients.

Primary objective is to characterize the cellular and immunologic mechanism of anti-platelet HSR and describe a lymphocyte toxicity assay (LTA) for the laboratory confirmation of diagnosis in affected individuals. We also aim to understand the immune and patho-physiological mechanism(s) of platelet-inhibitors HSRs.

Methods: Blood samples from 19 patients with a clinically confirmed diagnosis of clopidogrel HSR, 2 individuals with prasugrel-HSRs and 6 tolerant controls were analyzed for immuno-toxicologic response. LTA was performed after a minimum of 12 months post drug discontinuation. Pro-inflammatory cytokine and anti-inflammatory cytokine profiles and apoptotic and necrotic assays were performed in all patients.

Results: The hematological screen identified a significant increase in circulatory neutrophils and a significant decrease in circulatory lymphocyte counts with no change in the eosinophil counts. The immunohistochemistry of the affected areas showed a predominance of $CD4^+$ and $CD1^+$ cells, with few $CD8^+$ and CD 68^+ cells. The mean percentage LTA values were significantly higher for clopidogrel and prasugrel HSR compared to control patients (20±6 vs. 7±4%, p=0.0004). The levels of tumor necrosis factor (TNF)-α levels were also significantly higher for the HSR group compared to controls (116±49 vs. 29±9 pg/mL). ROC curves for LTA demonstrated excellent utility for the diagnosis of platelet-inhibitor HSR (AUC=0.97, p=0.003) and an LTA >16% showed a sensitivity of 86% and a specificity of 100% for the diagnosis of platelet-inhibitor HSR. In addition we have been able to predict that the change of the clopidogrel to prasugrel, that was tolerated clinically, had a low toxicity as demonstrated by LTA.

Conclusion: Anti-platelet HSR is a significant immune-mediated phenomenon. This adverse event may be diagnosed and prevented by the use of the laboratory LTA, *in vitro* cell assay that is useful for patient diagnosis and drug monitoring. LTA use will create unprecedented opportunities for profiling of individual risk factors for a variety of IDHRs including anti-platelet HSRs. The implementation of LTA, an immunogenetic test, leads to personalize treating of the patients, and preventing adverse reaction thus improving human health.

67. Physiological Cell Density Culture in a Self-oxygenating and Self-cleaning Scaffold

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Purpose: Sufficient O_2 supply as well as effective waste removal is essential for high density cell culture. Chinese hamster ovary (CHO) cells are the most commonly used mammalian host for bioproduction of therapeutic proteins. The main obstacles for large scale culture of CHO cells in bioreactors include the O_2 deficiency, accumulation of ammonia wastes and limited cell densities. We intended to develop an encapsulation system that is capable of culture high-density CHO cells by in situ O_2 delivery and in situ metabolic waste removal.

Methods: We developed a self-oxygenating scaffold with a controlled O_2 release rate consisted of calcium peroxide (CaO_2), polycaprolactone (PCL) and alginate hydrogel. We managed to prepare CaO_2-PCL microparticles using a phase separation method. Subsequently, we incorporated the microparticles into the alginate hydrogel to form a self-oxygenating scaffold. We used this scaffold to culture physical-density CHO cells (2×10^8 cells/ml) with the addition of zeolites pretreated with culture medium. Calcium peroxide served as the O_2-genearting agent, and polycaprolactone was used to control the O_2 release rate from CaO_2. Pretreated zeolites were used to remove nitrogenous wastes generated by CHO cells and to supply nutrients to the cells at the same time. Cell viability in the scaffold was examined using the fluorescent staining. Ethidium homodimer-1 was used to stain dead cells into red and Hoechst 33258 stain all the cells into blue. The changes in the culture medium composition were measured using a Nova BioProfile analyzer (BioProfile 400, US) to evaluate cell metabolic activities.

Results: Our preliminary results show that the self-oxygenating scaffold with zeolites successfully maintained high cell viability for up to 10 d, while CHO cells culture in hydrogel alone died rapidly and barely survived for one day. Moreover, the addition

of CaO_2-PCL microparticles caused a higher glucose consumption rate and a lower the lactate generation rate of the encapsulated CHO cells than that of cells encapsulated in alginate hydrogel. And the presence of zeolites resulted in a lower concentration of ammonia in the culture medium compared with control.

Conclusion: In this work, we developed a self-oxygenating scaffold consisted of CaO_2, PCL and alginate hydrogel. We further added zeolites into the scaffold and encapsulated high density CHO cells into the system. Our system successfully maintained high cell viability, alleviated glycolysis and reduced the accumulation of metabolic wastes in the culture medium.

68. FoxO3a Transcription Factor Activation by 1α,25 vitamin D_3 During Osteoblast Differentiation: A Tale of Two Transcription Factors

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Purpose: It is known that active vitamin D (1α,$25D_3$) plays an important role in mineralization of remodeling bone via direction action on osteoblast cell populations. Conversely, inflammatory cytokines such as TNFα (and glucocorticoids such as prednisolone) induce rapid bone loss, due to increased apoptosis of osteoblasts and osteocytes. Those events are associated with progressively increased levels of Reactive Oxygen Species (ROS) capable of inducing oxidative stress in those cells, which ultimately result in decreases in both osteoblast number and bone mass. On the other hand, FoxO (Forkhead Box O) transcription factors are involved in bone cell proliferation, differentiation, apoptosis and survival from degenerative stimuli such as inflammation and oxidative stress. FoxO3a transcription factor is of special interest due to its tissue-specific properties. We hypothesized that in the healthy osteoblast, where FoxO3a resides mostly in an inactive phosphorylated form, the administration of 1α,$25D_3$-vitamin D receptor complex will readily bind RXR (retinoid X receptor) response element and promote bone mineralization. Conversely, in situations of oxidative stress, ROS will enhance retention of unphosphorylated FoxO3a in the nucleus and transcriptional activation, leading to the competitive reduction in available RXR for activation of the vitamin D response element for bone formation.

Methods: Undifferentiated and differentiated MC3T3-E1 osteoblastic cells were treated with 10^{-7}M 1α,$25D_3$ for 24 hours and subsequent FoxO3a expression was assessed with Western Blotting using specific antibodies. MC3T3-E1 cells were differentiated with the ascorbic acid and β-glycerophosphate. Cells were exposed to oxidative stress to generate ROS and FoxO3a expression was reassessed. Cell proliferation was measured by trypan blue cell counting, MTT assay and mineralization potential was determined by alkaline phosphatase activity and Alizarin Red S Staining followed by spectrophotometric quantification.

Results: 1α,$25D_3$ enhanced FoxO3a expression in MC3T3-E1 osteoblast-like cells during cell mineralization. Basal levels of FoxO3a protein expression were detected in undifferentiated MC3T3-E1 osteoblastic cells, with increased FoxO3a expression following temporal growth in cell culture. In contrast, significantly increased levels of FoxO3a protein expression were measured in differentiated MC3T3-E1 cells treated with 1α,$25D_3$. Conversely, oxidative stress decreased cell viability and expression of alkaline phosphatase, collagen I and osteocalcin. In the presence of oxidative stress, FoxO3a expression was decreased in the cytoplasm and increased FoxO3a expression was detected in the nucleus.

Conclusion: We demonstrated that 24h of treatment with 1α,$25D_3$ upregulated nuclear FoxO3a activity in MC3T3-E1 cells. The transcription factor FoxO3a is known to play an important role in rescuing cell damage following oxidative stress via its antioxidant actions, and as such, upregulation of FoxO3a activity may serve to reduce osteoblast damage during the course of cellular metabolism, but at the expense of new bone formation.

69. Antimicrobial Properties of Phosvitin Hydrolysates with Mineral Chelating Capacity to Improve Oral Hygiene

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Purpose: Phosvitin (PV) is a powerful chelating agent found in egg yolk. This natural

phosphoprotein can be hydrolyzed by enzymes to produce phosphopeptides. There are mineral chelating agents to detoxify the poisoning minerals for oral hygiene; however, there is no natural chelating agent available. Since PV has shown bacteriostatic effect, its application may meet for chelating minerals and inhibiting bacterial biofilms or dental plaques causing periodontal diseases and dental caries. Thus, natural mineral chelating agents are in-direct need for oral hygiene in immune-compromised patients.

Method: Phosphopeptides are short peptides with several phosphate groups that enable them to chelate different minerals. In the current research, phosphopeptides were produced from enzymatic hydrolysis of egg yolk phosvitin which is known as one of the main phosphorylated proteins in nature. Calcium-binding capacity of two peptide fractions (targeting peptides with smaller than 1 and larger than 1 kDa) obtained from different phosvitin hydrolysates (hydrolyzed for 8, 24 and 48 h) was determined.

Results: Short peptide fractions displayed mild calcium binding capacity (18-21 μg Ca^{++}/mg peptide), while the large peptide fractions showed higher range of binding capacity (38-88 μg Ca^{++}/mg peptide). These results verified the fact that peptides bind the mineral ions through cheation bonding. Thus, the longer peptides possess more phosphate groups to surround the multivalent metal ion and entrap it inside a cage-like structure.

Conclusion: Phosvitin-derived phosphopeptides have shown promising capacity in binding calcium which can be improved by further purification of the potent peptides. The oral rinse formulation containing mineral-binding peptides has the potential to retard or inhibit microbial growth as well as chelating heavy metals. Understanding the mechanism of mineral binding by these peptides enhances the application of peptides in oral rinse to improve periodontal health and prevent dental plaques.

70. Systems Genetics Approach Identifies Nicotine Receptor Antagonist as a Potential Therapeutic Target in COPD

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Rationale: The SpiroMeta-CHARGE consortium undertook the largest genome-wide association study (GWAS) for forced expiratory volume in one second (FEV1) and its ratio to forced vital capacity (FEV1/FVC) in ~48,000 individuals. We hypothesized that a subset of SNPs associated with FEV1 and FEV1/FVC act as expression quantitative trait loci (eQTL) to change the level of mRNA in lung tissue, and that the identification of lung function eQTLs will uncover the actual genes and molecules regulating lung function and provide insights into potential therapeutics for chronic obstructive pulmonary disease (COPD).

Methods: The lung eQTLs were derived from genome-wide genotyping and gene expression analysis of 1,111 non-tumor lung tissue samples from three sites: UBC, Laval and Groningen universities. The eQTL study identified ~470,000 SNPs related to the level of gene expression in *cis* (within 1 Mb from transcription start site) and ~17,000 SNPs in *trans*. We integrated SNPs associated with either FEV1 or FEV1/FVC at $P <$ 0.001 from the GWAS with eQTLs (at 10% FDR) to identify lung function eSNPs. The lung mRNA levels of lung function eSNPs regulated genes (LFERG) were tested for association with COPD in individuals from the eQTL study using logistic regression adjusted for age, gender, and smoking status. COPD association results were used as input for Connectivity Map (https://www.broadinstitute.org/cmap/) to identify drugs/compounds that could reverse or augment the COPD gene signature.

Results: For FEV1, 3419 *cis*-eSNPs mapping to 271 genes, and 1568 *trans*-eSNPs mapping to 29 genes were identified. For FEV1/FVC, 2214 *cis*-SNPs mapping to 275 genes and 442 *trans*-eSNPs mapping to 21 genes were identified. These figures

represent a significant over-representation for eQTLs; enrichment ranged from 2.5 fold for *cis* to 32 fold for *trans* acting eSNPs. The identified genes were enriched in biological processes related to lung development and inflammation. When lung mRNA levels of these genes were tested for association with COPD, 53 genes showed association at P<0.05. CMap analysis using the 53 COPD associated genes identified a number of compounds that reversed the COPD gene signature, including adiphenine; a local anesthetic which also acts as a nicotinic receptor antagonist.

Conclusion: A large number of SNPs associated with lung function affect the expression of genes in lung tissue. In silico therapeutics analysis identified adiphenine as a drug that reverses the COPD associated gene signature. Future in vitro and in vivo studies are warrented to validate the findings.

Clinical Sciences & Pharmacy Practice

71. Differences in Benzodiazepines (BDZ) Systemic Exposure during Alcohol Withdrawal Syndrome (AWS) in Patients with or without Alcoholic Liver Cirrhosis

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Purpose: Benzodiazepines (BDZ) are considered to be the drugs of choice in treating alcohol withdrawal syndrome (AWS), a serious medical problem with about 5% of patients progressing to grand mal seizures and delirium tremors. Since guidelines for treatment dosing and timing vary quite widely, it is perhaps not apparent to the physician what the rationale and the BZD drug levels will be at any given time after the initiation of treatment. In addition, BZD drugs that are used for AWS are metabolized differently (Phase I oxidative metabolism versus conjugation) and therefore the presence of alcoholic liver cirrhosis will affect drug levels of BDZ with Phase I oxidative metabolism but not those with conjugation metabolism. Therefore, the purpose of this study is to demonstrate the differences in the expected exposure to BDZ during AWS treatment using different treatment regimens available in the literature, in patients with or without alcoholic liver cirrhosis, using as an example two frequently used BDZ: diazepam and lorazepam.

Methods: Diazepam and lorazepam-based alcohol withdrawal treatment protocols were obtained from the literature. Each protocol discussed in this work in fact represents a range of treatment regimens. For simplicity, only maximal (highest-dose) treatment regimen from each protocol has been analysed and simulated in this work. The simulation of the predicted plasma concentration-time profiles of diazepam and lorazepam in healthy individuals versus patients affected by alcoholic liver cirrhosis was performed using ADAPT 5 software (Biomedical Simulations Resource, University of Southern California). The pharmacokinetics of diazepam and lorazepam was assumed to follow a 2-compartmental model.

Results: In all treatment regimens prediction shows substantially higher concentrations of diazepam in patients affected by alcoholic liver cirrhosis versus healthy individuals. In addition, there is considerable variability in the exposure to diazepam between different protocols even within the same patient population category (healthy individuals or alcoholic liver cirrhosis). The predicted plasma concentration-time profiles of lorazepam are nearly identical in healthy individuals and alcoholic liver cirrhosis patients. However, similar to diazepam protocols, there is a substantial variability in exposure to lorazepam between different protocols.

Conclusions: There is considerable variation in the blood levels with the different dosing schedules for both BZDs assessed. In addition, the metabolism of diazepam but no lorazepam is affected by liver cirrhosis.

72. Determination of Epinephrine Isomers in Pharmaceutical Formulations

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Purpose: Epinephrine, a life-saving medication, exists in two isomers of which the *l*-isomer is the pharmacologically active form. Epinephrine base is very slightly soluble in water; therefore it is available as either a water-soluble and chemically stable chloride (racemic mixture) or a bitartrate (*l*-epinephrine) salt. Availability of formulations containing epinephrine as a racemic mixture can lead to potential dose calculation errors. The objective of this study was to evaluate the isomeric purity of epinephrine in commonly used formulations.
Methods: A normal phase HPLC method using Chirex 3022 (S)-ICA and a (R)-NEA chiral column (250 mm × 4.6 mm) at ambient temperature and UV detection at 282 nm was adapted for the separation and determination of *l*- and *d*-epinephrine in eight products manufactured by different pharmaceutical companies. Epinephrine solutions tested included *Asthmanefrin*™ containing racemic epinephrine 22.5 mg/mL, *Allerject*® and *EpiPen*® epinephrine autoinjectors containing 1 mg/mL, and *Xylocaine*® containing epinephrine 0.005 mg/mL as bitartrate salt. Each product was diluted with an organic mobile phase, hexane/tetrahydrofuran/ethanol-trifluoroacetic acid (60:25:15, v/v/v), and 20 µL was injected into the HPLC system at a flow rate of 1 mL/min for a run time of 30 min. Stock solutions of racemic epinephrine hydrochloride and pure *l*-epinephrine bitartrate were prepared by dissolving each compound in water and further diluting with mobile phase for use as standards to identify the corresponding peaks of *l*- and *d*-epinephrine.
Results: Five of the epinephrine-containing products evaluated yielded single peaks representing pure *l*-epinephrine. *Asthmanefrin*® showed two peaks representing *d*- and *l*-epinephrine. One product claimed to contain the chloride salt of epinephrine (racemic mixture), but yielded a single peak of *l*-epinephrine. No peaks for either of the epinephrine isomers could be detected for *Xylocaine*® and other local anesthetic formulations containing 0.01 mg/mL of epinephrine, because the epinephrine in these diluted solutions were below detection limits.
Conclusion: Despite the well-known pharmacological activity of *l*-epinephrine, pharmaceutical products containing the racemic mixture still exist. Lack of information about the isomeric status on label claims of epinephrine-containing products can potentially lead to confusion in clinical situations, especially when dose calculations are necessary.

73. Medication Use after Laparoscopic Sleeve Gastrectomy: One Year Results from the Newfoundland and Labrador Bariatric Surgery Cohort Study

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Purpose: Patients undergoing bariatric surgery for treatment of obesity may experience improvement or resolution of type II diabetes (DM2), hypertension, and dyslipidemia. However, there is limited medication use data for the laparoscopic sleeve gastrectomy (LSG) patient population. This study aims to determine whether LSG patients require fewer medications for management of diabetes, hypertension, and dyslipidemia post-surgery.
Methods: Inception cohort (n = 201) of patients ≥ 19 years of age living in NL with BMI ≥ 35kg/m^2 with comorbid condition(s) or BMI ≥ 40kg/m^2, undergoing LSG for treatment of obesity at Eastern Health, the largest regional health authority. A nurse practitioner collects data on medication use using standard medication reconciliation and study protocol data extraction forms. Use of antidiabetic, hypotensive, and antilipemic medications pre-surgery will be compared to 1, 3, 6, and 12 months post-surgery.
Results: The sample is 81.6% female, mean age 44 (SD 9.6), BMI 48.8 kg/m^2 (6.8), and weight 135.1 kg (23.6). Comorbid conditions include DM2 (42.9%), hypertension (47.9%), and dyslipidemia (47.9%). A statistically significant reduction in medication use is observed, as early as one month after LSG. More specifically, patients are less likely to be taking antidiabetic [OR = 0.18 (95% CI 0.11-0.29)], hypotensive [OR = 0.13 (95% CI 0.06-0.28)], and antilipemic agents [OR = 0.53 (95% CI 0.39-0.72)].

Conclusion: Study findings suggest that patients with a history of diabetes, hypertension,
and/or dyslipidemia who undergo LSG are less likely to require disease specific medications post-surgery.

Acknowledgement: Matthew Hamlyn is the MUN recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

Drug Delivery & Pharmaceutical Technology

74. Effect of Intestinal Digestion and Drug Load of SNEDDS on Absorption of Halofantrine

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Purpose: The purpose of this study was to a. determine the effect of intestinal digestion on absorption of halofantrine from self-(nano)emulsifying drug delivery systems (SNEDDS) and super-saturated SNEDDS (super-SNEDDS) in the presence or absence of the lipase inhibitor Orlistat following administration to rats and b. to evaluate if the super-SNEDDS please re phrase concept is also valid in rats.

Methods: SNEDDS pre-concentrates were prepared as previously described with or without the addition of 1 w/w % Orlistat (1). The SNEDDS contained halofantrine at 75% equilibrium solubility (S_{eq}) whereas the super-SNEDDS contained the drug at 150% S_{eq}. Male Sprague Dawley (SD) rats received the SNEDDS preconcentrates containing 6.7 mg/kg halofantrine by gavage.. The plasma samples were analyzed using a Waters Acquity UPLC system. Analysis was based on a previously validated method by Humberstone et al. (2).

Results: The t_{max} is significantly longer for the SNEDDS (6.3±1.2 hr.) and the super-SNEDDS (6.0±1.0 hr.) containing Orlistat compared to the formulations without the lipase inhibitor (2.8±1.2 hr. and 1.3±0.1 hr. respectively) (P 0.0023). Moreover, the presence of Orlistat had a tendency to decrease C_{max} for both SNEDDS. Interestingly the bioavailability (F_a) did not change significantly between the groups although it seems like the F_a for the super-SNEDDS with Orlistat is higher. The super-SNEDDS have a significantly higher C_{max} (964±167 ng/mL) than the SNEDDS (506±112 ng/mL) ($P<0.05$).

Conclusion: The super-SNEDDS principle is also effective in rats. Also the incorporation of Orlistat into a SNEDDS results in a longer T_{max}, a decreased C_{max} and a seemingly prolonged absorption phase for the administrated drug. The AUC does however not change thus Orlistat co-dosing can lead to changed PK parameters, without changing the F_a.

Acknowledgements: Funding provided by the Canadian Institutes of Health Research to KMW

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75. Liposome and Co-spray-dried PVP / *o*-carborane Formulations for BNCT Treatment of Cancer

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Purpose: Boron neutron capture therapy (BNCT) is a method for selectively destroying malignant (normally glioma) cells whilst sparing normal tissue. Irradiation of ^{10}B (large neutron capture cross-section) with thermal neutrons effects the nuclear fission reaction: $^{10}B + {}^{1}n \rightarrow \rightarrow {}^{7}Li^{+} + \alpha + \gamma$; where the penetration of α-particles and $^{7}Li^{+}$ is only 8 and 5 μm, respectively, i.e., within a single cell thickness. Poor selectivity is the main reason why BNCT has not become a mainstream cancer therapy. Carboranes. a third generation of high ^{10}B -containing, low-toxicity, BNCT compounds, are currently being investigated. Towards the aim of producing new BNCT formulations, monodispersed dipalmitoylphosphatidylcholine and 1,2-distearoyl-*sn*-glycero-3-phosphocholine small unilammellar vesicle (SUV) liposomes and spray-dried powders both containing the model boron agent *o*-carborane were produced. The particle sizes were designed to pass through the blood brain barrier (< 100 nm) and target bronchioles in the lungs (1 – 2 μm), respectively, for the effective BNCT treatment of gliomas and lung cancer. Malignant cell preferential targeting was also investigated using carboranes derivatised with delocalised lipophilic cations (DLCs), specifically the dequalinium *bis nido* carborane (DC) salt.
Method: SUV liposomes were successfully produced by thin film rehydration method. Atomic force microscopy (AFM) was used to investigated morphology and stability. Liposome integrity, in serum, was ascertained using as %latency and %retention experiments using a fluorescent hydrophilic marker (calcein). The Nile Red fluorescence method and IC-MS were used to measure *o*-carborane entrapment. Uptake of DC by IN699 (glioma, WHO grade IV) and SC1800 (non-neoplastic astrocyte) cells was investigated using live cell (fluorescence) imaging. Polyvinylpyrrolidone (PVP) / *o*-carborane co-spray-dried microparticles were produced using spray drying (Buchi B-290).
Results: Liposomes were monodispersed (PDI < 0.5) and had particle diameters of *ca.* 80 – 100 nm. AFM studies showed the loaded liposomes were stable (63 days, 4°C, if re-probing was employed). The liposome membrane integrity in serum was found to be high for both types of liposomes prepared using cholesterol. Carborane was successfully entrapped in the liposomes. Specific targeting glioma cells, rather than non-neoplastic astrocyte cells, with DC was demonstrated using live cell (fluorescence) imaging. ^{1}H NMR studies revealed the high temperatures (180 °C) of the spray drying process did not degrade PVP. Mean particle diameters (x_{90}) were in the 2 – 10 μm range, with finer fractions being present ($x_{10} \cong 1 - 2$ μm). SEM imaging showed the particles to be spherical, with dimples and cavities caused by the spray drier nozzle characteristics.
Conclusion: Liposomes containing *o*-carborane had sizes suitable for transit across the BBB. The cationic moiety of DC allowed selective targeting of glioma mitochondria, thought to be due to differences in mitochondrial membrane potentials between malignant and non-neoplastic cells. PVP / *o*-carborane co-spray-dried particles had diameters suitable for delivery to the lungs.

76. Development of Vitamin A-coupled Liposomes for the Targeted Delivery of BMP4-siRNA to Hepatic Stellate Cells

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Purpose: Hepatic stellate cells (HSCs) are the main cell type in the liver responsible to development of liver fibrosis, which features the excessive secretion of extracellular matrix in the liver. Our previous study indicated that there was an elevated mRNA level of bone morphogenetic protein 4 (BMP4) in the liver and activated HSCs. The most efficient method to inhibit mRNA level is to use RNA interference (RNAi), which is a new therapeutic tool to silence almost any gene in the body. However, RNAi therapy is encountering some challenges including safety, stability and effective delivery of small interfering RNA (siRNA). The purpose of this study is to develop and characterize vitamin A (VA)-coupled liposomes for the targeted delivery of siRNA directed against BMP4 to cultured human HSCs (LX-2 cells), because HSCs contain most VA in human body.
Methods: DOTAP/DOPE liposomes surfaces were prepared by thin film hydration method. Liposomes surfaces were conjugated with VA. After that, VA-coated liposomes were mixed with BMP4-siRNA at specific ratio (10:1 mol/mol) forming lipoplexes. Particle size and zeta potential were determined using ZetaPALS. In addition, the siRNA binding efficiency and siRNA release were determined by ultra-centrifugation technique and fluorescence

assay of liposomes with fluorescence labeled siRNA. The amount of VA conjugated to liposomes surface was determined by Reverse Phase HPLC assay. The cytotoxicity of VA conjugated liposomes was evaluated by WST-1 cytotoxicity assay. Inhibition of BMP4 mRNA was determined by real time RT-PCR.

Preliminary Results: The average particle size for VA-coupled liposomes was in the range between 100-130 nm with average zeta potential around +65 mV. Lipoplexes were in the range of 200 nm and their zeta potential was around +25 mV. In addition, siRNA binding efficiency was around 80%, and VA binding efficiency was around 80 % determined by HPLC assay. Cytotoxicity assay revealed that VA-liposome at concentration of 150μg/mL, there was no significant effect on cell viability. Moreover, at dose 3μg of BMP4-siRNA, BMP4 mRNA level was reduced by around 85%.

Conclusion: VA-coated liposomes were successfully produced and they are able to deliver BMP4-siRNA to HSCs. Moreover, at no cytotoxic range of liposome concentration, VA-conjugated liposomes with BMP4-siRNA could decrease BMP4 level in LX-2 cells.

77. Mimicking the Cholera Toxin to Enhance the Intestinal Absorption of Poorly Permeable Drugs

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Purpose: In order to increase the bioavailability of poorly permeable drugs, a biomimetic approach was investigated. This approach replicated the internalisation of the cholera toxin by the intestinal cells. The cholera toxin is composed of two subunits. The A subunit causes the toxic effect while the B subunit promotes the internalisation through its specific binding to the ganglioside GM1 which is present at the surface of the enterocytes. The proposed approach was to synthesize a prodrug consisting of a low permeability therapeutic molecule and a GM1-binding peptide. The hypothesis of this work was that the peptide sequence of the prodrug will enhance the intestinal absorption of the therapeutic molecule and that the complex will be metabolised rapidly after absorption.

Methods: As a proof of concept, piperacillin, an intravenous antibiotic was chosen. This therapeutic molecule was linked to a GM-1 binding peptide (G23: *HLNILSTLWKYRC*) using a spacer. The precursor of this spacer was synthesized by reacting chloromethyl chlorosulfate with pentenoic acid in phase transfer conditions and then replacing the chlorine for an iodine using a Finkelstein reaction. Afterwards, piperacillin sodium reacted with iodomethyl pentenoate (spacer) to afford an intermediate which was conjugated to G23 using thiol-ene click chemistry. The stability of the acyloxyalkyl ester was evaluated by incubation in various media followed by LC-MS/MS quantification. The affinity of G23 with GM1 was evaluated by two different methods, which are isothermal titration calorimetry (ITC) and competitive ELISA with cholera toxin B-subunit (CTB). A third method, surface plasmon reasonance (SPR), is ongoing.

Results: Iodomethyl penteonate was obtained in a yield of 54% and piperacillin prodrug was obtained in a yield of 17%. Stability results showed that the prodrug was rapidly metabolised in human plasma (metabolism of 95% within 5 minutes) liberating the intact therapeutic molecule. Moreover, the prodrug demonstrated a pH dependant stability, with higher conservation in acidic media (pH $\leq$ 6.5). The dissociation constant resulting from ITC measurements was 2 μM at pH 6.5. In competitive ELISA tests, G23 showed an IC50 around 16 μM against CTB.

Conclusion: The results of this study demonstrated the possibility of preparing prodrugs from a GM1-binding peptide and a drug demonstrating poor bioavailability. The initial *in vitro* study highlighted promising binding and pro-drug activation properties. Future studies will involve cellular and animal absorption studies as well as preparation of other prodrugs using the same approach.

Acknowledgement: Nour Ghazal is the Université de Montréal recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

78. Hepatocellular Carcinoma Cancer Stem Cells Show Enhanced Sensitivity to Low-Density Lipoprotein Mediated Delivery of Docosahexaenoic Acid

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Purpose: Hepatocellular carcinoma (HCC) is one type of human malignant cancers with high mortality, and current standard therapies could only be successful in one third of patients. Recent evidence suggests that cancer stem cells (CSCs) within HCC are largely responsible for the observed chemoresistance. Therefore, understanding of CSC of HCC would help to develop a successful therapy for treatment of HCC. Docosahexaenoic acid (DHA) is a natural omega-3 polyunsaturated fatty acid and has been documented to possess anticancer properties. However, delivery of DHA directly into human cancer cells especially CSC is a challenge. In the present study, we isolate CSC from human HCC cells to examine the delivery and anticancer activity in isolated CSC from Huh-7 human HCC cell line.
Methods: CSCs were isolated from human HCC cell line - Huh-7 cells with cellular markers of CD133 and epithelial cell adhesion molecule (EpCAM) by fluorescence-activated cell sorting (FACS). Low-density lipoprotein (LDL) was isolated from human plasma and DHA was incorporated into LDL particles by the core reconstitution methods (here on referred to as LDL-DHA). Finally, cell proliferation was determined by WST-1 cell proliferation reagent.
Results: From $1x10^7$ Huh-7 cells, 45.4% of cells were EpCAM+CD133- (i.e. CSCs), while 11.7% were EpCAM-CD133- (i.e. "mature" malignant cells). EpCAM+CD133- HCC cells were able to proliferate and remained 70% EpCAM+ for 20 days. DHA was successfully packaged into LDL with 1453±92 of DHA molecule per LDL particle. After treatment of EpCAM+CD133- Huh-7 cells with LDL-DHA nanoparticle, there were a dose and time dependent effects on cell proliferation. At concentration of 40μM, LDL-DHA significantly inhibited EpCAM+CD133- cell proliferation within 24 hours of exposure. However, Huh-7 cells appeared less sensitive to LDL-DHA treatment because significant inhibition of Huh-7 cell proliferation was only observed at 60μM over the same time period.
Conclusion: EpCAM+CD133- Huh-7 cells were successfully isolated and maintained in culture for up to 20 days. LDL-DHA nanoparticle was able to inhibit both EpCAM+CD133- Huh-7 cells and Huh-7 cell proliferation. However, EpCAM+CD133- Huh-7 cells were more sensitive to LDL-DHA than Huh-7 cells.

79. Nanoparticles Encapsulated with Serpin A1 and LL37 Promote Wound Healing *in vitro* and Possess Antibacterial Properties

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Introduction: Chronic wounds (CWs) are a serious health care concern as they fail to resolve and are characterized by a "non-resolving" chronic inflammation. Most current therapies target only a single aspect of the CW process making it difficult to achieve effective healing hence, a new paradigm towards the development of combination therapies for CWs is urgently required. Serpin A1 (α1 anti-trypsin), is a neutrophil elastase inhibitor and a powerful anti-inflammatory agent in CW healing. LL37is a host defense peptide possessing anti-infective and wound healing properties. Thus, the combination of these peptides (A1:LL37) may show synergistic potential in accelerating healing as well as establish control over the bacterial bio-burden, when delivered to the wound site simultaneously over a prolonged period using nanoparticles.
Objective: To evaluate nanoparticle encapsulated with A1:LL37 as a new potential combination strategy for accelerated wound healing and antibacterial properties.
Methods: Solid lipid nanoparticle (SLNs) encapsulated with A1:LL37 were made using solvent-diffusion double emulsion technique. The *in vitro* release study was performed in artificial wound fluid (AWF) pH 7.4 as release media, incubated in an orbital shaker at 37 ºC. Aliquots were withdrawn at fixed intervals and analyzed by HPLC. The *in vitro* wound healing assay and cytotoxicity studies were performed on fibroblast and keratinocyte cells. Minimum inhibitory concentration of A1:LL37 against E.coli and S.aureus and synergy of the drug combination was evaluated using checkerboard microtiter plate assay by determining ΣFICI.
Results: SLNs had an average particle size of 210 ± 5.6 nm and encapsulation efficiency of 85.4 ± 3.2. *In vitro* release studies in AWF demonstrated a daily release of ~15.2% of the initial loaded concentration of A1:LL37, over a 72h study period. *In vitro* scratch assay showed high level of healing in fibroblasts treated with A1:LL37 loaded SLNs

(500µg each, in comparison to untreated cells. No cytotoxicity was observed when fibroblasts and keratinocytes were treated for 24h with drug-free SLNs as well as A1:LL37 SLNs. ΣFICI of A1:LL37 was ≤ 0.5, demonstrating synergy against E.coli and S. aureus in comparison to individual drugs alone.
Conclusion: This is the first study to develop a nanoparticle formulation for the combination delivery of serpin A1 and LL37 as a novel strategy for the treatment of chronic wounds.

80. Evaluating the Impact of Hydoxychloroquine-Loaded Polyurethane Intravaginal Rings on Lactobacilli

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Introduction: Intravaginal ring (IVR) drug delivery systems have been extensively evaluated for the delivery of hormones for contraception and in the development of microbicides. In this study, we investigated the impact of polyurethane IVRs loaded with the immunomodulatory drug hydroxychloroquine (HCQ) on the major flora found within the FGT specifically Lactobacillus Jensenii, Lactobacillus Cryspatus and Lactobacillus Hansen and Mocquoct.
Methods: IVR segments were fabricated using medical grade polyurethane HP-60D-35 by hot-melt injection molding. The lumen of the IVR segments were filled with HCQ mixed with hydroxypropyl methylcellulose at a 1:1 weight ratio. The impact of free HCQ alone on lactobacilli was evaluated using the microplate dilution method. The toxicity of drug-free IVR segments was evaluated on bacteria using an elution assay method. The impact of HCQ-loaded IVR segments on lactobacilli growth was evaluated by incubating the IVR segments in MRS broth for various time points, followed by the addition of bacteria (10^5 CFU/mL) into the media. In vitro cytotoxicity of the IVR segments was assessed using the vaginal epithelial cell line VK2/E6E7 and the ectocervical cell line Ect1/E6E7 using the MTS assay.
Results: Sustained and controlled release of HCQ was achieved from the IVR segment for up to 2 weeks with an average release of 40 µg/mL/day. HCQ concentrations of up to 1.8 mg/mL appeared to have no significant impact on lactobacilli growth. Furthermore, drug-free IVR segments had no significant impact on the growth of lactobacilli or the viability of vaginal and cervical epithelial cells when compared to controls.
Conclusions: We describe for the first time, an IVR drug delivery system that can provide controlled release of HCQ for 14 days and is non-cytotoxic towards lactobacilli and vaginal/cervical epithelial cells.

81. Identification of Target Receptor Protein for P160-based Cancer Targeting Peptides

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Purpose: To identify the target receptor protein for P160-based cancer targeting peptides, which have shown to specifically bind to breast cancer cells and consequently reduce the number and severity of side effects that are normally associated with the use of chemotherapeutic agents such as Doxorubicin.
Methods: P160 was synthesized using Fmoc-SPPS. MCF7, MDA-MB-435, and MCF10A were grown in cell culture, lysed and centrifuged to cytoplasmic and microsomal compartments thought to contain the soluble and integral membrane proteins, respectively. Following affinity chromatography using competitive elution, the eluates were collected and run on SDS-PAGE. In-gel digestion was performed on the suspected bands from the SDS-PAGE, likely to contain the putative receptor(s), to prepare samples for Mass Spectrometric (MS) analysis.
Results: Molecular weights of three proteins were matched between the list of hits from the MS and the SDS-PAGE results.
Conclusion: A modified affinity chromatography protocol has been established for eluting the specific P160 receptor. Three proteins have been identified that may be the cancer-specific receptor of interest. Further investigation is required and will be performed to confirm the identity of this receptor using receptor-specific antibodies.
Acknowledgement: Kamran Bahadorani is the University of Alberta recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

82. An Amphipathic, Cationic Peptide mediated siRNA Delivery in 3D Culture

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Purpose: RNA interference is a natural regulatory process where small, double-stranded RNA molecules (typically 21-25 nucleotides) turn off specific genes in a biological cell. siRNA therapeutic has been hampered by its hydrophilicity, negative charge, and sensitivity to enzyme degradation. Thus, a safe and effective delivery system is mandatory. Our group has designed and characterized an amphipathic, cationic peptide (STR-H16R8), which can achieve pronounced siRNA uptake and gene silencing in monolayer culture. However, due to the lack of characteristics present in live tissues, the preliminary data generated in monolayer cultures may not be predictive as that *in vivo*. Therefore, a 3D cell culture model mimicking the *in vivo* tumors is utilized here to evaluate the cytotoxicity and gene silencing efficiency of STR-H16R8-siRNA complexes.
Methods: The 3D culture model of A549 cells was established using hanging drop method and characterized with light microscopy, confocal microscopy, and scanning electron microscopy. The cytotoxicity and gene silencing efficiency of STR-H16R8-siRNA complexes were quantified with WST-1 assay and qRT-PCR respectively.
Results: A549 cells in 3D culture closely mimicked the properties of tumors proven through microscopic characterizations. STR-H16R8-siRNA complexes induced significant Bcl-2 gene silencing efficiency with minimal cytotoxicity. Note that the complexes achieved lower gene silencing efficiency in 3D than that in 2D, possibly because of the complex microenvironment in 3D culture.
Conclusion: This data provides evidence of the differences in delivering siRNA to 3D cell culture model, comparing to monolayer cell cultures. The simple and reproducible 3D cell culture model introduced here is a useful system for evaluating the future gene delivery systems.

83. PBPK Modeling of Dextromethorphan and its Application in Developing Drug Delivery System

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Purpose: Physiological based pharmacokinetic (PBPK) *in silico* modeling has become an integral tool in formulation development especially in Quality by Design approaches. PBPK models offer an approach in which direct relationship between in vitro release and in vivo release can be made without requiring a linear relationship. PBPK provides the frame work for the integration of physiological environments and in vitro data to construct mechanistic models that better represent absorption, distribution, metabolism and excretion processes. The current study investigated how PBPK modeling can assist the formulation scientist in developing a drug delivery system especially for drugs, which undergo metabolic variations in healthy individuals. Dextromethorphan was selected as a model drug which undergoes extensive metabolism or poor metabolism in humans due to their CYP2D6 & CYP3A4 phenotypes.
Methods: Gastroplus™ 8.5 (Simulation Plus, Inc.) Advanced Compartmental Absorption and Transit (ACAT™) model and PBPKplus modules were used to build the dextromethorphan (DM) model. The model was validated using a clinical study. We simulated different in vitro drug dissolution profiles at a fix dose of 30mg DM.
F_1 formulation had a zero order release kinetic.
F_2 formulation had a sustained order release kinetic.
F_3 and F_4 were a combination of an immediate release and zero order release dosage form.
F_3 released 35% drug in 30 min followed by the zero order release kinetic to 100% until 24 hours.
F_4 released 70% drug in 30 min followed by a zero order release kinetic to 100% until 24 hours.
F_5 released 50% drug initially followed by pulse release of 50% drug at 2hours.
Results: There was a significant difference in drug plasma profiles between extensive metabolizer and poor metabolizer which matched well with the reported clinical studies. The difference was due to variations in drug metabolizing capacity which was included in the PBPK model. There was a gradual decrease in C_{max}, shortening of T_{max} and comparable AUC_{0-24} as the formulation release profiles were

altered from the F_1, F_2, F_3 and F_4 tablets, respectively. However the F_5 formulation showed some promise and had comparable drug plasma profiles that matched well a desired drug plasma profile with constant drug plasma levels.

Conclusion: I*n s*ilico PBPK modeling is able to help the formulation scientist to predict the drug plasma profiles of dosage forms with different release profiles. The F_5 formulation showed a desired drug plasma profile. The use of PBPK will help the formulation scientist to design and develop new dosage forms with desired drug plasma profiles. This will be important for Quality by Design approaches.

84. Targeted Chemotherapy: Trastuzumab Modified Docetaxel Loaded PLGA Nanoparticles for HER2 Positive Breast Cancer

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Purpose: Human epidermal growth factor receptor (HER2) overexpresses in around 30% of breast cancers that creates a rational and logical argument for the idea of designing HER2 targeted drug delivery system. The main purposes of this study is to develop Trastuzumab tailored Docetaxel loaded poly(D,L-lactic-co-glycolide (PLGA) nanoparticles (NPs) and to investigate the effects of this delivery system against HER2 overexpressed (SKBR-3) and moderate expressed (MCF-7) breast cancer cells.

Methods: Docetaxel loaded PLGA NPs were prepared using emulsification-solvent evaporation method. Trastuzumab was covalently attached with freeze-dried bis(sulfosuccinimidyl) suberate (BS3) pre-activated NPs. Targeted therapeutic NPs were characterized for size, surface charge, drug loading, antibody quantification and afterwards, the effects of these NPs were evaluated following uptake study, levels of HER2 expression, and apoptosis study in the respective cell lines.

Results: The average size of targeted NPs was found below 500 nm and surface charge shifted towards positive after antibody conjugation. Docetaxel encapsulation efficiency reached to 85% for drug loaded formulation and amount of drug loaded per mg of NPs was found to be maximum 7.13 ± 0.89 μg. Antibody attachment efficiency was found in a range between 50% to 60% of initial amount of antibody to be attached. Targeting efficiency of antibody was determined by clear cellular localization of Coumarin-6 loaded NPs. Covalently attached Trastuzumab modified NPs showed comparatively better cellular uptake compared to physically adsorbed antibody modified and unmodified NPs. Uptake studies were also performed with Trastuzumab pre-incubated cells which resulted in significant reduction in NP uptake into overexpressed SKBR-3 cells compared to moderate expressed MCF-7 cells. In addition, different levels of HER2 expression were measured following flow cytometry analysis, western blot and fluorescence microscopic studies. A significant reduction for the level of HER2 expression was observed for targeted drug loaded NPs in HER2 overexpressed SKBR-3 cells. However, both cell types did not show any significant percentage of cell death or early apoptosis for antibody modified drug loaded NPs compared to free drug.

Conclusion: Notably, a discernible reflection was observed for the presence of Trastuzumab onto the NP formulation compared to all control groups such as Herceptin®, Trastuzumab, free drug, drug free unmodified and modified NPs, and drug loaded unmodified and modified NPs. Overall, *in vitro* data for Trastuzumab modified Docetaxel loaded PLGA NPs have demonstrated a prospective potentiality to be applied as targeted chemotherapy against HER2 overexpressed breast cancer.

85. Externally-mediated Enhanced Pulsatile Drug Release from Injectable, Magnetic 'plum pudding' Hydrogels Nanocomposites with Embedded Microgels

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Purpose: Numerous therapies could benefit from better control of both the site and rate of drug release, particularly for therapeutics that require multiple injections (insulin) or where localizing the drug is advantageous (chemotherapeutics). As a result, "smart" materials that are capable of externally-actuated drug release would be highly desirable to allow for a high degree of control in a patient-compliant manner.

Method: An injectable nanocomposite was developed that is comprised of thermosensitive microgels and superparamagnetic iron oxide

nanoparticles (SPIONs) embedded within an injectable thermosensitive hydrogel matrix derived from *in situ* gelation of PNIPAM-hydrazide and dextran-aldehyde polymers. SPIONs can generate heat in response to an external alternating magnetic field (AMF) that is then transferred to the thermosensitive microgels to induce deswelling, creating free volume for enhanced drug release. This is a reversible process that is entirely controlled by the external AMF. Control over pulsatile release was achieved with an AMF system developed to maintain nanocomposites at a specific baseline temperature (typically set at 37°C), while the temperature of the nanocomposites can be increased to ~6°C above this baseline temperature when an AMF is applied.

Results: Dynamic light scattering showed that the microgels within the nanocomposites experience a ~90% decrease in volume when heated from 37°C to 43°C. The composites were also confirmed to be superparamagnetic in nature, degradable, and to exhibit minimal cytotoxicity *in vitro*. The proposed mechanism of release was confirmed by showing that release from these nanocomposites is dramatically enhanced over control composites lacking either the microgel or SPION component for long AMF exposures and intermittent 10 minute AMF pulses over multiple days (with up to a four-fold enhancement in the rate of release due a pulse). The effects of microgel composition, microgel content, the baseline temperature, the hydrogel thermosensitivity, and whether the SPIONs are embedded or crosslinked into the network on the AMF-mediated pulsed drug release over multiple days will be demonstrated.

Conclusion: The externally-mediated drug release shown from these injectable, degradable hydrogel nanocomposites could benefit a multitude of current therapies. The major factors influencing AMF-controlled drug release were studied to ultimately fabricate nanocomposites that experience a 4-fold increase in the rate of release in response to a short, 10 minute AMF pulse.

86. Design of Stable Block Copolymer Micelle-Based Drug Formulations using AB Copolymers Synthesized by Living Anionic Polymerization: Effect of Pendant Functional Groups on Aggregation Behaviour and Drug Retention

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Purpose: Block copolymer micelles have emerged as a viable delivery technology with drugs relying on formulation in these systems now in late stage clinical development. One of the major challenges facing block copolymer micelle-based drug formulations is that they often only serve to solubilize the drug and have poor stability and limited drug retention. In this study, new copolymers were designed with the aim of improving the affinity between a hydrophobic drug and the core of the micelle.

Methods: Living anionic polymerization of oxirane derivatives on methoxy-polyethylene glycol (MePEG) was used to obtain amphiphilic block copolymers that varied in terms of the nature of their hydrophobic blocks. Polymerization of allyl glycidyl ether (AGE) enabled functionalization with pendant alkyl chains (C_6 and C_{12}) by thiolene click chemistry (mPEG-*b*-(AGE-$C_{6 \text{ or } 12})_n$) while polymerization of phenyl glycidyl ether (PheGE) resulted in a polyaromatic hydrophobic block ((mPEG-*b*-$(\text{PheGE})_n$). In aqueous media, these block copolymers self-assemble to form micelles that include a hydrophobic core surrounded by a hydrophilic shell or corona driven by the alkyl chain interactions or π-stacking.

Results: Characterization by gel permeation chromatography revealed narrow molecular distributions (PDI<1.04) for the copolymers and ^1H NMR spectroscopy confirmed successful polyalkylation of mPEG-*b*-(AGE-$C_{6 \text{ or } 12})_n$. The three copolymers were found to have critical micelle concentrations of less than 30 µg / mL. The aggregation behaviour of the copolymers was further evaluated by dynamic light scattering, transmission electronic microscopy, atomic force microscopy and fluorescence spectroscopy techniques. Depending on

the nature of the core-forming block, the copolymers were found to form aggregates of different morphologies including worm-like, vesicular and spherical with D_h < 50 nm. The materials were evaluated in terms of ability to solubilize the anti-cancer drug doxorubicin (DOX). For each copolymer, stable micelle formulations were prepared that included high DOX to copolymer ratios of up to 20 % (wt%) with drug loading efficiencies > 60 % (w/w). *In vitro* drug release studies revealed sustained release profiles and assessment of cytotoxicity in MDA-MB-468 breast cancer cells confirmed that the activity of the drug was conserved.
Conclusion: The promising preliminary results obtained for these copolymers, which include alkyl and aromatic functional groups, encourage additional evaluation of stability and drug delivery *in vivo*.

87. Flipids: From a Molecular Switch to pH-triggered Delivery of Gene and Drugs

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Purpose: It has been shown that it takes less than one hour for newly endocytosed liposomes to reach highly acidic lysosomes, meaning that the degradation of the content occurs rapidly, thus limiting the bioavailability of the delivered gene or drug. We propose to use new pH-sensitive lipids able to quickly change their conformation upon protonation at endosomal pH values, leading to the disruption of the lipidic bilayer and thus to the fast release of the liposomal content. We propose to use the designation “flipid” (conformational flip + lipid) to describe these molecules.
Methods: Cationic (gene delivery) and non-cationic (drug delivery) flipids were synthesized and characterized. pH-sensitive fliposomes incorporating our molecules and the commercial co-lipid DSPC were prepared using manual extrusion. We performed sulforhodamine B leakage and R-18 lipid-mixing experiments to characterize the pH-sensitive destabilization and fusogenic behaviours of the PEGylated non-cationic fliposomes. The endosomal escape properties of these non-cationic PEGylated fliposomes were studied on HeLa cells using fluorescent microscopy, observing the intra-cytosolic release of the highly polar sulforhodamine B dye. Gene silencing delivery of cationic fliposomes was assayed performing anti-GFP *si*RNA delivery on stable HeLa-GFP cells. Transfection efficiency was measured using flow cytometry. Finally, the cytotoxicity of the fliposomes formulations was assessed using a resazurin-based toxicology assay.
Results: Sulforhodamine B leakage experiments of non-cationic fliposomes confirmed the anticipated fast pH-sensitive behavior. The best formulation was able to release 88% of its content at pH 5 in only 15 minutes, whereas no leakage was observed at pH 7.4. Lipid-mixing experiments against empty POPC vesicles showed a 5-fold increase in R-18 dequenching fluorescence intensity at pH 5, as compared with dequenching intensity at pH 7.4. This confirms the fusogenic properties of the fliposomes at endosomal pH values. Fluorescent microscopy experiments showed that our best non-cationic fliposome formulation was able to efficiently deliver the highly membrane-impermeable sulforhodamine B dye in the cytoplasm of HeLa cells, *via* an endosomal escape pathway. The cationic fliposomes formulations were able to efficiently silence GFP expression on HeLa cells (50% knockdown). Resazurin-based toxicology assays showed that cationic and non-cationic fliposome formulations are non-toxic for HeLa cells (at least 85% of viability following a 48h incubation period).
Conclusion: We successfully reported the elaboration of pH-sensitive fliposomes, incorporating synthetic pH-sensitive cationic or non-cationic flipids we designed. Fluorescence experiments (leakage of an entrapped hydrophilic dye according to the pH, R-18 lipid-mixing assays and microscopy experiments) showed us that our non-cationic fliposomes seem suitable for intra-cytosolic pH-sensitive delivery of polar drugs. Cationic fliposomes showed strong gene silencing efficiencies on HeLa cells.

88. Novel Anti-Cancer Compounds for Cervical Cancer

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Purpose: In this study, we investigate the cytotoxicity effect of a number of novel compounds for cervical cancer cell lines. These compounds were

designed as thiol alkylating agents. In the case of NC 1153, the potential pharmacophoric group namely an alpha, beta-unsaturated keto moiety will be formed on deamination. In the other cases, the biologically-reactive group is the 1, 5-diaryl-3-oxo-1,4-pentadienyl substituent which can act sequentially with cellular thiols.

Methods: Four novel compounds were synthesized in Dr. Dimmock's laboratory and coded as NC1153, NC2140, NC1910 and NC 2095. The compounds were tested against the cervical cancer cell line, Hela cells. Different numbers of cells were treated with various concentrations from solutions of NC1153, NC2140, NC1910 and NC2095 in DMSO. Docetaxel was used as a positive control in the experiments. After incubation for different time points, MTT assay was performed to evaluate the toxicity of the compounds on Hela cells.

Results: These results indicated that compounds NC1153 and NC1910 showed very high toxicity to cervical cancer cells, comparable to docetaxel. NC2095 demonstrated moderate toxicity, whereas no toxicity was observed with NC 2140. 48 hours was found to be the optimum incubation time for our study. At the experimental conditions, increasing the number of cells did not reduce the effect of compounds on cervical cancer cells, while docetaxel showed lower effect at higher number of cells. The preliminary studies have shown that NC1153 could inhibit JAK kinase pathway through STAT3 inhibition.

Conclusion: Three of these novel compounds showed promising results on cervical cancer cells, which could be further investigated for the mechanism by which they inhibit the cell growth.

Acknowledgement: Matthew Walliser is the recipient from University of Saskatchewan of the GSK/CSPS National Undergraduate Student Research Program Award.

89. Study of the Permeability of Cellular Membranes using Proteoliposomes

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Purpose: Membrane permeability regulates the absorption of xenobiotics through the intestinal barrier via two main processes: passive diffusion and active transport. In order to predict how new molecules will be distributed in the cellular compartments of the body, two main assays are currently used: Caco-2 and PAMPA. Unfortunately, the Caco-2 assay is limited by data variability and is very time consuming. The PAMPA assay only enables us to study passive diffusion. The aim of this study is to evaluate a new tool for measuring permeability, which does not have the drawbacks of current techniques. Thus, we propose to use proteoliposomes to develop non-cellular models of membrane permeability including efflux and uptake transporters. This novel model of permeability will be utilized to study the underlying mechanisms of membrane permeability to xenobiotics. During this project we will focus on the extraction and the purification of the human membrane transporter P-glycoprotein (P-gp).

Methods: To begin, plasma membranes enriched in P-glycoprotein from MDCK-MDR1 cells (Madin-Darby canine kidney cells transfected with the human MDR1 gene) were extracted by sucrose cushion. These plasma membranes were then solubilised using 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and yielded partially purified human P-glycoprotein (P-pg).

P-glycoprotein purification was monitored by SDS-PAGE and immuno-dot blotting using the monoclonal antibody C219. ATPase activity of the final product was determined by measuring the release of inorganic phosphate from ATP, using a colorimetric method.

Results: The extraction method is functional but did not allow us to obtain large quantities of the membrane transporter. In addition, the P-gp extracts were not totally purified. The final material contains many other proteins that could interfere with future experiments.

Conclusion: It was decided that we would explore a new method of obtaining purified P-glycoprotein using another expression system. The yeasts *Pichia Pastoris* present the advantage of being able to produce P-glycoprotein in a large amount at a high level of purification. Using this method, it might be possible to work with other membrane transporters using different expression vectors and to build different permeability assays.

90. Lipids Potentiate the Immune Response Produced by Targeted Nanoparticulate Vaccines

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Purpose: Polylactide co-glycolide (PLGA) is an FDA approved polymer to be used in human. The PLGA vaccine delivery system prepared with the antigen (ovalbumin)-adjuvant (MPLA) combination is particularly promising for clinical application due to their low immunogenicity, low toxicity, biocompatibility and biodegradability. Dendritic cells (DC) targeting vaccines devoid of nanoparticulate system are limited by insufficient antigen uptake by DCs, low internalization and instability of soluble materials in-vivo. Thus the concept of utilizing nanoparticulate system to deliver the cargo arises. We hypothesize that lipid carrying vaccine significantly enhances the immune response compared to the non-lipidic vaccine. This research aims to formulate a delivery system that would enable DCs to efficiently capture tumor antigens that are released from tumor cells.
Methods: Double emulsification solvent evaporation method was used to prepare the nanoparticles. The DC targeting ligand was attached on the nanoparticle surface through covalent binding in presence of the spacer molecule and physical adsorption method. Flow cytometry experiments were done to assess the upregulation of maturation markers on the DC surface. Enzyme linked immunosorbent assay (ELISA) was done to quantify the levels of different cytokines.
Results: Results indicated that several groups of nanoparticles with different viscosity grades had suitable physicochemical properties for in-vitro biological experiments. DC uptake study shows when ligand is covalently attached, higher uptake of nanoparticles was observed compared to ligands that are adsorbed. This indicates the targeted nanoparticles carrying both MPLA and ovalbumin will be better uptaken by DCs. Following evaluation of DCs maturation, targeted nanoparticles showed upregulation of maturation markers such as CD40, CD86 and MHCII molecules. In 0 .18 i.v. COOH terminated nanoparticles, DCs showed 4, 6 and 7 fold increase in mean fluorescent intensity for MHCII molecules when treated with MPLA-nanoparticle, ova-MPLA nanoparticle or ligand-ova-MPLA nanoparticle, respectively. Therefore, presence of lipid in the formulations can potentiate the maturation of DCs, which is necessary to induce activation of T cells. Treatment of DCs with MPLA containing nanoparticles released higher amount of Th1 cytokines (IFN-gamma, IL-12, IL-2) than control groups.
Conclusion: Therefore, the delivery of ovalbumin in PLGA nanoparticles to DCs induced potent in vitro antigen-specific primary Th1 immune responses that were furthermore enhanced with co-delivery of the lipid MPLA along with the antigen in the nanoparticle formulation.

91. Nystatin Nanosuspensions: Comparative Investigation of Production Methods and Stability Study

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Purpose: Pharmaceutical nanosuspensions can be produced by particle size reduction of drugs. Several technologies can be used for this purpose such as high pressure homogenization (HPH), low energy media milling (LEM) or high energy media milling (HEM). The objective of this study is to compare three different technologies for the production of a nystatin nanosuspension while taking into account process efficiency and product quality.
Methods: A commercial nystatin suspension was used as the reference formulation. The formulation was processed using HPH, LEM and HEM where the batch sizes ranged from 0.5 to 2.5L. Nanosuspensions were characterized for particle size, zeta potential, viscosity, crystallinity, *in vitro* activity against *C. albicans* using disk diffusion tests and assayed for content by HPLC. The nanosuspensions were evaluated for one month at two stability conditions: 25°C/60%RH and 40°C/75%RH.
Results: All processes were efficient in respect to the time needed to obtain adequate particle size and production yield. It should be noted though that production process flow had to be modified to accommodate LEM and HEM, whereas HPH could be used as is. Nanosuspensions obtained by LEM or HEM had a smaller particle size distribution than the ones obtained by HPH: median of 169 ± 12 nm and

433 ± 46 nm respectively. However, LEM and HEM showed significant aggregation after one month (micron scale), whereas HPH suspensions did not. Viscosity of nanosuspensions was lower than the viscosity of the commercial suspension and remained unchanged after one month. Zeta potential reflected the stability of the nanosuspensions. Nanosuspensions produced by LEM and HEM had a smaller zeta potential than HPH suspensions: -26.6 ± 4.5 mV and -36.5 ± 4.9 mV respectively. Zeta potential decreased after one month, which indicated that the nanosuspensions adopted compact conformation or aggregated. For all nanosuspensions, nystatin particles retained their crystalline structure. *In vitro* tests demonstrated that LEM and HEM had a higher diameter inhibition of *C. albicans* versus HPH suspensions due to smaller particle size. HPLC analyses revealed no degradation and no change in assay.

Conclusions: The commercial nystatin formulation could be nanosized using the 3 techniques evaluated. With respect to the current commercial formulation, LEM and HEM yielded a smaller particle size distribution at manufacture than HPH nanosuspensions, but were found to be less stable physically.

92. Towards Glaucoma Gene Therapy: Minicircle Gemini-surfactant Phospholipid Nanoparticles for the Delivery of Brain-derived Neurotrophic Factor in Retinal 3D Co-culture Models

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Purpose: Brain-derived neurotrophic factor (BDNF) is a protein in the neurotrophin family that is essential for the development, maintenance, and survival of neurons. Glaucoma is a multifactorial neurodegenerative disease where stressors such as neurotrophin deprivation and oxidative stress play a pivotal role in the disease progression that leads to retinal ganglion cell apoptosis. Herein, we have developed minicircle (encoding BDNF) - gemini surfactant -phospholipid nanoparticles (GL-NPs) as a non-viral gene delivery system, and a three-dimensional co-culture model of retinal cells to evaluate BDNF gene expression.

Methods: Nanoparticles were prepared using 16-3-16, and 18-3-18 gemini dicationic surfactant (m-s-m; m= alkyl tail; s= spacer) through complexation with either parental plasmid or minicircle in conjunction with dioleoylposphatidylethanolamine (DOPE) neutral helper phospholipid. Therapeutic plasmid was constructed by cloning BDNF gene into MN5301A-1 parental plasmid vector (SBI). Parental/minicircle plasmids were produced by growing parental plasmid transformed ZYCY10P352T E.*coli* without/with induction period of 5.5 hours, respectively. Physicochemical characterization (size and zeta potential) of GL-NPs was performed using dynamic light scattering. Transfection efficiency of GL-NPs was evaluated in A7 rat optic nerve-derived astrocyte cells cultured as monolayers based on green fluorescent protein (GFP) expression. Retinal co-culture was established using a model retinal cell line (RGC-5) cultured in both scaffold and scaffoldless 3D culture systems and was characterized using confocal laser scanning microscopy.

Results: The 16-3-16 and 18-3-18 GL-NPs formulated with regular parental plasmid and minicircle exhibited average particle sizes of 144.43±1.06 nm and 192.57±1.16 nm with zeta potential of +43.43±0.60mV and +45.50±0.53mV for the regular plasmid; and 122.30±1.25 nm and 203.83±2.74 nm with zeta potential of +40.53±4.05mV and +45.90±1.71mV for the minicircle using 16-3-16 or 18-3-18 GL-NPs, respectively (n=3). Transfection efficiency using minicircle increased twofold compared to regular plasmid (4.23±0.99% vs 2.34±0.77% and 1.83±0.77% vs 1.40±0.67% for 16-3-16 and 18-3-18 GL-NPs, respectively). Preliminary studies in retinal co-cultures constructed using Alvetex and RAFT culture models show that these two platforms mimic the *in vivo* retinal architecture, providing suitable proximity of different cell populations for the evaluation of transgene-mediated effects with high *in vivo* relevancy.

Conclusion: The development of minicircle GL-NPs and three-dimensional co-culture is beneficial at two levels: GL-NPs formulated with minicircle plasmid may express sufficient level of BDNF to provide neuroprotection of retinal ganglion cells; and the three-dimensional retinal co-culture model could provide a useful platform for the evaluation of gene delivery systems for the treatment of glaucoma.

93. A Flow Cytometric Approach to Study the Mechanism of Gene Delivery to Cells by Gemini-lipid Nanoparticles: An Implication of Cell Membrane Nanoporation

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Purpose: The aim of this study was to evaluate gene delivery by a series of non-viral dicationic gemini surfactant-phospholipid nanoparticles (GL-NPs) and to explore their mechanism of interactions with the cell membrane of PAM212 epidermal keratinocyte cells.

Methods: GL-NPs containing pCMV-tdTomato plasmid coding for red fluorescent protein (RFP) were prepared using 12 different gemini surfactants (12, 16 and 18C alkyl chain length series) and dioleoylphosphatidylethanolamine (DOPE) helper lipid. RFP gene expression and cell viability status were evaluated using MitoTracker Deep Red mitochondrial stain as an indicator of cell viability and the cell impermeable Sytox red nuclear stain as an indicator of cell membrane integrity by flow cytometry.

Results: No significant viability loss was detected in cells transfected with 18-3-18, 18-7-18, 18-7NH-18, and 18-7NCH3-18 NPs, whereas a significant reduction of viability was detected in cells treated with 12-3-12, 12-7-12, 12-7NH-12, 16-7NH-16, or 16-7NCH3-16 GL-NPs. Compared to Lipofectamine plus, 18-3-18 GL-NPs showed higher transfection efficiency (13.58± 1.33% *vs.* 7.13±2.37%) and similar viability profile (84.03 ±12.36% *vs.* 86.84±18.06%) by evaluation using MitoTracker Deep Red in PAM212 cells. Flow cytometric analysis of PAM212 cells stained with Sytox red revealed two cell populations with low and high fluorescent intensity, representing cells with partially-porated and highly-porated membranes, respectively. Additional combined staining with MitoTracker and ethidium homodimer (a membrane impermeable DNA dye) showed that that 18-3-18 GL-NPs disturbed cell membrane integrity, while cells were still alive and had mitochondrial activity.

The SSC (side scatter) parameter, as an indicator of cell granularity and cellular uptake of nanoparticles, evaluated in RFP positive cells transfected with 18-3-18 GL-NPs or Lipofectamine Plus, showed that in spite of NP uptake, the majority of RFP positive cells transfected with 18-3-18 GL-NPs or Lipofectamine Plus (10.54% and 3.23%, respectively), did not show high SSC intensity. This result suggests that an increase in SSC intensity may not be a solid proof for NP uptake, as the visibility of nanoparticles in SSC depends on their size and density (refractive index).

Conclusions: This study found higher transfection efficiency and comparable viability profile of 18-3-18 GL-NPs to Lipofectamine Plus. The interaction of 18-3-18 GL-NPs with PAM212 cell membranes may lead to the formation of nanoscale pores, which could be a possible explanation of the efficient gene delivery. This novel nanoconstruct appears to be a promising nanocarrier candidate for further skin gene therapy studies *in vivo*.

94. Neurotrophic Factor Gene Therapy in Glaucoma by Minicircle DNA Biopharmaceuticals

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Purpose: The fast-growing field of gene/cell-based therapy is rapidly expanding as the ultimate approach to prevent and treat neurodegenerative disorders such as glaucoma. Among different classes of vectors for gene therapy, plasmid DNA (pDNA) is one of the promising classes of biopharmaceuticals for gene transfer because the therapeutic agent of interestis generated by the cell's transcriptional machinery. The newest development is the pDNA-derived bacterial-sequence-depleted minicircle DNA (mcDNA) vector, a miniaturized non-viral and non-integrating DNA vector that carries only the therapeutic gene expression cassette. These vectors show improved performance due to smaller size and higher immunocompatibility due to the avoidance of bacterial sequences relative to traditional pDNA vectors. In this study, we have constructed and evaluated several mcDNA vectors encoding brain derived neurotrophic factor (BDNF) with rational and versatile gene expression cassette design to find the optimal construct for *in vitro* and *in vivo* ocular non-viral neutrophic factor-based gene therapy applications.

Methods: We have constructed pDNA vectors to

encode *BDNF* cDNA under the control of EF1/CMV promoters with *GFP/RFP* reporter gene that were expressed as (cis) either as a separate protein (separated from BDNF transgene by IRES) or as a tagged protein. These constructs were replicated in genetically engineered phiC31-integrase^{+} *E.coli* cells (System BioScience, USA) that carried out the production of mcDNA derivatives. pDNA and mcDNA vectors were complexed with K2 cationic lipid (Biontex, Germany) to form nanoparticles and transfected into A7 rat optic nerve-derived astrocyte cells.Gene expression was assessed by flow cytometry and confocal laser scanning microscopy.Physicochemical characterization of K2 nanoparticle was performed using dynamic light scattering.

Results: Several different mcDNA vectors were constructed to test the effect of size, promoter, and structure. Minicircle mc*EF1-BDNF-IRES-GFP* (4.7 kb) and its respective parent construct p*EF1-BDNF-IRES-GFP/RFP* (8.7 kb) were compared by forming nanoparticles with K2 cationic lipids. Zetasizer measurements demonstrated a construct size-dependent change in particle size (102.5±0.9nm vs. 86.2±0.5nm) with zeta potential of 52.9±0.8mV vs 53.3±0.4mV. The smaller mcDNA vector showed higher RFP expression levels relative to their pDNA precursors (4.7±0.3 vs 1.3±0.3%) and higher cell viability (98±3 %vs80±1.5). ELISA results confirmed higher expression and secretion level of therapeutic BDNF protein by mcDNA-mediated gene delivery.

Conclusion: Reducing the vector size and removing the bacterial sequences from nonviral vectors is a promising approach to develop more effective neuroprotective therapeutics to treat glaucoma. The increased efficiency and safety of mcDNA biopharmaceuticals provide significant opportunity to develop non-viral gene therapiesfor retinal and potentially other neurodegenerative disorders.

95. Long Circulating Nanoemulsions for the Delivery of Poorly Water-Soluble Drugs

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Purpose: Drug carrier systems are being developed with the aim of changing the biodistribution of an active substance to increase its pharmacological efficacy or reducing its toxicity. Emulsions are promising carriers due to their biocompatibility, ability to solubilize large quantities of lipophilic drugs and relative ease of manufacture at commercial scale. The purpose of this work was to develop and evaluate oil-in- water nanoemulsions (NE) using pharmaceutically acceptable excipients. These NE could represent a valuable carrier delivery for lipophilic compounds such as taxane derivatives used in cancer chemotherapy.

Method: NE composed of medium chain triglycerides (core) and hydrogenated soybean phosphatidylcholine/Solutol® HS15 (shell) were prepared by a solvent-free phase inversion process. Three types of medium chain triglycerides were evaluated. Preformed NE were then coated with a distearoyl phosphatidylethanolamine polyethylene glycol (DSPE-PEG) conjugate by incubation. Mean hydrodynamic diameter (peak) and size distribution (width) were measured for uncoated and coated NE. The stability of all formulations was assessed at 4 and 25°C. Atomic Force Microscopy experiments were performed in tapping mode for uncoated NE. Aqueous NE were free-dried with glucose as cryoprotectant (10% w/w) and then redispersed. The polydispersity was evaluated as well as the size distribution.

Results: Oil-in-water NE were obtained using three different pharmaceutically acceptable medium chain triglycerides. Size distribution was slightly affected by this difference with a size of 70 to 75 nm. Inserting DSPE-PEG into preformed NE was found to be a convenient and effective method to obtain controlled size NE (80-95 nm) with a high PEG density at their surface. AFM experiments confirmed the size range and nanocarriers of 70 nm were observed. NE are stable at both 4 and 25°C since 12 weeks, samples are still in stability. Aqueous NE were successfully free-dried and an increase in size was observed.

Conclusion: With a simple solvent-free process and using pharmaceutically acceptable excipients, it was possible to produce stable nanoscale delivery carriers. These nanocarriers represent a valuable alternative to polymeric micelles and liposomes for site-specific delivery of hydrophobic anticancer drugs.

96. Characterization and Drug-permeation Profile of Porous Ethylcellulose Membrane

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Purpose: A controlled release tablet of Oxybutynin.HCl was recently developed at John Abbott College's Pharmaceutical technology department, using a programmable rupturable film coating. A mixture of commercial polymer coating dispersion of Ethylcellulose (Surelease®) and Polyvinyl Alcohol (PVA) was used to control the release of Oxybutynin.HCl. PVA was incorporated into the Ethylcellulose membrane to increase its porosity and therefore induce the rupture of the membrane as a result of tablet swelling. The purpose of this present work is to characterize the drug-permeation and the mechanical properties of Ethylcellulose-PVA membranes.

Method: Ethylcellulose-PVA membranes were prepared in a pan coater (LDCS, Vector, USA) by spraying an aqueous polymeric mixture on a hard plastic sheet attached to the interior side of the coating pan. The thickness of the membrane was measured using a digital micrometer. Membrane strength was measured at different thicknesses and porosities using a TA-XT2i texture analyser (Texture Technologies Corp., USA). Drug permeation was evaluated at 37°C using a horizontal diffusion-cell with an effective diffusion area of 1.5cm^2. A sample (1mL) of phosphate buffer (pH 6.8), deionized water and HCl solution (pH 1.2) were taken from the donor chamber at a predetermined time, then appropriately diluted and measured on UV-Vis spectrophotometer at 230 nm. The apparent permeability was then calculated according to the lag-time model.

Results: Aqueous dispersions of pure Ethylcellulose form strong membranes with very low porosity. Incorporation of PVA into Ethylcellulose membranes significantly affects the permeation and mechanical properties of the membrane. As the concentration of PVA increases, the resulting PVA-Ethylcellulose membrane becomes more porous and therefore less strong and highly permeable. However, at high concentration of PVA (50%), the resulting membrane dissolves quickly in the diffusion medium, leading for no controlled release of Oxybutynin.HCl.

Conclusion: Incorporation of PVA into Ethylcellulose membrane can be used to modulate the permeation and mechanical properties of the membrane and therefore the lag-time of Oxybutynin.HCl release.

97. Gemini Nanoparticle Formulation Design for Non-Invasive Cutaneous Gene Delivery: Effect of Neutral Helper-Lipids on Transfection Efficiency *in vitro*

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Purpose: Gemini surfactant-phospholipid-based nanoparticles (GL-NPs) are promising carriers for topical gene delivery. Their transfection efficiency (TE) is influenced by the type and concentration of neutral helper lipids used such as dioleoylphosphatidylethanolamine (DOPE) and dipalmitoylphosphatidylcholine (DPPC). The objective of this study was to evaluate the transfection efficiency and biocompatibility of five different gemini surfactant series (m-s-m; m = 12, 16 and 18C and pyrenyl substituted chain length; s = 3 methylene-unit and imino-substituted 7 methylene (7NH) spacer group) with various DOPE:DPPC co-lipid ratios. Additionally, the established 24-well transfection assay format was converted into a 96-well high-throughput format and the miniaturization parameters were characterized.

Methods: GL-NPs were prepared in a two-step process. First, aqueous solution of gemini surfactants, 12-3-12, 12-7NH-12, Py-3-12, 16-3-16 and 18-3-18 (300µg/mL) were precomplexed with pCMV-tdTomato plasmid DNA (0.5µg and 0.1µg pDNA for PAM 212 keratinocyte transfection in 24- and 96-well plates, respectively) at 10:1 gemini/DNA charge ratio and incubated for 15 minutes at room temperature. Second, GL-NPs were obtained by adding the helper lipid vesicles to the gemini surfactant-plasmid complex (1:1 volume ratio) followed by incubation for 30 minutes at room temperature. Helper lipid vesicles with five different DOPE:DPPC ratios (DD100/0, 75/25, 50/50, 25/75 and 0/100) were prepared in sucrose solution (9.25% w/v) by high pressure homogenization using Microfluidizer LV1. Particle size distribution of GL-NPs was measured using dynamic light scattering

(DLS). TE and cell viability of GL-NPs in PAM 212 cells seeded in 24- and 96-well plates were analyzed using flow cytometry.

Results: Twenty five different GL-NP compositions were formulated and characterized. TE results from 24-well plate revealed that 16-3-16 (961.1 ± 108.4 nm), 18-3-18 (602.6 ± 2.8 nm), and 12-7NH-12 (183.4 ± 1.6 nm) GL-NPs with DD75/25 ratios had the highest mean TE (23.81% ± 11.38%, 6.5% ± 1.57% and 8.46% ± 0.7%, respectively, n=3) among other GL-NPs and Lipofectamine3000 (5.64% ± 0.75%). Increasing DPPC ratio in all GL-NPs resulted in decreasing TE. It was also demonstrated that by adjusting the GL-NP dose - cell number per well ratio transfection efficiency in PAM 212 cells using 96-well plate can provide reproducible results comparable to the 24-well format.

Conclusion: This study showed that GL-NPs with the highest TE had the optimum DD75/25 ratio and high compatibility with keratinocytes suitable for applications in cutaneous gene therapy. The miniaturized 96-well assay can be used as a labor-, time- and cost-saving alternative in transfection studies by flow cytometry.

98. Gene Delivery Nanoparticles Targeting Ovarian Cancer using Folic Acid

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Purpose: Ovarian cancer is often diagnosed at a late stage when multiple organ systems are involved and the chance of successful therapy is low. Following treatment, many patients continue to experience recurrence of the disease and encounter resistance to traditional chemotherapeutics. Given the tendency for ovarian cancers to rapidly develop resistance, and the underlying genetic factors involved, a gene therapy approach to ovarian cancer treatment is a viable addition or alternative to current methods.

To reach the full potential of a gene therapy, one must ensure effective and safe delivery to the desired tissue(s). Non-viral delivery systems are a safer alternative to viral vectors, which are associated with severe immune responses and oncogenesis, but are less efficacious and require rational, bottom-up design. Incorporation of folic acid in the formulation of gemini surfactant and Pluronic block copolymer self-assembling nanoparticles is intended to 1) enhance transfection efficiency through increased cellular uptake, and 2) reduce systemic toxicity through decreased delivery to non-target cells by targeting ovarian cancer cells with an overexpression of folate receptor α (FRα).

Method: The dicationic N,N'-bis(dimethylalkyl)-**α,ω**-alkanediammonium gemini surfactant, 16-3-16, was complexed with plasmid DNA encoding an enhanced green fluorescent protein reporter gene at a +/- charge ratio of 5:1. This complex was then combined with Pluronic F87 and folic acid, at varying concentrations, to form self-assembling, FRα-targeting gene delivery nanoparticles. *In vitro* transfection efficiency and cytotoxicity assays in human ovarian adenocarcinoma cells (OVCAR-3) were analyzed using flow cytometry to obtain reporter gene expression and propidium iodide staining results. Physical characterization of the resultant nanoparticles such as particle size and zeta potential were measured through dynamic light scattering and laser Doppler micro-electrophoresis, respectively.

Results: Plasmid DNA-loaded nanoparticles exist within the desired size and charge range (below 200nm; above +30mV) expected to favour transfection. *In vitro* transfection assays showed potential for successful gene delivery and expression, and low cytotoxicity. Formulations with F87 concentrations equal to half its CMC in the presence of folic acid provided the greatest gene expression (equal to 0.5 fold-change relative to the Lipofectamine control) and 100% normalized cell viability. However, there was no statistically significant increase compared to formulations without folic acid.

Conclusion: While the preliminary results are promising, optimization of the nanoparticle formulations is required. Upcoming efforts will include incorporation of the folate-functionalized phospholipid DSPE-PEG(2000)-Folate, which is expected to aid in both targeting and membrane destabilization. Furthermore, i*n vivo* studies are needed to confirm toxicity, bioavailability, and tissue-targeting.

99. Temperature-Induced Assembly of Monodisperse and Degradable Poly(N-isopropylacrylamide) Microgels based on Oligomeric Precursors

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Purpose: Temperature-responsive microgels and nanogels based on poly(N-isopropylacrylamide) (PNIPAM) have attracted significant research interest as triggerable, on-demand drug carriers due to their ability to change their diameter, hydrophobicity, pore size, and surface charge as a function of temperature. However, conventional PNIPAM nanogels are limited in this context in that there is no way to ensure the degradability of the nanoparticles over time. In response, we have designed a method to create degradable thermosensitive nanogels by controlled precipitation of well-defined linear hydrazide and aldehyde-functionalized PNIPAM precursor polymers.
Methods: Heating a hydrazide-functionalized PNIPAM polymer precursor to a temperature above its lower critical solution temperature (LCST) results in nanoaggregate formation, after which the addition of the aldehyde-functionalized PNIPAM polymer precursor enables the formation of a hydrolytically-labile hydrazone bond to cross-linked the aggregate into a nanoparticle. Subsequent characterization of the formed particles was completed using dynamic light scattering, Nanosight particle tracking analysis, and transmission electron microscopy (TEM) to determine particle shape and size. The generated particle size can be controlled by altering polymer ratios (hydrazide:aldehyde polymer fraction), reaction temperature, and reaction time. Long-term colloidal stability as well as cytotoxicity were also assessed.
Results: The nanogels created exhibit a narrow polydispersity, retain their thermosensitivity, and behave similarly to PNIPAM nanogels made through traditional monomer-based methods. TEM imaging confirmed the particles were monodisperse and spherical in nature. The most predictable parameters for controlling nanogel size include the amount of both polymer phases (base polymer and cross-linking polymer), the method of mixing the precursor polymers, and the temperature of the reaction. At physiological pH, the particles exhibit long-term colloidal stability, although degradation of the hydrazone cross-links is observed over the period months to enable ultimate clearance of the nanoparticles *in vivo*. Cell cytotoxicity studies show that the nanogels and their degradation products elicit low toxicity after short-term exposure. The capacity for temperature-triggered drug release from these nanogels is currently being assessed and will be presented.
Conclusion: We anticipate this novel approach to nanogel fabrication has significant potential to enable translation of thermoresponsive nanogels to *in vivo* biomedical applications.

100. The Effect of pH on Size of Human Serum Albumin Nanoparticles

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Purpose: Human Serum Albumin nanoparticles (HSA-NPs) are widely used for drug-delivery applications including targeted cancer therapy and cardiovascular diseases. HSA-NPs have several advantages that distinguish them from other drug carriers including high biocompatibility, biodegradability and non-immunogenicity. To effectively prepare and characterize HSA-NPs, different parameters like HSA concentration, desolvation agent, cross-linker etc., must be optimized. Of these, the pH of solution, while preparation of HSA-NPs, is a critical parameter. Our aim was to optimize the pH and study its effect on the size of HSA-NPs.
Methods: Five solutions of 20 mg HSA dissolved in 1 ml deionized water were prepared, and titrated to different pH levels with 0.1 M NaOH: 7, 7.5, 8, 8.5 and 9. To each of the solutions, 2-4 ml ethanol was added dropwise until the solution became turbid. Subsequently, Glutaraldehyde was added as the cross-linking agent. This was followed by purification of the HSA-NPs by three rounds of ultracentrifugation and re-suspension of the pellet in PBS (pH 7.4). Finally, HSA-NPs were ultrasonicated and their sizes and zeta-potentials were measured by photon correlation spectroscopy and zeta potential analyser.
Results: The smallest HSA-NP size was obtained at pH 7 with a mean diameter of 214±3.1 nm with

Polydispersity Index (PI) < 0.2 and a mean zeta potential of -23.92 mV. The largest HSA-NP size was obtained at pH 9, for which the HSA-NP diameter was 304.3±3.1 nm with PI<0.2 and zeta potential of -28.66. The HSA-NP sizes obtained for pH 7.0-9.0 have been summarized in Table 1 and represented in Fig. 1.

Conclusion: The pH of HSA solution strongly influences HSA-NP size. Previous studies optimized pH in the range 8.5-9 for nanoparticle sizes 150-250 nm. However, our results differ from previous observations as the smallest HSA-NP sizes of 214±3.1were obtained at pH 7. Further studies are required to completely understand these pH-related variations in nanoparticle size.

Table1: Effect of pH of HSA solution on HSA-NP size

pH of HSA solution	**HSA-NP Diameter (nm)** (mean±SE)
7.0	214±3.1
7.5	303.9±5.2
8.0	284.2±3.4
8.5	300.6±5.9
9.0	304.3±3.1

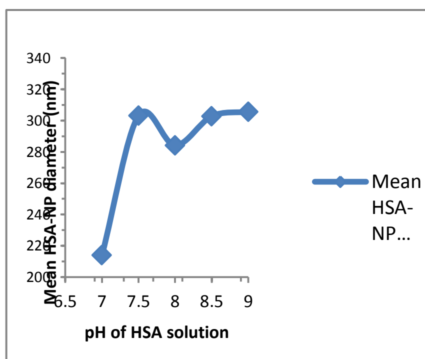

Fig. 1: Diameter of HSA-NPs at different pH values, ranging from 7.0-9.0, titrated with 0.1M NaOH, (mean; n=10), prepared from 20 mg/ml HSA solution.

101. Determinants of Liposome Distribution at the Bulk and Intratumoral Levels: Impact of Time, Region and Vascular Density in an Orthotopic Tumor Model

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Purpose: The limited potency of nano-based therapeutics remains a significant challenge in nanomedicine. Despite an enhanced tumor uptake relative to conventional chemotherapeutics, signifincant inter- and intratumoral heterogeneities limit anti-tumor response to nanomedicines. A spatio-temporal characterization of the intratumoral distribution of a liposomal formulation is proposed for the guidance of current and future interventions.

Methods: A multi-scalar liposome formulation for computed tomography (CT) imaging and optical microscopy was developed for concomitant assessment of macro- and microdistribution, respectively, in an orthotopic tumor xenograft model of cervical cancer. CT enabled a quantitative and non-invasive assessment of liposome macro- and microdistribution within the same tumor. A computational methodology was applied to fluorescence images of whole tumor sections as an objective and robust means to characterize the local deposition of liposomes relative to the tumor vasculature.

Results: *In vivo* formulation stability was demonstrated by HPLC via the co-retention of CT and fluorescence contrast agents within the liposomes. Bulk tumor uptake was found to be concordant with temporal trends of macroscopic tissue quantification (i.e., decreasing levels as a function of time). However, microdistribution analysis exposed variable trends in uptake as a function of region in the tumor. In particular, highest levels of liposome uptake were achieved and maintained in the rim of the tumor over the 5-day study period, with no change in liposome levels found in the tumor core. Furthermore, liposome concentration was positively correlated with tumor MVD ($p<0.01$).

Conclusions: Our study investigates the distribution of liposomes both at the bulk tumor and intratumoral levels. These findings suggest tumor region and corresponding microenvironmental properties as potential determinants in liposome transport and deposition. Such parameters are expected to complement conventional metrics of nanomedicine candidacy (e.g., biodistribution). A characterization

of heterogeneity, both in liposome distribution and microenvironmental parameters, may elucidate key determinants of nanomedicine efficacy.

102. Lysine-Functionalized Nanodiamonds as Gene Carriers: Development of Stable Colloidal Dispersion for*in vitro* Gene Delivery Application and Cellular Uptake Studies

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Purpose: This study is aimed at synthesizing amino acid (AA) functionalized nanodiamonds (NDs) as potential nanocarriers for gene delivery. Lysine-NDs are synthesized to introduce primary amines on the surface that can bind negatively charged genetic materials, and can also interact with the cell membrane to facilitate cellular uptake.
Methods: Positively charged NDs were synthesized by covalent conjugation of lysine on the surface. Interaction with serum proteins and stability in aqueous and tissue culture medium were analysed for fNDs by size and zeta potential measurements with respect to time. Binding capacity of fNDs with pDNA and SiRNA at different weight ratios was analysed by gel electrophoresis. Uptake of fNDs in HeLa cells alone and as complexes with siRNA was evaluated using flow cytometry and scanning transmission X-ray microscopy (STXM). Cellular toxicity was analyzed using flow cytometry and MTT assay.
Results: Lysine functionalization was able to create highly uniform ND dispersion (PDI: 0.14) with a positive zeta potential of +26.5 mV and majority of particles having size of ~50 nm. Aqueous dispersion of lysine-NDs showed minimum aggregation and was found to remain stable over a period of 25 days. Lysine NDs were able to bind pDNA at 1:1 and SiRNA at 1:20 weight ratios. STXM and flow cytometry analysis revealed dose dependent cellular uptake of lysine NDs alone. Flow cytometry results also showed a fluorescence shift and an increase in relative fluorescence intensity for the cells treated with diamoplexes compared to untreated cells, indicating the delivery of siRNA within cells. Lysine NDs did not induce any apoptosis in the cells when compared to a traditionally accepted apoptotic agent melphalan at varying concentrations.
Conclusion: Covalent functionalization of NDs with AAs can reduce aggregation and produce stable dispersion. Moreover, AA functionalized NDs are capable of binding genetic materials and shows a potential to protect and deliver therapeutic genes in mammalian cells.

103. Transdermal Delivery of Bone-Targeting Peptide Hormone Conjugates for Treatment of Tibial Bone Stress Fractures Using Liposomal Cream Base

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Purpose: Stress fractures (shin splints) are common painful bone injuries in athletes or military personnel. The management of pain with systemic NSAIDs or narcotic analgesics is associated with side effects and may interfere with cellular bone repair. Nasal or parenteral calcitonin (CT) and parathyroid hormone (PTH) trigger bone repair and/or reduce pain burden. Several limitations, such as the invasive route of administration, high dosage requirements and side effects in tissues other than bone, preclude their application. Our ***hypothesis*** is that transdermal delivery of CT and PTH/or their bone-targeting conjugates will improve bone availability, especially in tibial stress fractures, as the desired site of bone action is in close vicinity to the applied transdermal cream.
Method: Using commercially available liposomal cream base, several dosage forms of test compounds (CT, PTH and their bone-targeting conjugates) were prepared and permeability tested against artificial hydrophilic membranes or epilated hairless skin from rats on Franz static cell apparatus, using acetate (pH 5.0) or phosphate buffer (pH 7.4) as receiving medium. The accumulated amounts of test compounds were calculated after 6 hr using HPLC. Transdermal dosage forms of test compounds are currently being assessed *in vivo* on the tibial bone of rats, with serial plasma sampling at 0, 1, 2, 4 and 6 hr post-dose application in order to determine their systemic bioavailability vs sub-cutaneous application. Post-mortem analysis of tibial bone will subsequently be conducted to gauge the success of transdermal delivery and localization of these compounds to the bone surface.

Results: The results of *in vitro* studies indicated that hydrophilic CT and PTH and their conjugates are readily incorporated in the liposomal cream base. Using HPLC detection, CT was eluted at 3.99 min and there was no interference with other component of the transdermal formulation. The results indicated that phosphate buffer 7.4 yielded maximal peptide hormone flux compared to acetate buffer pH 5.0. Optimal release and permeability of CT through the artificial membrane into acetate and phosphate buffers were measured at 58% and 98%, respectively. Continuing *in vitro* and *in vivo* studies involving rats are in progress.

Conclusion: Transdermal delivery may serve as a feasible route for the administration of small peptide hormones and their conjugates to treat superficial bone injuries. This approach offers increased local bone access of peptide hormone compounds that enhance bone repair, and may also reduce pain burden, whilst further decreasing off-site side effects when those drugs are used systemically.

104. Local Delivery of Zoledronate Mitigates Bone Destruction in a Mouse Model of Bone Metastasis

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Background: Bone metastases are the most common cause of cancer-related pain and often lead to other complications such as pathological fractures and spinal cord compression. Surgery is the main treatment option for bone cancers and metastasis. However, proximity to vital structures often prevents complete tumor resection. Bisphosphonates are potent antiresorptives commonly prescribed to retard osteoporosis progression. Interestingly, BPs have been suggested to have anti-tumor properties through interacting with macrophages, endothelial cells and matrix metalloproteinase (MMP). However, the use of bisphosphonates for cancer therapy is generally restricted to high dose intravenous infusion, which may in some instances be associated with devastating side effects. The purpose of this study was to investigate the effects of local delivery of zoledronate at a dose below systemic chemotherapeutic levels, directly to the tumour site in a mouse cancer model.

Methods: An intra-tibial xenograft model of bone metastasis was established in severe combined immunodeficiency (SCID) mice by injecting 1×10^5 of breast cancer cells (MDA-MB-231) in the right and left tibia (n=17). Five days after implantation, mice were treated with a local injection of 2µg Zoledronate in the right tibia only. Zoledronate treatments were repeated 3 times a week, for a period of 3 weeks. Following treatment, the mice were sacrificed, and micro-CT images of the right and left tibia were obtained. Bone Volume (BV) and bone volume to tissue volume ratio (BV/TV%) were determined using µ-CT biomarkers. Histological analyses of the tibia were performed using Von Kossa, tartrate-resistant acid phosphatase (TRAP) stain and alkaline phosphatase stain (ALP) staining of non-decalcified resin embedded sections.

Results: There was a statistically significant ($P<0.001$) increase in the mean bone volume (BV) in the treated tibia (8.9 mm^3) compared to the untreated tibia (7.03 mm^3). The ratio of BV to tissue volume (TV) was also significantly greater ($P<0.001$) in the treated leg (BV/TV=4.8%) as compared to untreated leg (BV/TV=3.8%). Histological analysis correlated well with Micro-CT.

Conclusion: These preliminary results suggest that local injection of zoledronate can lead to a significant inhibition of tumor-induced osteolysis. It remains to be seen how long-lived the effect is and the minimum dose required. This may have application to situations where only partial tumour resection is feasible. Future experiments on regrowth of partially resected tumours will be performed using live animal fluorescence to better standardize tumour size.

Pharmaceutical & Analytical Chemistry

105. An LC-MS/MS Method for the Analysis of Urinary Organic Acid Metabolites Differentially Expressed in Asthmatic Patients

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Purpose: Asthma is a complex syndrome with symptoms that can overlap with other respiratory conditions such as chronic obstructive pulmonary disease (COPD). A simple, non-invasive diagnostic test can facilitate the differentiation between asthma and other respiratory conditions especially in pediatric population. The goal of this study is to develop a sensitive and selective liquid chromatography-tandem mass spectrometric (LC-MS/MS) method to analyze 17 urinary organic acid metabolites that were differentially expressed in asthma patients.
Methods: p-dimethylaminophenacyl (DmPA) is used as a labeling agent for the analysis of the organic acid metabolites. Light ^{12}C and heavy ^{13}C DmPA labeling agents are used. The ^{13}C isotope labeled derivatives were used as an internal standard. Derivatized metabolites were separated on a C_{18} column using a gradient system of 0.1% formic acid in 5% ACN/water (solvent A) and 0.1% formic acid in ACN (solvent B). The analysis was conducted on an Agilent HPLC system interfaced with an AB SCIEX 4000 QTRAP® mass spectrometry instrument (Qq-LIT-MS). The system was operated in the positive ion mode using multiple reaction monitoring (MRM) scans.
Results: The fragmentation patterns of the 17 isotopically labeled metabolites showed various diagnostic product ions that were selected for targeted MRM-MS analysis. The product ion of the labeling agent (^{12}C-DmPA) at *m/z* 180 was one of the common product ions that have been detected with the tested metabolites. Target carboxylic acid-bearing metabolites were selectively detected and separated with a run time of 35 minutes. Each metabolite showed the desired linearity that will be applied for the analysis of patient urine samples.
Conclusion: A qualitative and quantitative targeted LC-MRM-MS/MS method was developed for the analysis of 17 urinary organic acid metabolites. The method is selective and is currently being validated according to USFDA guidelines. Expression of target metabolites will be investigated in respiratory illness patients.

106. The Design and Synthesis of Neuroprotectants Based on an Endogenous Platform

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Purpose: Stroke is an ever-increasing problem in the aging Canadian population. It is the third leading cause of death, however, treatment of stroke is greatly limited. There is a pressing need for the development of a neuroprotective therapy that limits neuronal injury in ischemic stroke patients. A diversity of neurotoxic factors are released during ischemia that result in brain damage. An important group of neurotoxic mediators are divalent metal cations, such as Ca^{2+} and Zn^{2+}. The goal of this research is to develop a method to mitigate the highly toxic influxes of these divalent metal cations (focusing on Zn^{2+}), thus limiting neuronal necrosis during an ischemic stroke.
Methods: This study makes use of *in silico* predictions, organic synthesis, and *in vitro* testing to rationally design, synthesize, and evaluate new chemical entities that are able to cross the blood-brain barrier, chelate neurotoxic divalent cations, and either "tie them up" or preferably, transport them out of the brain entirely. Histidine is an endogenous compound known to chelate divalent metal cations, and has been used as a starting design platform for this research. Selected molecules, based on *in silico* predictions, were synthesized and their capacity to interact with Zn^{2+} was assessed qualitatively using NMR spectroscopy. *In vitro* protection against Zn^{2+}-induced toxicity was also assessed.
Results: *In silico* modeling predicts histidine-based analogues will have a strong interaction with Zn^{2+} and resulting metal complexes will be stabilized.

NMR spectra of synthesized histidine-based analogues show changes in chemical shift as Zn^{2+} is titrated into samples. This suggests an interaction is occurring between the cation and compound, as predicted *in silico*. A preliminary *in vitro* assay shows histidine and several analogues have protective effects against Zn^{2+}-induced cell death.
Conclusion: Histidine may represent a platform around which to design new chemical entities to be used as putative therapeutics for the treatment of ischemic stroke.

107. Developing a Lead Compound for the Inhibition of Bacterial DNA Gyrase

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Purpose: As reported by the Centers of Disease Control (CDC), multi-drug resistance bacteria have an estimated cost of $40 billion on health-care systems globally. In the past 20 years, developments of therapeutics aimed at killing infectious strains of bacteria have been very limited. Bacterial DNA gyrase is a complex enzyme that is used to relieve strain from DNA unwinding and introduces supercoils for various cellular processes including DNA compaction, replication and transcription. Inhibition of bacterial DNA gyrase provides an avenue to develop compounds that can be optimized for clinical applications. The present study focuses on using computational chemistry predictions and *in vitro* enzymatic activity assays to search for a lead compound.
Methods: The rationale for the study employs molecular dynamic simulations and binding energy calculations using the London dG scoring function to predict potential lead compounds from a library containing 800 substituted pyrrole and thiophene analogues. The cumulative binding energy predictions and ligand geometries in binding pockets of gyrase subunits were used to select 25 compounds. These compounds were then tested in an *in vitro* DNA supercoiling gel-based assay.
Results: *In silico* predictions demonstrated favorability of compounds to interact with the ATPase binding site of Gyrase subunit B. Ligand binding scores suggested a preference for tetrazole-substituted thiophenes that adopted pi-stacking interactions with hydrophobic side chains and formed hydrogen bonds with donor side chains. *In vitro* DNA gel-based assays indicated dose-dependent reduction of supercoiling activity of DNA gyrase at a concentration of 80μM for compounds 0599, 0607 and 0608. Out of 25 compounds, only 15 were tested due to insufficient solubility in 2%DMSO. Future research is directed towards quantifying IC_{50} values using a fluorescence-based high-throughput assay.
Conclusions: Molecular modeling predictions were able to select compounds that show potential as inhibitors of bacterial DNA gyrase *in vitro*. Further characterization is required using quantitative enzyme activity measurements.

108. Evolution and Identification of Substrate Specific Sialidase from *Micromonospora viridifaciens*

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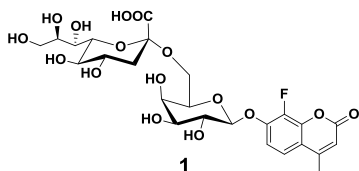
Purpose: Sialic acids are often found at the terminal positions on the glycan chains that adorn all vertebrate cells and glycoproteins. This prominent position confers an essential role to sialic acid residues in biology, evolution and disease propagation. Glycoside hydrolase family 33 (GH33) contains exo-sialidases (E.C. 3.2.1.18, neuraminidases), from both eukaryotes and prokaryotes, that catalyze the hydrolysis of sialic acid from glycoconjugates. Interestingly, subtle differences exist in both the structure of the particular sialic acid and its position of attachment to glycoconjugate chains between humans and other mammals. These differences are indicators of the unique aspects of human evolution, and are relevant to understanding an array of human conditions. We will be exploring multiple routes to further unravel the importance of sialic acids.
Methods: We are developing tools to probe for various sialic acid structures such as Kdn, a sialic acid family member that is poorly understood. To this end, we have constructed a random mutant library of the neuraminidase from the soil bacterium *Micromonospora viridifaciens* (*Mv*NA) and identified a number of recurring mutations in the sialidase gene which lead to a more efficient

hydrolysis of synthetic natural substrate analogues such as 8FMU α-Kdn-(2→6)-β-d-Galp(1). We have also using the available structure of wild type *Mv*NA bound to the natural inhibitor, DANA identified amino acids potentially involved in recognition and binding to acetylated sialic acids and generated genetic libraries which we will use to identify clones with enhanced cleavage activity. We will now further induce mutations in the *Mv*NA gene and aim to identify clones with enhanced Kdnase activity under positive and negative evolutionary forces.

Results: We have identified several clones with enhanced cleavage activity of synthetic natural substrate analogue 8FMU α-Kdn-(2→6)-β-d-Galp and we will be testing these clones to determine the specific enzyme activity of each clone. This will in turn aid in choosing a clone for further active site mutagenesis.

Conclusion: Despite their documented role of sialic acids in numerous diseases, our knowledge of the functions and metabolism of certain sialoglycans, linkage specific attachment or cleavage of sialic acids to cellular glycans are scant and require further in depth research to delineate the role of various sialic acid structures. Evolution of an enzyme with substrate specific hydrolysis activity is essential in elucidation of the importance of specific sialidation of glycoproteins and their role in disease propagation.



109. Development of a High Performance Liquid Chromatographic Method for Determination of Dronedarone and its Dealkylated Metabolite in Rat Plasma

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Purpose: Dronedarone is a Class II antiarrhythmic benzofuran derivative of amiodarone. Previously we had developed an HPLC assay for dronedarone with a lower limit of quantitation of 25 mg/mL based on 0.1 mL of rat plasma. However, dronedarone is metabolized to desbutyldronedarone (DBD), a metabolite with some antiarrhythmic activity, and the assay was not capable of measuring it. Here we described an adapted method to concurrently measure dronedarone and DBD in rat plasma.

Methods: Plasma was collected from the blood by centrifugation at 3000 rpm for 10 min. dronedarone, DBD and internal standard (IS, ethopropazine) were extracted from 100 µL rat plasma using liquid-liquid extraction. 100 µL of water was added to the plasma followed by 50 µL of 1N NaOH. Thereafter, 3mL of methyl tert-butyl ether was added. After vortex mixing for 1 min and centrifugation for 3 min, the organic layer was transferred into clean tubes and dried *in vacuo*. The dried residue was reconstituted in 150 µL of mobile phase and 130 µL was injected. Separation was accomplished using a C18 analytical column (150 mm×4.6 mm with 5µm particle size). The mobile phase consisted of acetonitrile: [25 mM KH_2PO_4: 3 mM H_2SO_4: 3.6 mM triethylamine] in a combination of 55:45 v/v was pumped at an isocratic flow rate of 1 mL/min. Detection was by UV absorption at 288 nm.

Results: The components eluted within 15 min. The peaks were symmetrical with no interference from endogenous compounds in plasma. Calibration curves were highly linear (>0.99) over the range of 25-5000 ng/mL of dronedarone and DBD in rat plasma. The intraday CV% were <14% and the mean error value were <11% for both dronedarone and DBD. The validated lower limit of quantitation were 33 ng/mL based on 100 µL of rat plasma for both compounds. The assay was tested using 4 Sprague Dawley rats administered 55 mg/kg of dronedarone base orally. At 6 h post-dose, the animals were euthanized and their plasma were collected and then assayed.

Conclusion: This developed chromatographic method was successfully capable of measuring dronedarone and DBD concentrations in rat plasma.

Pharmacokinetics & Pharmacodynamics

110. Effect of Tigecycline on ATP Concentrations in Red Blood Cell in Rats

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Purpose: Tigecycline (Tygacil®) is a tetracycline which has been shown to attenuate break down of ATP synthase in macrophages in response to inflammation induced by lipopolysaccharide suggesting that it may preserve intracellular concentration of ATP. The purpose of the research was to investigate the effect of tigecycline on concentrations of ATP and its catabolites in red blood cell (RBC) in a rat model under general anesthesia.
Methods: Tigecycline (TIG; 25 mg/kg) or placebo (normal saline) was administered intravenously to Wistar rats (n = 20 in total). Blood samples were collected with "Stopping Solution" at 1 and 2 hours (hrs) after injection and centrifuged immediately to separate plasma and RBCs. RBCs were suspended in phosphate buffer saline (PBS), and lysed by adding cold trichloroacetic acid. Samples were centrifuged again to collect the RBC lysates which were analysed for ATP and its catebolites (ADP and AMP) by a previously described HPLC. Differences between the tigecycline treated and control groups were compared by Student's t-test and considered significant at $p < 0.05$.
Results: The RBC ATP concentrations were significantly higher in the TIG treated group than in the control (1 hr post dose 2.88 ± 1.07 vs 1.74 ± 0.88 mM; 2 hr post dose 2.95 ± 1.05 vs 1.54 ± 0.53 mM, $p < 0.05$ for both). There was no difference in the ADP concentrations (1 hr post dose 0.33 ± 0.26 vs 0.34 ± 0.10 mM; 2 hr post dose 0.34 ± 0.18 vs 0.31 ± 0.10 mM, $p > 0.05$ for both), or AMP concentrations (1 hr post dose 0.032 ± 0.009 vs 0.023 ± 0.021 mM; 2 hr post dose 0.031 ± 0.013 vs 0.019 ± 0.013 mM, $p > 0.05$ for both) between the drug treated rats and control.
Conclusion: TIG increases ATP concentrations but not the concentrations of ADP or AMP in RBC in an anaesthetized rat model.

111. Appropriateness of Traditional Bioequivalence Metrics to Infer Therapeutic Equivalence for Follow-on Long Acting Injectables with Complex Pharmacokinetic Profiles

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Purpose: C_{max} and AUC_t are standard metrics used to establish the bioequivalence of test and reference products. To enable inference of therapeutic equivalence (TE) and switchability of products exhibiting complex, multi-phasic release (e.g. long-acting injectable (LAI) products such as Risperdal Consta (RC) (Janssen)), it is critical that the entire PK profile is equivalent. In 2013, the FDA issued a draft BE guidance for Risperdal Consta and in 2014, the EMA issued a final guidance on the evaluation of medicinal products with modified drug release. The objective of this assessment is to evaluate the appropriateness of traditional BE metrics to predict TE for follow-on products of RC.
Methods: Different risperidone LAI formulations were evaluated in accordance with the FDA and the EMA guidances to determine the adequacy of their *in vitro* release and *in vivo* BE metrics to ensure TE to RC. To this end, *in vitro* dissolution and *in vivo* PK data obtained for risperidone LAIs were compared to RC to assess the impact of manufacturing and formulation changes. In addition systemic exposure was predicted when switching between RC and a similar drug product exhibiting either a shorter or longer lag phase.
Results: *In vitro:* Small changes in the manufacturing process (cold drying step) or excipient impurity profile were observed to significantly alter the initial release and lag phase. Such initial release may not be detected through evaluation of C_{max} and AUC_t alone but may increase systemic exposure significantly.

In vivo: A single dose PK study with a risperidone LAI with a slightly different polymer (different copolymer ratio) showed a substantially different PK profile but exhibited a C_{max} and AUC_t

similar to RC.

Simulation studies suggest that a switch from RC to a follow-on product with an identical C_{max} and AUC_t but shorter lag phase of 1 or 2 weeks could expose a patient to significantly higher peak levels of risperidone (~80% and 25%, respectively) and that a switch from RC to a follow-on product with a longer lag phase of 4 or 5 weeks could expose a patient to significantly lower trough levels of risperidone (~57% and 69%, respectively) upon switching.

Conclusion: The use of C_{max} and AUC_t alone to establish the BE of follow-on LAI with complex PK profiles such as Risperdal Consta is not sufficient to establish therapeutic equivalence. It is recommended that additional PK metrics, such as pAUC, be utilized in the current Health Canada guideline to ensure therapeutic equivalence of a follow-on risperidone LAI to Risperdal Consta.

112. Development of a High Performance Liquid Chromatographic Assay for the Simultaneous Determination of Posaconazole and Vincristine in Rat Plasma and Its Application in a Drug Interaction Study

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Purpose: To develop a high performance liquid chromatographic assay for the simultaneous quantitation of posaconazole (PSZ) and vincristine (VCR) in rat plasma, to utilize in a drug interaction study.

Method: PSZ, VCR and itraconazole (internal standard) were extracted from 200 µL plasma using diethyl ether in the presence of 0.1N NaOH. After vortexing, and centrifugation, the organic layer was transferred to clean tubes and evaporated *in vacuo*. The dried residue was reconstituted in methanol and injected into the HPLC through a C18 column. The mobile phase consisted of acetonitrile: 15 mM potassium dihydrogen orthophosphate, (30 : 70 to 80 : 20, linear over 7 minutes) pumped at 1.5 mL/min. VCR was detected at λ=220 nm while PSZ and itraconazole at λ=262 nm. Sprague Dawley rats (n=2) were orally dosed PSZ 40 mg/kg followed by iv dosing of 0.1 mg/kg VCR 30 minutes later. Serial blood sampling was performed at 0.5, 0.75, 1, 1.5, 2, 3.5, 6, 8, 12, 24 and 48 h post oral dose.

Results: The components eluted within 11 min; calibration curves were linear (r^2≈0.995) over the range of 50-5000 ng/mL of PSZ and VCR concentrations. The CV and mean error were <20% for both drugs. The validated lower limit of quantitation was 50 ng/mL for both drugs based on 200 µL rat plasma. Rat plasma concentrations of PSZ and VCR were simultaneously measured up to 72 h and 48 h, respectively. The pharmacokinetics parameters calculated for both drugs were found to be comparable to literature.

Conclusion: The assay was validated as per ICH guidelines and was shown to be rapid, sensitive and appropriate for a pharmacokinetic study. This project was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 5575.

113. Impact of Overweight on Anthracycline and Trastuzumab-Induced Cardiotoxicity: Experimental Study in Mice

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Purpose: Trastuzumab (TRZ), a humanized monoclonal antibody against Human Epidermal Growth Factor Receptor 2 (HER2) oncogene, is believed to potentiate doxorubicin (DOX) cardiotoxicity, resulting in left ventricular dysfunction. Few data indicate that overweight could influence DOX-induced cardiotoxicity, and no study has already evaluated the impact of moderate overweight on the cardiotoxic effect of DOX alone or in combination with TRZ.

Methods: Immediately after birth, litters of C57BL/6 mice were either maintained at 10 (normal litter, NL), or reduced to 3 (small litter, SL) in order to induce programming of ~15% overweight through postnatal overfeeding. At 4 months, in order to evaluate the potentiation of DOX cardiotoxicity by

TRZ, NL and SL mice received a single intraperitoneal injection of either saline, DOX (6 mg/kg), TRZ (10 mg/kg) or the combination of both (DOX-TRZ). Transthoracic echocardiography was performed 24 hours before, 10 and 20 days after treatments, in order to evaluate the evolution of cardiac function.

Results: Twenty days after DOX administration, systolic dysfunction was observed only in overweight-SL group, while NL mice group kept a preserved left ventricular ejection fraction (LVEF). Moreover, in NL group, the function impairment appeared when TRZ was co-administrated. 48 hours after drug administration, gene expression of Erb-B2, the murine analog of HER2, was induced in the myocardium of DOX-treated mice, and its induction was potentiated by co-treatment with TRZ. Expression of natriuretic peptides (ANP, BNP) appeared to be potentiated in DOX-TRZ mice of both NL and SL groups, whereas the expression of β-MHC increased significantly in overweight-SL mice.

Conclusion: In an acute model of DOX cardiotoxicity, moderately overweighed adult mice are more sensitive to cardiac systolic impairment. Moreover, our results show an early myocardial induction of TRZ-receptor after DOX and/or TRZ, and confirm the potentiating action of TRZ on DOX-induced cardiotoxicity in mice.

Acknowledgment: This work was supported by the French Government through a fellowship granted by the French Embassy in Egypt (Institut Francais d'Egypte).

114. A Comparison of Model Independent and Dependent Pharmacokinetic-Pharmacodynamic Methods

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Purpose: Pharmacometric methods have been used to describe pharmacokinetic (PK)-pharmacodynamics (PD) relationships, often with no test of superiority over the conventional approaches. We used our reported data on the L-type calcium channel blocker, verapamil, to test the hypothesis that pharmacometric is superior to the conventional method in predicting changes in the PD of the drug. Verapamil's PK-PD relationship has been studied in patients with different severity of Crohn's disease. In patients with clinically active disease, the drug potency is decreased despite an increase in the concentration of verapamil enantiomers. However, in patients who are in clinical remission, the PK and PD of verapamil are within the normal range. The aims of this study were to 1) find out if pharmacometrics can predict covariates better than conventional methods, 2) describe the PK and PD of verapamil in patients with different severity of Crohn's disease, and 3) identify factors involved in inter-subjective variability.

Methods: 45 volunteers including 9 healthy, 14 with active Crohn's disease (Harvey-Bradshaw Score; HBS>5) and 22 patients in remission (HBS<5) was included to develop a population PK-PD model using the Monolix software 4.3.1. In the software, a combination of the Stochastic Approximation EM algorithm with a Markov Chain Monte Carlo procedure for maximum likelihood estimation in nonlinear mixed-effects models without linearization was applied.

Results: An indirect PK model (Ke0) with a two-compartmental open model and a first-order input best described the base model for S-verapamil concentration. The PD response (maximum % of PR interval prolongation from baseline) was best described by an Emax model with S-verapamil concentrations at the effect site. The HBS was identified as the most powerful predictor of the changes in PK–PD model. While the conventional model-independent methods could not detect the significant difference in response to the drug between three groups ($p>0.05$), the model-dependent pharmacometric analysis identified significant difference between the three groups ($p= 0.039$).

Conclusion: Verapamil PK and response are related to disease severity in Crohn's disease. This work demonstrates that the model-dependent pharmacometric approach was a more reliable predictor of the observed physiological changes in Crohn's patients than the model-independent method.

115. Calebin A: Analytical Development for Pharmacokinetic and Content Analysis of Natural Health Products and Elucidation of Pharmacological Activities

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Purpose: Calebin A is a curcuminoid derived from turmeric (*Curcuma Longa* L.).The purpose of these studies was to develop a bioanalytical assay using RP-HPLC to quantify calebin A, to characterize its pharmacokinetics in rats after intravenous (IV) and oral (PO) administration, to identify its pharmacological activities and to evaluate its content in natural health products.
Methods: RP-HPLC method was developed for detection of calebin A. Separation was carried out using a Phenomenex® Kinetex® C18 column with UV detection at 339 nm. Isocratic mobile phase consisting of acetonitrile and 10 mM ammonium formate (pH 7.0) (40:60, v/v) with a flow rate of 0.8 mL/min was employed. Linear standard curves were established and applied in a pharmacokinetic study. Calebin A was administered to male CD (Sprague-Dawley) rats IV (20 mg/kg) or PO (500 mg/kg) (*n*=4/route of administration). Serum and urine samples were collected for 72h post dose. *In vitro* antioxidant activity, anti-inflammatory activity (cyclooxygenase and lipoxygenase inhibition), dipeptidyl peptidase-4 (DPP-4) inhibition and cytochrome P450 inhibitory activties of calebin A were examined using commercial assay kits. Content analysis of calebin A in 14 natural health products advertised to contain turmeric were carried out using methanolic extraction.
Results: The HPLC method was successfully applied to a pharmacokinetic study of calebin A in rats. After IV and PO administration of calebin A, the compound was detected as the aglycone and glucuronide metabolite. Oral bioavailabitily was found to be ~0.5% with serum half-life was determined to be 1-3h. Calebin A appears to be primarily excreted via non-renal routes. Calebin A possessed concentration-dependent antioxidant activity and DPP-4 inhibition. Calebin A appears to be a non-selective cyclooxygenase inhibitor and poor lipoxygenase inhibitor. The curcuminoid displayed interactions with CYP2D6 and CYP1A2. Calebin A was inconsistently found in the tumeric-contaning natural health products investigated.
Conclusion: A sensitive, accurate and reproducible assay was developed for the detection of calebin A using RP-HPLC. Preliminary pharmacokinetic studies indicate that calebin A has poor oral bioavailability. Calebin A appears to exhibit a variety of pharmacological activities. Calebin A was found in some tumeric-contaning natural health products. Studies are ongoing in our laboratory to further characterize the pharmacokinetic and bioactivity of calebin A and related compounds.

116. Regulation of Breast Cancer Resistant Protein (Bcrp) by Peroxisome Proliferator-Activated Receptor Alpha (Pparα) at the Blood-Brain Barrier (BBB)

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Purpose: We have previously demonstrated that PPARα, a ligand-activated transcription factor primarily involved in lipid metabolism, can regulate the functional expression of BCRP in human cerebral microvascular endothelial cells (hCMEC/D3), an *in vitro* model of the BBB. In this study, we investigated, *ex vivo* and *in vivo,* the contribution of Pparα in the regulation of Bcrp functional expression at the rodent BBB.
Methods: *Ex vivo*: brain capillaries isolated from wild-type male CD-1, C57BL/6 and Pparα knockout (KO) mice (B6.129S4-Pparatm1Gonz N12 strain) were treated with Pparα ligand clofibrate (10-125μM), inhibitor GW6471 (0.5uM) or vehicle for 4-6hr. Samples were analyzed by qPCR, immunoblotting and transport assays to evaluate Abcg2/Bcrp expression and function.

In vivo: CD-1, C57BL/6 and Pparα KO mice were injected with clofibrate (125-500mg/kg) or vehicle intraperitoneally for 2-4 days. Following treatment, brain capillaries were isolated and analyzed by immunoblotting. To assess Pparα function, BODIPY FL Prazosin (Bcrp substrate) fluorescence intensity in capillary lumen was measured in absence or presence of Ko143 (Bcrp

inhibitor).

Results: For ***ex vivo*** experiments, our qPCR and immunoblot analyses demonstrated significant upregulation of Abcg2/Bcrp mRNA and protein levels in CD-1 brain capillaries treated *ex vivo* with clofibrate (~180% and ~140%, respectively) compared to controls (100%). In addition, brain capillaries (isolated from CD-1 and C57BL/6 mice) exposed to clofibrate (50-125µM, 4h) showed significantly higher luminal BODIPY FL prazosin fluorescence intensity compared to control. Such accumulation was not observed in capillaries isolated from Pparα KO mice. For ***in vivo*** experiments, our immunoblot analysis showed: 1) significant Bcrp protein upregulation in clofibrate treated CD-1 and C57BL/6 capillary lysates (~160% and 130%, respectively) compared to controls (100%); 2) no difference in Bcrp protein expression in clofibrate versus vehicle treated brain capillaries isolated from Pparα KO mice; 3) significantly higher BODIPY FL prazosin accumulation in the capillary lumen isolated from clofibrate treated animals compared to vehicle control, and such accumulation was significantly inhibited when capillaries were incubated in the presence of Ko143 (5µM), a selective Bcrp inhibitor.

Conclusion: Together these data suggest that Bcrp functional expression can be induced by Pparα selective ligand at the BBB. Since Bcrp is recognized to play a major role in brain drug permeability, these findings may provide new strategies for limiting brain penetration of neurotoxins. *(Supported by CIHR).*

117. Jadomycins B, S, and F are Aurora B Kinase Inhibitors and are Cytotoxic to Multiple Breast Cancer Subtypes

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Purpose: Jadomycins are a group of natural product molecules that are known to be cytotoxic to select cancer cell lines. However, the mechanism of their cytotoxicity has yet to be elucidated. The purpose of this study was to investigate the cytotoxicity of jadomycins in several breast cancer cell lines *in vitro*, and to investigate the inhibition of Aurora B kinase as a potential mechanism of jadomycin cytotoxicity.

Methods: The cytotoxicities of jadomycin B, jadomycin S, jadomycin F, and the positive control drugs mitoxantrone and lapatinib were assessed in MCF-7, BT-474, SKBR3 and MDA-MB-231 breast cancer cell lines using a standard MTT assay protocol.

A direct, extracellular kinase activity assay measuring the inhibition of Aurora B kinase by jadomycin B, jadomycin S, jadomycin F, and the positive control drug staurosporine was conducted using the commercially available ADP-Glo™ Kinase Assay from Promega.

Results: Jadomycin B, S, F, and mitoxantrone reduced cell viability without discrimination in all of the tested breast cancer cell lines. The HER2 inhibitor lapatinib was a potent cytotoxic agent towards the proliferating MCF-7, SKBR3 and BT-474 cells, with IC_{50}s in the high nM to low µM range. The potency of lapatinib was significantly reduced in the triple negative MDA-MB-231 cells. In the extracellular ADP-Glo™ assay, it was found that jadomycins B, S, F, and the positive control drug staurosporine inhibit Aurora B kinase.

Conclusion: Jadomycins were found to be cytotoxic to all of the tested breast cancer cell lines at similar concentrations *in vitro*. Jadomycins were also found to directly inhibit Aurora B kinase *in vitro*, suggesting that a potential mechanism for jadomycin toxicity is via Aurora B kinase inhibition. The results of this study warrant further investigation into the use of jadomycins as a chemotherapeutic treatment for breast cancer.

Acknowledgement: Matthw Ladda is the Dalhousie University recipient of the GSK/CSPS National Undergraduate Student Research Program Award.

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