Conference 2017:

From Innovation to Patient Solution

May 10 - 12, 2017
Hyatt Regency Hotel, Montréal, QC Canada

A joint conference of:

Canadian Society for Pharmaceutical Sciences
Canadian Chapter of Controlled Release Society

Conference Co-Chairs:

Frank Abbott, University of British Columbia, Vancouver, BC
Catherine Lau, Janssen Inc., Toronto, ON

Conference Organizing Committee:

Denis DeBlois (Université de Montréal), Marie Di Maso (COREALIS Pharma), Rudi Erlemann (InSymbiosis), Marc Gauthier (INRS), Fakhreddin Jamali (University of Alberta)

Canadian Society for Pharmaceutical Sciences

Welcome to Montréal and to the celebration of the **20th** Anniversary of CSPS, **150th** Anniversary of Canada, and **375th** Anniversary of Montréal!

We are pleased to once again collaborate with the Canadian Chapter of the Controlled Release Society to bring you an exciting program - *From Innovation to Patient Solution.* We hope you find the conference sessions to be valuable and thought-provoking.

The Canadian Society for Pharmaceutical Sciences (CSPS) is a non-profit organization established in 1997 to foster excellence in pharmaceutical research. Our members are scientists and educators involved in all aspects of pharmaceutical sciences including academia, industry and government. A major objective is to build partnerships and develop a strong voice to encourage government, academia, and industry to advance pharmaceutical R&D innovation in Canada.

The electronic *Journal of Pharmacy and Pharmaceutical Sciences* is the official, international journal of CSPS and can be accessed on our website.

Enjoy the program while meeting old friends and making new ones!

Frank Abbott, Ph.D.
President, CSPS (2016-2017)

CSPS Board of Directors includes representatives from industry and academia and government.
Current members of the board are **Frank Abbott**, President 2016-2017 (University of British Columbia), **Catherine Lau,** President Elect (Janssen Inc.), **Raimar Loebenberg**, Past President (University of Alberta), **Christine Allen**, Treasurer (University of Toronto), **Noriko Daneshtalab**, Secretary (Memorial University of Newfoundland), **Jane Alcorn** (University of Saskatchewan), **Denis deBlois** (Université de Montréal), **Ron Boch** (BIOTECanada), **Fakhreddin Jamali** (University of Alberta), **Agnes Klein** (Health Canada), **Elisabeth Kovacs (**Apotex), **Elizabeth Kwong** (Kwong Eureka Solutions), **Ted Lakowski** (University of Manitoba), **Fethi Trabelsi** (BioPharma Services), **Matthew Lamont (**Memorial University of Newfoundland)

CSPS 2017 Awards:

CSPS Leadership Award: Marcel Bally, BC Cancer Agency
CSPS Fellow: Christine Allen, University of Toronto
GSK/CSPS Early Career Award: Mohsen Sadatsafavi, University of British Columbia
Gattefossé Canada/CSPS Lipid-Based Drug Delivery Award: Mays Al-Dulaymi, University of Saskatchewan
GSK/CSPS National Undergraduate Student Research Program Awards:
Pawan Gill (University of Alberta), **Jovan Gill** (University of British Columbia), **Camille Thibault** (Laval University), **Lyndon Walker** (University of Manitoba), **Catherine Grandy** (Memorial University of Newfoundland), **Valérie Long** (Université de Montréal), **Kayla Wharton** (University of Saskatchewan), **Steven Choi** (University of Toronto)

Poster Awards (Winners to be announced Friday afternoon):

- Cedarlane Award of Excellence
- Antoine A. Noujaim Award of Excellence, sponsored by the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta
- 7 New CSPS Poster Awards

Special thanks to our Sustaining Partner: Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta.

Canadian Chapter of Controlled Release Society

On behalf of the Canadian Chapter of the Controlled Release Society (CC-CRS), it is my pleasure to welcome you to our Annual Chapter Symposium in Montreal. This year, we once again join forces with our CSPS pharmaceutical sciences colleagues to bring together a critical mass of academic researchers and industry professionals to offer an outstanding conference program and research forum focused on "From Innovation to Patient Solution".

CC-CRS represents over 250 Canadian academics and industry professionals across scientific, engineering, and medical fields involved in the science and technology of controlled delivery of drugs and therapeutic agents in human and animal health, and of other active agents in environmental, consumer and industrial applications. We encourage you to join our Society (membership is free!) and participate in the networking opportunities provided among Canadian controlled release specialists and promote education and student involvement in this field. In particular, we would welcome you to attend our regional events that bring together local students, professionals, and industry leaders in the area of controlled release – in the past, we have held events in Winnipeg, Toronto, and Montreal and plan to broaden these events in the coming year across Canada. Finally, we encourage you to attend the Annual Meeting & Exposition of our parent body, the international Controlled Release Society (CRS), taking place in Boston, Massachusetts, U.S.A, July 16-19, 2017 – an ideal spot to access the latest in delivery science worldwide!

Thank you for your attendance and we look forward to serving you and interacting with you in the coming months! If you'd like to get involved in CC-CRS, we are always looking for new and motivated individuals to be involved – please contact us! Visit our website at http://cc-crs.com for updates on all our activities throughout the year.

CC-CRS Board of Directors

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Student Board Members: Michael Majcher (McMaster), Soudeh Fakhari Tehrani (Montreal)

Sincerely,

Emmanuel Ho

CSPS/CC-CRS
Conference Program

WEDNESDAY, MAY 10, 2017	
12:00 PM	***CSPS Board Meeting*** (*Symphonie Room 2, Level* 5)
2:00 PM	***Registration for CSPS/CC-CRS Conference*** *(Grand Salon Foyer, Level 4)*
4:00 - 7:00 PM	**Industry Day:** **Innovation and Development of Modern Pharmaceuticals** *(Soprano Rooms A/B, Level 4)*
4:00	**Opening and Welcome:** Frank Abbott, CSPS President & Conference Co-Chair
4:10 – 5:00 PM	**Bettina Hamelin,** Vice-President, Research Partnerships Directorate, Natural Sciences and Engineering Research Council (NSERC) *Positioning Canada to Lead: Come Together, Right Now!*
5:00-5:20 PM	**Frank Béraud**, CEO, Montréal InVivo *Competitiveness of Quebec Life Sciences: How do we Compare to the Best?* *Chair:* Denis DeBlois (Université de Montréal)
5:20-7:00 PM 5:20 5:35 5:50 6:05	Showcase of companies in Quebec that are truly innovative in their approaches to drug development *Chair:* Denis deBlois, Université de Montréal **enGene:** Anthony Cheung, CEO & President *Gene Therapy 2.0: Taking Gene Medicine to the Masses* **Inception Sciences**: Alicia Levey, Senior Director *Towards Innovating the Biotech Business Model* **IRICoR Montreal**: Nadine Beauger, Chief Executive Officer *From Drug Discovery Research to Innovative Therapeutics in Partnership with Industry: a Seamless Path* **Ilkos Therapeutic Inc.**: Monique Champagne, President & CEO *Ilkos Therapeutic Inc.: A Different Business Model*
6:20	***Panel Discussion*** (Government support, academic/industry partnerships, outsourcing, venture funds) *Chair:* Rudi Erlemann, InSymbiosis
7:00 – 8:00 PM	***Welcome Reception: CSPS, CC-CRS, Vaccine Conference*** *(Grand Salon Foyer)*

THURSDAY, MAY 11 - MORNING SESSIONS

7:00 AM	***Registration*** *(Grand Salon Foyer)* ***and Poster Set-Up*** *(Grand Salon C)*	
7:00 - 9:00 AM	**SPONSORED BY FACULTY OF PHARMACY, UNIVERSITÉ DE MONTRÉAL** **Trainee Breakfast:** ***Success During and After Graduate School*** Presented by: **Nana Lee,** Depts. of Biochemistry & Immunology, University of Toronto (Ovation Room, Level 5)	
9:00 -12:00	**SPONSORED BY: ASTRAZENECA / MERCK / ROCHE** **SESSION 1: IMMUNO-ONCOLOGY** *Chair*: Barbara Melosky, BC Cancer Agency *(Grand Salon Opera A)*	**SESSION 2: CROSSING BIOLOGICAL BARRIERS** *Co-Chairs:* Michael Doschak, University of Alberta, and Marc Gauthier, INRS *(Grand Salon Opera B)*
9:00	*Overview of Development of Check-Point Inhibitors and Impact on Oncology - Now and Future* **Barbara Melosky**, BC Cancer Agency	*Real-time In Vivo Drug Values using Electrochemical Aptamer-Based Sensors* **Kevin Plaxco**, University of California Santa Barbara
9:30	*Principles of Immuno-Oncology – An Immunologist Oncologist Perspective* **Linh Nguyen**, Princess Margaret Cancer Centre	*Shuttling Brain-impermeable Therapeutic Agents Across the Blood-brain Barrier by Nanoparticles* **Shirley Wu**, University of Toronto
10:00-10:10		Trainee Presentation: *Assembling DNA Nanostructures using External Stimuli: Towards Modular Polyspecific Antibodies* **Andrea A. Greschner**, Institut National de la Recherche Scientifique – Centre EMT, Varennes, Quebec
10:10	***Coffee & Tea Break: Posters, Exhibitors, and Networking*** *(Grand Salon Foyer & Grand Salon Opera C)*	
10:40	*Impact of Immuno-Oncology on Clinical Management of Lung Cancer, Current and Future Trends* **Normand Blais**, Université de Montréal & CHUM	*Transdermal Peptide Hormone Delivery using PLO Gel to Treat Superficial Bone Surfaces* **Michael Doschak**, University of Alberta
11:10	*Optimizing Treatment of Melanoma: Impact of Immuno-Oncology on Current and Future Clinical Management* **Wilson Miller**, B. Davis-Jewish General Hospital & McGill University	*Non-Invasive Delivery of Drugs across the Dermis: Key Formulation Considerations* **Jasmine Musakhanian**, Scientific & Marketing Director. Gattefossé USA
11:40	**Panel Discussion**	
12:00	**CSPS Leadership Award Lecture: Marcel Bally**, BC Cancer Agency, UBC (Pathology & Laboratory Medicine; Pharmaceutical Sciences), CDRD *Lipids, Drugs and Metals: Ingredients for Promising Anticancer Drugs* *Chair*: Christine Allen, University of Toronto *(Grand Salon Opera B)*	
12:30 - 1:30	***12:30 - Annual General Meetings:*** ***CSPS AGM (Grand Salon Opera A)*** ***CC-CRS AGM (Grand Salon Opera B)*** ***Lunch break (on your own)*** ***Posters, Exhibitors, and Networking*** *(Grand Salon Foyer and Grand Salon C)*	
1:30 - 2:30	***Poster Session, Exhibitors*** *(Grand Salon Opera C and Grand Salon Foyer)*	

THURSDAY, MAY 11 - AFTERNOON SESSIONS

1:30 - 2:30	***Poster Session, Exhibitors*** *(Grand Salon Opera C and Grand Salon Foyer)*	
2:30 - 5:10	**SESSION 3: TRANSLATIONAL MEDICINE** *Co-Chairs*: Janet Dancey, Canadian Cancer Trials Group and Queen's University, and Catherine Lau, Janssen Inc. *(Grand Salon Opera A)*	**SESSION 4: KEY REGULATORY ISSUES** *Chair*: Fakhreddin Jamali, University of Alberta *(Grand Salon Opera B)*
2:30	*2:30 PM* *Recent Advances in the Development of Companion Diagnostics and Predictive Biomarkers in Oncology* **Janet Dancey**, Canadian Cancer Trials Group, and Queen's University	*2:30 PM* *Process Validation Lifecycle Stages (Stage 1, 2 and 3)* **Naheed Sayeed-Desta**, Apotex
	2:50 PM *A Clinical Diagnostic Lab Perspective on the Incorporating Immunotherapy Biomarkers, TMB and MSI, into Cancer Genome Profiling* **Garrett Frampton**, Foundation Medicine Inc.	*3:00 PM* *A Risk-based Approach to Development and Manufacture of a New Chemical Entity using PCMM* **John Groskoph** and **Daniel Blackwood**, Pfizer
	3:10 PM *Health Technology Assessment of Companion Diagnostics: A Canadian Perspective* **Sohail Mulla,** CADTH	
3:40-4:10	***Coffee & Tea Break: Posters, Exhibitors, and Networking*** *(Grand Salon Foyer and Grand Salon C)*	
4:10 PM	*4:10 PM* *Best Practices and Lessons Learned in Integrating Biomarkers/Companion Diagnostics in Clinical Trials; A Global Central Laboratory's Perspective* **Patrice Hugo,** Q2 Solutions	*4:10 PM* *Therapeutic Equivalence of Second Entry Products of Long Acting Bronchodilators or Inhaled Corticosteroids: The International Council for Harmonisation Guidelines* **Irvin Mayers**, University of Alberta
	4:30 PM **Panel Discussion** - *Panel Members include the 4 speakers plus: Monette Greenway, Precision Rx-Dx; Raffi Tonikian, Merck*	*4:40 PM* **Panel Discussion**
Thursday 6:00 PM	***Conference Gala and Awards Dinner*** *6:00 PM Cash Bar, 7:00 PM Dinner* *(Imagination Room, Level 6)*	

FRIDAY, MAY 12 - MORNING SESSIONS		
7:00 AM	***Registration and Poster Set-up*** *(Grand Salon Foyer and Grand Salon Opera C)*	
8:30 - 9:30	**PLENARY: Jason Moffat**, Canada Research Chair in Functional Genomics of Cancer, University of Toronto *Genome-scale CRISPR Screens and Protein Engineering for Target Discovery and Translation* *Chair*: Frank Abbott, UBC *(Grand Salon Opera A/B)*	
9:30--10:00	***Coffee & Tea Break: Posters, Exhibitors, and Networking*** *(Grand Salon Foyer and Grand Salon C)*	
10:00 - 11:40 AM	**SESSION 5: WHAT IS HAPPENING IN ANTIBACTERIALS?** *Chair*: Bastien Castagner, McGill University *(Grand Salon Opera A)*	**SESSION 6: GENE THERAPY** *Co-Chairs:* Jeanne Leblond Chain, Université de Montréal, and Azita Haddadi, University of Saskatchewan *(Grand Salon Opera B)*
10:00	*To Kill a Bacterium, You Need to Think Like a Bacterium* **Eric Brown**, McMaster University	*Overcoming Barriers to Nucleic Acid Delivery* **Tom Anchordoquy**, University of Denver
10:30	*Using the CoA Biosynthetic Pathway in the Activation of Antimicrobial Agents* **Karine Auclair**, McGill University	*Development of Gene Pill™ for Gut-Localized and Systemic Delivery of Protein Drugs* **Anthony Cheung**, enGene
11:00	*Clostridium difficile Infection: How to Trick a Toxin* **Bastien Castagner**, McGill University	*Smart Lipid Nanoparticles for Nucleic Acids Delivery* **Jeanne Leblond Chain**, Université de Montréal
11:30-11:40	Trainee Presentation: *Protease-mediated Suppression of DRG Neuron Excitability by Commensal Bacteria* **Jessica L. Sessenwein**, Queens University	Trainee Presentation: *Towards the Fabrication of a Bioinspired Synovial Fluid for Viscosupplementation Treatment in Osteoarthritis* **Jimmy Faivre**, Université de Montréal
11:40-12:10	**GSK Early Career Award Lecture: Mohsen Sadatsafavi**, University of British Columbia *Respiratory Evaluation Sciences Program (RESP): Innovations in Analytic Approaches to Improve Efficiency in Respiratory Care* *Chair*: Frank Abbott, UBC *(Grand Salon Opera B)*	
12:10 - 1:00	***Lunch Break (on your own)*** ***Posters, Exhibitors, and Networking*** *(Grand Salon Foyer and Grand Salon C)*	
1:00-2:00	***Poster Session, Exhibitors*** *(Grand Salon Opera C and Grand Salon Foyer)*	

FRIDAY, MAY 12 - AFTERNOON SESSIONS		
1:00-2:00	***Poster Session, Exhibitors*** *(Grand Salon Opera C and Grand Salon Foyer)*	
2:00 - 4:30 PM	**SESSION 7: BIOSIMILARS** *Co-Chairs*: Agnes Klein, Health Canada, and Catherine Lau, Janssen Inc. *(Grand Salon Opera A)*	**SESSION 8: ORPHAN DISEASES** *Chair*: Durhane Wong-Rieger, Canadian Organization on Rare Diseases *(Grand Salon Opera B)*
2:00	*Framework Guidelines for Biosimilars: Recent Updates, Future Considerations* **Agnes Klein**, Health Canada	*Orphan Disease Overview* **Durhane Wong-Rieger**, Canadian Organization on Rare Diseases
2:30	*Clinical Considerations for Authorization of Biosimilars* **Jian Wang**, Biologics and Genetic Therapies Directorate, Health Canada	*Exploring the Canadian Ecosystem of Rare Disease* **Cate McCready**, BIOTECanada
3:00	***Coffee & Tea Break: Posters, Exhibitors, and Networking*** *(Grand Salon Foyer and Grand Salon C))*	
	***** NOTE CHANGE OF ROOMS AFTER BREAK to SOPRANO ROOMS *****	
	SESSION 7: BIOSIMILARS *(Soprano Room A, Level 4)*	**SESSION 8: ORPHAN DISEASES** *(Soprano Room B, Level 4)*
3:30	*Physicians' Evaluation of Biosimilars on Multiple Indications* **Brian Feagan**, Robarts Clinical Trials	*Challenges and Opportunities for Biotech Companies Developing Treatments for Rare Diseases in Canada* **Michael Harvey**, Clementia Pharmaceuticals
4:00	*Disruption and Maturity: Commercial Implications for the Next Evolution of Biologics in Canada* **Mark Omoto**, QuintilesIMS	*Rare Diseases Regulatory Pathway in Canada, a Health Canada Perspective* **Fiona Frappier**, BGTD, Health Canada
4:30	***Trainee Poster Awards - Announcement and Presentation of Awards*** *(Soprano Room A)*	
4:45 PM	***Conference Concludes***	

See you next year!

May 22-25, 2018

Chelsea Hotel, Toronto, ON

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Clinical Sciences & Pharmacy Practice

Pharmacokinetics & Pharmacodynamics

Pharmaceutical & Analytical Chemistry

Biomedical Sciences

Drug Delivery and Pharmaceutical Technology

POSTER SESSION 2: CSPS AND CC-CRS
FRIDAY, MAY 12

Clinical Sciences & Pharmacy Practice

Pharmacokinetics and Pharmacodynamics

Pharmaceutical and Analytical Chemistry

Biomedical Sciences

Drug Delivery and Pharmaceutical Technology

Speaker Abstracts & Bios

Wednesday, May 10

Industry Day:

Innovation and Development of Modern Pharmaceuticals

Plenary Lecture 1

Chair: Frank Abbott, University of British Columbia

Positioning Canada to Lead: Come Together, Right Now!

Bettina Hamelin, Vice-President, Research Partnerships, Natural Sciences and Engineering Research Council (NSERC)

Despite comparatively lower levels of funding for research, Canada has excelled globally with respect to scientific productivity and publication impacts. On the other hand, the Conference Board of Canada has recently stated that "Canada ranks 9th of 16 peer countries and earns a "C" on innovation…. there are signs of emerging and persistent weakness …" How can Canada not only keep up with the rest of the world but lead and be an essential player in an inclusive and ambitious research and innovation ecosystem?

There is wide consensus that innovation takes place in a highly complex network. For example, the science and know-how behind drug discovery has become so complex that it cannot be contained within the confines of a single sector. In fact, drug discovery and development work best when the public university sector, biotech companies, big pharma, foundations, disease advocacy groups, health economists, health administrators and citizens all come together. Most often, we don't have an accurate map of all the players in the system and a government should create conditions that allow all relevant players to come together, to set ambitious goals and to excel. Research and innovation can only flourish in an inclusive, collaborative, multi-disciplinary, multi-sectoral and multi-cultural ecosystem. The Canadian federal government offers a series of programs that support many aspects of this ecosystem. Some national and international best practice examples and impact data will be shared.

This talk will explore the challenges and opportunities for Canada with respect to research and innovation, in the pharmaceutical sector and beyond, and in the context of Canada's Fundamental Science Review, its Inclusive Innovation Agenda and the ongoing consultations to create Innovate Canada.

Bettina Hamelin

As Vice-President of NSERC's Research Partnerships Directorate, Dr. Bettina Hamelin is responsible for a range of programs designed to stimulate increased public/private collaboration and technology transfer and to maximize the benefits that university and college research provides to Canada. Bettina has more than 15 years of experience in the biotech and international pharmaceutical industry as well as 10 years of academic experience as a tenured professor at the Faculty of Pharmacy at Université Laval.

Prior to joining NSERC Bettina held a variety of positions at Pfizer Canada, most recently as Canadian Medical Lead, Vaccines, and Head, Strategic Research Partnerships, Western Canada. Passionate about R&D, she excels at bringing together unlikely partners to attract and leverage funds from diverse sources. She is known for pioneering novel public-private partnership models, and breaking down barriers between federal, provincial and private sector stakeholders.

Early roots in biology and chemistry (Vordiplom, Universität Kaiserslautern, Germany) have led Bettina to complete a B.Sc. in pharmacy and a Doctor of Pharmacy, both from the University of Kentucky, U.S. and an EMBA in Healthcare from the University of British Columbia, Canada.

Wednesday, May 10

Plenary Lecture 2

Chair: Denis DeBlois, Université de Montréal

Competitiveness of Quebec Life Sciences: How do we Compare to the Best?

Frank Béraud, Chief Executive Officer, Montréal InVivo

Despite economic turbulences and global structural changes in the life sciences sector, the industry remains a very strong contributor to the Quebec economy and especially in Montreal, which represents 80% of the life sciences jobs in the province. The sector employs 56 000 people, of which 40 000 are direct jobs in about 450 companies and 150 public research groups in Montreal. These organisations account for 1.6% of the province GDP (or 5.6 B$) and for 2/3 of the life sciences GDP in Canada.

But one can legitimately ask: how good is that? How does it compare to other life sciences jurisdictions in North America? What are the lessons learned from other successful life sciences jurisdictions? This presentation will present answers to those questions.

Frank Béraud

Holding more than 25 years of experience in the life sciences sector, Mr. Béraud has particularly acquired a solid expertise in business development. With a background in sales and marketing within multinationals in the field of clinical diagnostic, his career path has led him to assume responsibility for business development for an SME in the domain of biotechnology, in addition to working as a consultant within the industry as well as a technology transfer organization. Mr. Béraud has also worked on managing the policies and strategic development of an industrial association in the life sciences sector before joining Montréal InVivo's team. Highly socially engaged with schools and the health community, he currently chairs on the board of a community organization working towards the social and economic reintegration of individuals in situations of homelessness in Montreal (Le Sac à Dos).

Wednesday, May 10

Showcasing Innovative Companies in Quebec

Chair: Denis DeBlois, Université de Montréal

Gene Therapy 2.0: Taking Gene Medicine to the Masses

Anthony T. Cheung, PhD, CEO and President, enGene, Inc., Montreal, QC

The first gene therapy was tested in humans in 1990, but the field has experienced several significant setbacks since then. Only until recently, gene therapies are finally seeing light at the end of the tunnel. The first gene therapy product - Glybera® - was approved in Europe in 2012 for the treatment of an ultra-rare genetic disease called familial lipoprotein lipase deficiency. Several other gene therapies are also being tested in patients with serious unmet needs and they are in the final stretch of pivotal trials. These successes have attracted significant capital flow into the sector, resulting in over US$11 billion in total financing that went into gene therapy companies in the last two years. Furthermore, the number of pharma and large biotech partnerships with gene therapy companies have also increased substantially with total corporate investment generated ballooned to US$2.4B in 2015 from a meager US$59M in 2013. Due to the high cost and immunogenicity of viral-based gene delivery vectors, the vast majority of gene therapy companies are targeting rare diseases with high unmet needs that can be treated with a few doses. Such a business model is inherently limited and relies on very high drug pricing to be profitable, which inevitably would increase the risk of drug reimbursement. For gene therapy to be adopted as a common therapeutic modality, the cost and safety limitations of gene delivery vector must be addressed. enGene is developing a gene delivery system that is more economical and easily scalable than the commonly used viral vectors. More importantly, this gene delivery platform is orally available and has the capability to deliver specific therepeutic gene to the gut mucosa, leading to its expression and secretion of the encoded protein drug into the bloodstream. enGene has demonstrated that this approach will only have a transitory action due to the natural turnover of gut mucosal cells. This unique short term action provides for a safe, easily managed treatment since the protein dose can be readily adjusted and treatment can be halted as desired.

Anthony Cheung
Dr. Anthony Cheung is the CEO and President of enGene, Inc. (Montreal, Canada). Prior to that, he served as the Chief Scientific Officer for the Company from 2004-2012, where he developed enGene's current platform technology for delivery of nucleotides to the gut. Dr. Cheung received his bachelor degree in Biochemistry from the University of British Columbia (Vancouver, BC) and doctorate degree in Physiology from the Tulane University School of Medicine (New Orleans, LA). Since his appointment as the CEO in 2012, Dr. Cheung has raised significant equity financing for enGene and completed two major pharma partnership transactions. Dr. Cheung has co-authored numerous book chapters, review articles and peer-reviewed journals on the topics of diabetes, gene therapy and autoimmune diseases. He has been invited to speak at many international scientific and biotechnology conferences - BIO, American Society for Gene & Cell Therapy, Diabetes Technology Meeting, Children with Diabetes - on topics related to gene therapy, drug development and bio-entrepreneurism. He also serves as Board Member and Advisor for several biotechnology companies and professional organizations including Bio-Industry Liaison Committee of the American Society for Gene & Cell Therapy.

Towards Innovating the Biotech Business Model

Alicia Levey, PhD, Vice President of Business Development, Inception Sciences, San Diego, CA

Despite the creation of multiple incubators, gap funding, accelerators and other mechanisms aimed at "bridging" the translation gap between academic science and biopharma there remains a need for

additional strategies to translate emerging science into bona fide drug discovery programs that can be further developed in to drugs. Inception Sciences has developed a business model aimed at addressing this gap while also meeting the needs of key stakeholders involved – academic contributors, founders, investors and downstream partners. We'll discuss the Inception model, program case studies and thoughts on how the model may evolve going forward.

Alicia Levey
Alicia Levey is Vice President of Business Development at Inception Sciences, a small molecule drug discovery company formed in collaboration with Versant Ventures, a global venture capital firm focused primarily on early stage investing and biotechnology company creation. Alicia leads business development and alliance management activities across companies under the Inception umbrella. While at Inception, Alicia has led or played key roles in new company incubation efforts and in establishing multiple enabling Pharma partnerships. Prior to Inception, Alicia was a Principal in the San Francisco office of Versant Ventures where she led the diligence on multiple deals including several that have been acquired (Novira) or are now public (Audentes). Before joining Versant, Alicia worked as a Project Leader in the San Francisco office of The Boston Consulting Group where she focused on primarily on biopharma and global health strategy. Alicia's academic background is in Cancer Biology, earning a PhD from the Stanford University School of Medicine where she focused on the development of novel activity based protease probes for non-invasive imaging in cancer, work that led to multiple peer reviewed publications and two US patents. Originally from Colorado, Alicia graduated Summa Cum Laude with dual degrees in Molecular Biology and Biochemistry from the University of Colorado at Boulder where she was a Boettcher and Norlin Scholar.

From Drug Discovery Research to Innovative Therapeutics in Partnership with Industry: a Seamless Path

Nadine Beauger, PhD, MBA, Chief Executive Officer, IRICoR, Montreal, QC

IRICoR (Institute for Research in Immunology and Cancer (IRIC) - Commercialization of Research), a key drug discovery and project maturation cluster for academic research, is a not-for-profit organization based at Université de Montréal's (UdeM's) Institute for Research in Immunology and Cancer (IRIC). Since its creation in 2008, IRICoR's mission has been to accelerate maturation of projects from IRIC/UdeM/other academic centres toward co-development partnerships with industry or creation of new spin-offs, for the development of patient-accessible innovative therapeutics in oncology, immunotherapy and related fields. IRICoR integrates under a single roof in academia access to state-of-the-art basic research; to IRIC's drug discovery technology platforms, with one of the largest industry-experienced academia-based medicinal chemistry groups in Canada; and to its dedicated in-house business expertise.

The proximity to leading edge biology and critical expertise in medicinal chemistry was central to IRICoR building a portfolio of close to 40 projects and 12 patents granted, establishing lasting public-private partnerships, bringing 5 projects to the clinic, creating 4 spin-off companies, including clinical-stage ExCellThera and revenue-generating Domain Therapeutics NA. IRICoR thereby actively contributes to the creation and retention in Quebec and in Canada of a unique expertise in drug discovery research translation.

After 9 years of existence in close association with IRIC / UdeM, IRICoR is increasingly at the heart of the ecosystem, efficiently transforming drug discovery research into high value innovations.

Nadine Beauger
Nadine Beauger is Chief Executive Officer at the Institute for Research in Immunology and Cancer – Commercialization of Research (IRICoR). Since 2009, Dr Beauger has been instrumental at building IRICoR's project portfolio and related intellectual property (IP) activities. With over 15 years of experience in technology transfer, venture capital and IP portfolio management with thorough experience in both the private and the academic sectors, she holds a Ph.D. in Biomedical Sciences, an M.Sc. in Cellular Biopathology (Université de Montréal) and a B.Sc. in Anatomy (McGill University). She also has an MBA degree from HEC Montréal (Excellence Admission Award).

Ilkos Therapeutic Inc.: A Different Business Model

Monique Champagne, B.Pharm., M.Sc., President & CEO, Ilkos Therapeutic Inc.

The quality of R&D and service infrastructures, combined with the availability of capital, make Quebec an optimal location for the development of new therapies and shows that Quebec can succeed in the life science sector. Innovation also involves coming up with different business models and new ways to invest by sharing the risk with pharmaceutical firms while capitalizing on their expertise. By leveraging collaboration between venture capital investors such as CTI Life Sciences and the Fonds de solidarité FTQ, and Servier, a French organization with a long-standing presence in the province, Quebec is at the vanguard of biomedical innovation.

Ilkos Therapeutic Inc. (Ilkos) is a new Québec biotechnology firm and the result of a combined $21-million investment by three equal partners: CTI Life Sciences Fund, the Fonds de solidarité FTQ and Servier. It is the first time Servier, an international pharmaceutical firm which is well established in Quebec, is partnering with VC funds in Canada. The creation of Ilkos makes it possible to continue the development of an innovative drug, S42909, which was deprioritized from Servier's R&D strategic priorities. This partnership ensures the development of an original molecule under a global exclusive licence granted by Servier to Ilkos in August 2016.

The target indication for S42409 is the oral treatment of venous lower limb ulcers, a disease that affects 1% to 1.5% of the population, mainly older people. Current treatment, which besides being only moderately effective for complete wound healing and limited to compression bandages and local wound care, is labor intensive, accounting for 1.5% to 2% of the health care budget in countries such as Canada. The new treatment, which seeks to become the first disease-modifying therapy for venous lower limb ulcers, is potentially a major clinical breakthrough.

The main project to be headed by Ilkos involves demonstrating the efficacy and safety of S42909 in a Phase 2a proof of concept multicenter trial to be conducted in Canada, in Europe and in the United States and involving more than 200 patients.

As part of a collaboration agreement with Ilkos, Servier will coordinate and oversee the trial through Servier Canada's Centre of Excellence in Clinical Research, ensuring expertise in the development of S42909 and transfer of knowledge.

Monique Champagne

Monique Champagne is a pharmacist with 29 years of experience in clinical research, drug development and regulatory affairs, acquired in the pharmaceutical, biotechnology and contract industries. A creative manager, she has extensive practical experience and expertise in the first phases of oncology drug development, in clinical development in various therapeutic areas, clinic operations management, regulatory affairs, integration and improvement of operational processes, as well as in managing international projects and professional relations with business partners.

Monique was appointed as President and Chief Executive Officer of Ilkos Therapeutic Inc. in January 2017. Prior to joining ILKOS, Ms. Champagne was Vice-President, Clinical Research and Regulatory Affairs, at Telesta Therapeutics. Before that, she held management positions at, among others, Xanthus Life Sciences, Supratek Pharma, PricewaterhouseCoopers and Wyeth-Ayerst (Pfizer).

Monique Champagne holds a Bachelor of Pharmacy (B.Pharm.), a master's degree in pharmaceutical sciences (M.Sc.) and a pharmacist license in Québec.

Panel Discussion

Chair: Rudi Erlemann, InSymbiosis

Thursday, May 11

Trainee Breakfast

SPONSORED BY:
FACULTY OF PHARMACY, UNIVERSITÉ DE MONTRÉAL

Chair: Nana Lee, University of Toronto

Success During and After Graduate School

Nana Lee, PhD, Director and Lecturer of Graduate Professional Development, Departments of Biochemistry and Immunology, Lead Coordinator, Faculty Development Program, Graduate Life and Science Education, Faculty of Medicine, University of Toronto

What are the secrets in succeeding as a trainee and being market-ready? Trainees are invited to discuss practical tips on strategic communications, individual development plans, marketing oneself and the resume which makes it to the top of the list with Dr. Nana Lee, a leader in graduate professional development and former industry scientist. All trainees are encouraged to submit in a resume and/or cover letter to receive interactive feedback during the workshop.

Nana Lee

Dr. Nana Lee completed her PhD in Biochemistry at the University of Toronto, was a Visiting Graduate Scholar at MIT, followed by a PDF at the University of Michigan. She brings her several years of biotech industry experience in roles as Director of Application Science, Product Manager, Senior Research Scientist to her current position of Director of Graduate Professional Development at her alma mater. Along with Dr. Reinhart Reithmeier, she developed and implemented the innovative Graduate Professional Development (GPD) course in the fall of 2012 which was published in Science Careers. GPD has been featured as a transformative initiative by the Conference Board of Canada's 2015 national report on PhD programs and highlighted in the 2017 Report on North American GPD programs by the Council of Graduate Schools, National Science Foundation. She is currently expanding the program at the faculty development level with the Faculty of Medicine in partnership with the School of Graduate Studies. She and Dr. Reithmeier just published their book "Success After Graduate School."

Thursday, May 11

SESSION 1:

Immuno-Oncology

SPONSORED BY:

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Chair: Barbara Melosky, BC Cancer Agency

Overview of Development of Check-Point Inhibitors and Impact on Oncology - Now and Future

Barbara Melosky, MD, FRCP(C), BC Cancer Agency, Vancouver, BC

Immuno-oncology (I-O) is recognized as the new pillar of oncology as a cancer treatment. The concept of immunotherapy as a means to control disease is not new. In the 18th century, Edward Jenner demonstrated the power of immunotherapy for infectious disease when he pioneered a vaccine for small pox. I-O therapy options have grown since then, with vaccines, tumour-directed monoclonal antibodies. Immune checkpoint inhibitors allow the patient to activate his or her own immune system to fight disease. The efficacy of these agents was first shown in immune sensitive cancers such as melanoma with CTLA-4 inhibitors such as ipilumub. The list of therapeutics and indications has grown tremendously since then.

It is recognized that a growing number of tumor types have benefit from this strategy.

This is a new class of therapeutics. The unique time and prolonged duration of response deserves attention. Pseudo-progression may be seen and challenges our traditional efficacy parameters.

The side effect profiles of checkpoint inhibitors are unique and education of both patient and physician is the key to successful delivery.

To allow more patients to benefit, the future of immuno-oncology is to combine these agents with other treatment modalities.

The emergence of this class of drugs has taken the oncology community by a storm and remains an incredible journey for both physician and patient.

Barbara Melosky, MD, FRCP(C)
Dr. Melosky is an Associate Professor of Medicine at the University of British Columbia and a Medical Oncologist at the British Columbia Cancer Agency. She graduated from medical school at the University of Manitoba and did a residency in internal medicine and an oncology fellowship at the University of British Columbia. She is currently a medical oncologist at the BC Cancer Agency with clinical interests in lung malignancies. She is the chairman of the annual Canadian Lung Cancer Conference and is chair of the Lung Cancer Biobank of BCCA.

Principles of Immuno-Oncology – An Immunologist Oncologist Perspective

Linh Nguyen, Head, Translational Immunotherapy Laboratory, Tumor Immunotherapy Program, Princess Margaret Cancer Centre, Toronto, ON

T cells can be effective mediators of anti-tumor immunity. Recognition of cancer cells by T cells occurs via interactions between the T-cell receptor and tumor-associated antigens. Various events occur during an anti-tumor immune response, many of which are subject to similar stimulatory and inhibitory mechanisms that govern T cell responses against pathogens. Potential cancer immunotherapies aimed at enhancing T cell-mediated elimination of cancer cells include strategies such as targeting tumor-associated antigens using monoclonal antibodies, vaccination with tumor antigens in various forms, blockade of immune inhibitory molecules, and infusion of tumor-reactive T cells. The current status of these strategies will be discussed from an immunological perspective, as well as their potential mechanisms of action and future directions.

Linh Nguyen
Dr. Nguyen obtained her PhD in Immunology at the University of Toronto and pursued postdoctoral studies at Harvard Medical School. Her current interests include translational work in tumor immunology and immunotherapy. Her team also manufactures clinical-grade cell products and prepares regulatory documents for investigational trials of products such as tumor-infiltrating lymphocytes (TILs), dendritic cells and TCR gene-transduced T cells.

Impact of Immuno-Oncology on Clinical Management of Lung Cancer, Current and Future Trends

Normand Blais, MD, MSc, FRCPC, Hematologist and Medical Oncologist, Associate Clinical Professor - University of Montreal, Montreal, QC

Lung cancer remains the most common cause of cancer death in men and women in Canada. The vast majority of patients challenged by lung cancer will succumb to metastatic disease. The rapidity of disease progression, the presence of major comorbidities in many patients and multiple challenges to investigate patients suspected to have lung cancer in a timely manner are but some of the obstacles to patient management. As such, a majority of patients with inoperable or metastatic disease do not even have the opportunity to discuss systemic treatments for their disease and even fewer patients are actually eligible for treatment. Current approaches to treatment are varied and are based on the presence of molecular and immune-histological biomarkers obtained on the tumor biopsy. As specific targeted treatment is possible in only 15-25% of patients, most patients are still being prescribed a platinum containing doublet as first line therapy. Immunotherapy, mostly directed at the PD-1/PD-L1 interaction, is being convincingly demonstrated to be superior to second line chemotherapy in most patients with NSCLC and has become the standard of care in that setting in the past few years. Recent findings also strongly suggest that immunotherapy is more active than standard first-line platinum containing chemotherapy in a subset of patients, particularly those whose tumors highly express PD-L1 as demonstrated by immunohistochemistry. This rapidly moving field is being extensively investigated and much more exciting practice changing data will be presented in this coming year. Important questions that remain to be answered concern the role of immune checkpoint inhibitors in first line treatment either in monotherapy, or as combination treatment with chemotherapy, other immunotherapy agents or radiation therapy. There are also hopes that this type of approach may be useful in the adjuvant setting and lead to an increase in the curability of this devastating disease.

Normand Blais
Dr. Blais is a Hematologist and a Medical Oncologist. He is currently a Clinical Associate Professor at University of Montreal, and an Assistant Professor at McGill University. He serves also as the Team Director for Interdisciplinary Thoracic Oncology Team, the Director of Hemostasis-Thrombosis Laboratories, and Co-Director of the Visiting Professors Program at CHUM - Notre-Dame Hospital. More recently he was appointed as the director of the clinical oncology research program at the CHUM Research Center.

His major interests are research in lung cancer, urological tumors, cancer and thrombosis, and running.

Optimizing Treatment of Melanoma: Impact of Immuno-Oncology on Current and Future Clinical Management

Wilson Miller, B. Davis-Jewish General Hospital & McGill University, Montreal, QC

The treatment of advanced melanoma has seen major breakthroughs in two distinct domains: targeted therapy and immunotherapy. Advances in both directions have dramatically improved response rates and overall survival in the past ten years. Not only do reports of further advances keep coming with almost every international meeting, but melanoma has been a model for successfully moving targeted therapy and especially immune therapy to many other malignancies, where we are also seeing durable responses and major survival benefits. This presentation will briefly review the history of immune therapy in melanoma then will concentrate of recent advances and the promise of more advances to come from ongoing clinical trials. A major additional research goal is to develop predictive clinical and molecular biomarkers for response to these new therapies, especially when we

must choose between immunotherapy and targeted therapy. Although generally well tolerated, toxicities to these new agents do occur, especially with combined immunotherapy regimens, and can be serious. They are also quite different from the toxicities of chemotherapy, and they require very different management, which will require education of ER staff and other medical specialists, as well as oncologists and patients. Thus, the advantages and disadvantages of our growing armamentarium of new treatments for melanoma will be discussed.

Wilson Miller

Wilson H. Miller, Jr. is the James McGill Professor in the Departments of Oncology and Medicine at McGill University, Montreal. He is Associate Director of the Lady Davis Institute, Deputy Director of the Segal Cancer Center, co-Director of the Clinical Research Program in the Department of Oncology at McGill and Clinical Lead of Rossy Cancer Network. Dr. Miller has a PhD from The Rockefeller University and an MD from Cornell University Medical College. He has held a faculty appointment in the Department of Medicine at Cornell and at the Memorial Sloan-Kettering Cancer Center in New York. Dr. Miller's laboratory at the Lady Davis Institute investigates molecular mechanisms underlying leukemia, breast cancer and melanoma, with a focus on the development of novel targeted therapies. He has received a number of research awards throughout his career, including the Medical Research Council of Canada Scientist Award and the FRSQ Chercheur national Award. Dr. Miller is a well-known speaker at national and international meetings and sits on peer review panels for the National Institutes of Health (US), the Canadian Institute of Health Research, the Ontario Cancer Research Network, and the Leukemia Lymphoma Society. Dr. Miller's laboratory uses several approaches to understand the mechanisms of action and development of novel anti-cancer therapies. Current projects include the role of nuclear co-regulators of transcription and changes in chromatin in response and resistance to epigenetic therapies in acute promyelocytic leukemia and other hematopoietic malignancies. Another area of interest for the lab is the development of novel therapeutics for breast cancer and melanoma, with a focus on new pathways regulating protein synthesis, modification and degradation. In his role as Director of Phase 1 studies at McGill, he has had a leading role in the development of immuno-oncology. As Clinical Lead of the Rossy Cancer Network — a partnership dedicated to integrating and improving quality of care in the McGill University-affiliated hospitals — Dr. Miller spearheaded the implementation of a multi-hospital disease site program and promoted collaboration between clinical trials throughout the network.

Thursday, May 11

SESSION 2:

Crossing Biological Barriers

Co-Chairs: Michael Doschak, University of Alberta, and Marc Gauthier, INRS

Real-time In Vivo Drug Values using Electrochemical Aptamer-Based Sensors

Kevin Plaxco, Professor, University of California, Santa Barbara

The development of technology capable of continuously tracking the levels of drugs, metabolites, and biomarkers in situ in the body would revolutionize our understanding of health and our ability to detect and treat disease. It would, for example, provide clinicians with a real-time window into organ function and would enable therapies guided by patient-specific, real-time pharmacokinetics, opening a new dimension in personalized medicine. In response my group has pioneered the development of a "biology-inspired" electrochemical approach to monitoring specific molecules that supports real-time measurements of arbitrary molecular targets (irrespective of their chemical reactivity) directly in awake, fully ambulatory subjects.

Kevin Plaxco
Kevin Plaxco is a Professor at the University of California, Santa Barbara, with shared appointments between the Department of Chemistry and Biochemistry, the Department of Mechanical Engineering, and the Biomolecular Science and Engineering Graduate Program. Prof. Plaxco also serves as Director of campus's Center for Bioengineering. Prior to joining UCSB in 1998 Dr. Plaxco received his Ph.D. from Caltech and performed postdoctoral studies at Oxford and the University of Washington. Dr. Plaxco's research focus is on the physics of protein folding and its many and varied engineering applications. A major aim of the group's applied research is to harness the speed and specificity of folding in the development of sensors, adaptable surfaces, and smart materials. Dr. Plaxco has co-authored nearly a dozen patents and more than 180 papers on protein folding, protein dynamics, and folding-based sensors and materials, and is recognized by Thomson Reuters at one of the most highly cited chemists of the prior decade. He serves on the scientific boards of a half dozen biotechnology firms (several of which are commercializing technologies developed by his group), and has also written a popular science book on Astrobiology.

Shuttling Brain-impermeable Therapeutic Agents Across the Blood-brain Barrier by Nanoparticles

Xiao Yu (Shirley) Wu, PhD, FAAPS, Director of Advanced Pharmaceutics & Drug Delivery Laboratory, University of Toronto, Ontario

Diseases in the brain and the central nervous system (CNS), including primary brain tumors, brain metastases of cancer, and neurodegenerative diseases, affect over two billion people worldwide. Unfortunately the majority of therapeutics intended for these diseases, including 98% of small molecules and almost all macromolecular biologics, are unable to cross the blood-brain barrier (BBB) at sufficient levels to achieve therapeutic effect. Thus effective and safe technologies for delivering therapeutic agents to the disease sites in the brain is pressingly needed. In this presentation, Dr. Wu will review the current strategies to overcome the BBB for drug delivery with a focus on nanoparticle systems that utilize receptor-mediated mechanism to gain entry to the brain. Dr. Wu will present a polymeric BBB-penetrating nanocarrier system, developed in her Advanced Pharmaceutics & Drug Delivery Laboratory, which works as a Trojan Horse to shuttle various brain-impermeable agents to the brain. Examples of delivering anticancer drugs and biologics using this nanocarier system for treatment of brain metastases of breast cancer and CNS diseases will be presented. Challenges and future perspectives will be discussed.

Xiao Yu (Shirley) Wu

Dr. Xiao Yu (Shirley) Wu is a full Professor and elected Fellow of American Association of Pharmaceutical Scientists (AAPS). She was trained in polymer science and engineering and earned her PhD degree in Chemical Engineering with a thesis on polyelectrolytes and stimulus-responsive nanohydrogels. She joined the Faculty of Pharmacy at the University of Toronto in 1994 as a tenure-track faculty member and progressed to Full Professor in 2006. During her 23 year academic career, Dr. Wu has directed a well-funded innovative research program and become an internationally recognized expert and leader in controlled release dosage forms and novel drug delivery strategies and delivery systems. She is also a dedicated educator who has played a leading role in teaching pharmaceutics and drug delivery in the faculty at both graduate and undergraduate levels over two decades. She has provided excellent multidisciplinary training in pharmaceutics, drug delivery and drug development to ~150 graduate students, undergraduate research students, and postdoctoral researchers working in her laboratory. She has extensive collaborations with scientists in academia and pharmaceutical industry. Dr. Wu has published >150 journal papers and book chapters, and ~270 conference proceedings and abstracts, and delivered >100 invited presentations. She is a co-inventor of 25 issued or pending patents worldwide. Her current research projects include the design and *in vitro*/*in vivo* evaluation of blood-brain barrier-penetrating nanoparticles for therapy and imaging of brain cancer and CNS diseases, synergistic drug combination nanomedicine for treatment of multidrug resistant and metastatic breast cancer, hybrid bioreactive nanoparticles for remodeling tumor microenvironment and improving tumor therapy, intelligent polymer and nanotechnology for closed-loop insulin delivery, and computer-aided design of controlled release drug delivery systems. She has received a number of awards including Astra Pharma-AFPC New Investigator Research Award (1999) and AFPC-Pfizer Research Career Award (2016).

Selected Abstract for Oral Presentation

Assembling DNA Nanostructures using External Stimuli: Towards Modular Polyspecific Antibodies

Andrea Greschner, Institut National de la Recherche Scientifique – Centre EMT, Varennes, Quebec
(See Abstract # 31)

Transdermal Peptide Hormone Delivery using PLO Gel to Treat Superficial Bone Surfaces

Michael Doschak, MSc, PhD, Associate Professor, Faculty of Pharmacy & Pharmaceutical Sciences, and Adjunct Associate Professor, Departments of Biomedical Engineering and Dentistry, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta

Current clinical use of bone-active peptide hormones, such as calcitonin and parathyroid hormone (PTH), involves systemic delivery by sub-cutaneous injection at supra-physiological dosages, in order to reach an effective therapeutic index in the desired target tissue, namely bone. However, systemic exposure results in unwanted side-effects of those often pleiotrophic peptide hormones in non-bone tissues which also express resident receptors. To improve bone drug delivery, we developed bone-targeting analogues of calcitonin and PTH, by conjugation to bone-seeking bisphosphonate drug moieties. When administered by sub-cutaneous injection, those analogues immediately seek and bind to bone surfaces, notably those undergoing active turnover or increased bone metabolism. However, for anatomically superficial bone sites (such as tibial bone), it may be possible to further refine drug delivery to bone by developing effective transdermal peptide hormone delivery systems. In this presentation, we show data from preliminary in vivo studies in rats, aimed at transdermal delivery of bone-targeting calcitonin analogues using alternating hydrophilic-hydrophobic pluronic lecithin organogel (PLO) drug delivery cream base, to treat sites of superficial bone injury in anatomically placed superficial bone sites (such as shin splints) in order to reach maximum efficacy and to minimize possible side effects involved with parenteral administration and systemic exposure.

Michael Doschak

Michael Doschak is an Associate Professor with the Faculty of Pharmacy & Pharmaceutical Sciences at the University of Alberta. His initial degree was a Bachelor of Science (in Medical Technology) from Curtin University in Perth, Western Australia (1984), then an M.Sc. (1998) and Ph.D.(2004) in Medical Sciences, both with the Faculty of Medicine at the University of Calgary, where his research centered on the mineral binding effects of the bisphosphonate drug family during the pathogenesis of Osteoarthritis. Following post-doctoral training in drug delivery in the lab of Dr. Hasan Uludag at Chemical & Materials Engineering at the U of A, and an NSERC Industrial Fellowship with the Canadian biotech company Millenium Biologix in Mississauga Ontario, he was recruited to the University of Alberta by the Faculty of Pharmacy & Pharmaceutical Sciences in 2005, where he established the first *in vivo* Micro-Computed Tomography imaging lab in Edmonton, to temporally assess novel bone drug compounds on the mineralized tissues of small laboratory animals, non-invasively and at very high resolution. His research efforts recently culminated with the synthesis and characterization of several novel peptide hormone drug conjugates as the "next generation of bone drugs" – namely, bone-targeting biologic agents that strategically coat bones immediately after systemic administration. He has served with the international Controlled Release Society (CRS) as the Canadian Chapter President (2011-13), as Chair of the International Committee (2014-16), and was elected to the CRS Board of Scientific Advisors for a 3 year term (2015-2018).

Non-Invasive Delivery of Drugs across the Dermis: Key Formulation Considerations

Jasmine Musakhanian, Scientific & Marketing Director. Gattefossé USA

The growing interest in percutaneous absorption is a reflection of a growing need for alternative routes of administration to facilitate delivery of challenging molecules that suffer from poor oral bioavailability. Assisting the passage of a molecule to or across the skin however comes with its own unique set of challenges, especially if the objective is to deliver the drug in a safe and non-invasive manner. This presentation explores the role of penetration and permeation enhancing excipients in modulating the delivery of different types of molecules. Providing examples and case studies, the presentation highlights the key issues; implications of drug solubilization vs dispersion in the vehicle; selection of vehicle type(s); and possible synergies between select combinations of excipients to drive molecules' diffusion, partitioning, penetration, and permeation.

Jasmine Musakhanian

Jasmine is Scientific & Marketing Director at the Pharmaceutical Division of Gattefossé USA. She has a multi-disciplinary career that has revolved around safety, regulatory, and functionality aspects of specialty ingredients and excipients. Special areas of interest to her are lipid-based solubilization technologies for enhancing the delivery of challenging molecules. She is author of technical and scientific reviews; active member of the AAPS; and resides on USP Expert Committees for excipient functionality and monograph modernization. Jasmine graduated from McGill University in Montreal with B.Sc. (Food Science) and M.Sc. (Protein Chemistry). Her résumé includes a variety of technical liaison positions at McGill University; Seiko Scientific Instruments; Ashland Chemical; Daminco Inc.; and Gattefossé since 1997.

Thursday, May 11

CSPS Award of Leadership in Canadian Pharmaceutical Sciences Lecture

Chair: Christine Allen, University of Toronto

Lipids, Drugs and Metals: Ingredients for Promising Anticancer Drugs

Marcel Bally, BC Cancer Agency, UBC (Pathology & Laboratory Medicine; Pharmaceutical Sciences), CDRD, Vancouver, BC

One of the research projects that I completed during my PhD concerned the use of safranine, a dye used to assess mitochondrial membrane potentials. When this dye was added to liposomes with a transmembrane potential, the dye redistributed to the inside of the liposomes and under appropriate conditions >98% of the dye became trapped within the liposomes. This observation led to "remote" drug loading methods, where drugs were added to pre-formed liposomes with a transmembrane gradient and subsequently redistributed to the liposome interior. This methodology has evolved, with one variation relying on use of encapsulated divalent metal ions that complex candidate drugs bearing appropriate binding ligands. Doxorubicin, as an example drug candidate, complexed manganese trapped within the liposome. A color change accompanied drug encapsulation as the solution went from an orange to purple. The technology described has provided a versatile method to form metal drug complexes within liposomes. The remote loading technology enabled development of many drug candidate formulations some of which were subsequently developed and approved by regulatory bodies as single agents (e.g. MyoCet, Marqibo) while others focused on development of fixed ratio drug combination formulations as exemplified by CPX-351 (Vyxeos); a formulation of cytarabine and daunorubicin. The drugs and drug combinations that have been developed to date represent successes and illustrate how academic research can lead to translational opportunities with commercial and therapeutic potential. However, these formulations are simply not able to overcome the challenges faced when trying to treat patients with metastatic cancers; the patient population that represent the greatest clinical challenge. The challenges that need to be overcome include: (i) the heterogeneous microenvironment within tumors that define barriers to optimal drug delivery as well as the genetic heterogeneity that contributes to cancer cells exhibiting differing sensitivities to drugs; (ii) cytoprotective responses that limit the effectiveness of drugs when first used; and (iii) the inability to deliver drugs in a manner that ensure appropriate exposure at all sites where tumors grow. To address these challenges we are now trying to integrate discoveries made in synthetic lethal screens with nano-particulate formulation technologies designed to sustain drug levels and duration of exposure of the selected combinations at all sites of tumor growth. This program is attempting to integrate discoveries made in chemical and genomic synthetic lethal screens with nano-particulate drug carrier formulations designed to ensure that optimally effective concentrations of selected agents are achieved where needed.

Marcel Bally

Dr. Bally received his BSc (1977) and MSc (1979) degrees in biology from Texas A&M University. He obtained his PhD from the Department of Biochemistry at the University of British Columbia (1984). Dr. Bally is an authority in drug delivery, anti-cancer drug combinations and drug evaluation in animal models of disease. His research interests focus on the development and characterization of novel lipid-based nanoparticle formulations for use in the treatment of cancer. Dr. Bally's is one of the founders of the Center for Drug Research and Development; an organization aimed at addressing the growing commercialization gap between discoveries made in academia and the opportunity to develop this technology to a stage where investments can be made to support clinical and commercial development. He has extensive training in the care and use of animals for research, and he is qualified to conduct preclinical safety studies under Current Good Laboratory Practices (cGLP). Further, he has completed training in Current Good

Manufacturing Practices (cGMP) as required for operating a cGMP compliant manufacturing facility at the BC Cancer Agency. He has published >400 citable articles; including peer-reviewed papers (>200), published abstracts (>100), book chapters (19) and patents (10 examples of US patents he is a co-inventor represent a total of 76 patents when considering international filings). His work has >18,250 citations (6,219 citations since 2012). His research can be linked directly to regulatory approved drugs, one product in late stage development and several others in early stage clinical trials and preclinical development. This would include (i) Myocet, a liposomal formulation of doxorubicin approved for use in treatment of metastatic breast cancer; (ii) Marqibo®, a liposomal vincristine formulation for treatment of Non-Hodgkin's Lymphoma and Acute Lymphoblastic; (iii) CPX-1; a first in class drug combination product comprising a fixed ratio liposomal formulation of Irinotecan (CPT-11)/Floxuridine (FUDR); and (iv) CPX-351 (Vyxeos); a first in class drug combination product comprising of cytarabine and daunorubicin. CPX- 351 is being evaluated by the FDA under a Fast Track Designation for the treatment of elderly patients with secondary acute myeloid leukemia.

Thursday, May 11

SESSION 3:

Translational Medicine

Chair: Janet Dancey, Canadian Cancer Trials Group, and Queen's University

Recent Advances in the Development of Companion Diagnostics and Predictive Biomarkers in Oncology

Janet Dancey, Director, Canadian Cancer Trials Group, and Professor, Queen's University

Research has advanced the understanding of the molecular determinants of cancer, directing the development of new therapeutic strategies against actionable molecular targets. Clinical trial design and pathways to drug registration have adapted to meets the needs for rapid evaluation of drugs and candidate biomarker tests. In vitro companion diagnostic tests have been integral in the successful development and implementation of targeted therapies. More recently, clinical trials utilizing next generation sequencing have been designed to efficiently screen patients for targeted therapies. In the near future, the biomarker testing will require evaluation of multiple genomic, epigenomic and proteomic features to determine optimal treatment of patients with cancer. This presentation will highlight how trials have evolved in light of advances in science and technology as well as the challenges and opportunities of precision medicine approaches in cancer therapeutics development.

Janet Dancey
Dr Dancey is a medical oncologist with over 20 years' experience in cancer clinical trials, experimental therapeutics and biomarker testing. She is also Scientific Director of the Canadian Cancer Clinical Trials Network. Prior to becoming Director of the Canadian Cancer Trials Group on September 1, 2014, Dr. Dancey was Director, Translational Research – Clinical at Canadian Cancer Trials Group and Director of the High Impact Clinical Trials Program at the Ontario Institute for Cancer Research. Prior to joining the Canadian Cancer Trials Group, Dr. Dancey was Associate Chief of the Investigational Drug Branch Senior Clinical Investigator in the Cancer Therapy Evaluation Program at the US National Cancer Institute. Dr. Dancey received her MD from the University of Ottawa and completed her residency training in internal medicine and medical oncology at the University of Toronto. In 1994-95, she was a research fellow with the Canadian Cancer Trials Group and continued her fellowship training at the Institut Gustave Roussy in France. Dr. Dancey has special expertise in new anti-cancer drug development, linking drug and biomarker development, and associated clinical trials methodology. She is also Professor in the Department of Oncology at Queen’s University.

A Clinical Diagnostic Lab Perspective on the Incorporating Immunotherapy Biomarkers, TMB and MSI, into Cancer Genome Profiling

Garrett Frampton, Associate Director of Cancer Genomics, Foundation Medicine Inc.

The ability of tumors to evade immune surveillance by overexpressing immune checkpoint proteins has been exploited for therapeutic intervention through antibodies designed to interrupt their signaling. A number of patients across a range of disease types, including melanoma, lung, renal and bladder cancer, have demonstrated robust and durable responses using checkpoint inhibitor therapies (CPITs). Identifying the most likely responders remains an urgent need for proper clinical management. Tumor mutational burden (TMB) measures the overall number of somatic protein coding mutations per area of sequence counted occurring in a tumor specimen. Microsatellite Instability (MSI) is a state of genetic hyper-mutability that results from impaired DNA mismatch repair. Both measures have been associated with both response and survival for multiple CPITs across an array of indications. It is hypothesized that immunotherapies are more effective for tumors with high TMB and MSI because these cells are more likely to express

immune-reactive neoantigens. In this study we describe Foundation Medicine's (FMI) work to develop and validate TMB and MSI results as part of the current FoundationOne (F1) and FoundationOne Heme (F1H) comprehensive genomic profiling assays.

Garrett Frampton

Garrett's career in science and medicine began when he worked as a pharmacy technician during high school. He attended college at the University of Chicago and graduated with honors in 2001, with a double major in chemistry and in biology. During his last two years of college he worked as the data manager in a Clinical Neuroscience and Psychopharmacology Research Unit at the University of Chicago Hospital, which performed clinical drug trials and academic research into impulsive aggression.

Immediately following college, Garrett was introduced to the field of genomics when he began working as the first laboratory technician in the newly formed microarray core facility at the Boston University School of Medicine. Garrett spent 3 years at Boston University, during which time he transitioned from doing primarily wet bench science to full time computational biology data analysis and bioinformatics. He took part in a number of collaborations, and authored 5 publications.

Garrett attended graduate school at the Massachusetts Institute of Technology, graduating with a doctoral degree from the department of Biology in 2010. During graduate school, he worked at the Whitehead Institute in the laboratory of Richard Young. His research focus was investigating the transcriptional regulatory circuitry that controls cell type specific gene expression programs, primarily using embryonic stem cells as a model system. With the development of the ChIP-seq method in 2007, Garrett primarily focused on ChIP-Seq analysis and developed a software package for the analysis of these datasets, which has been used widely. During his time at MIT he authored 13 publications.

For the past six years, since finishing graduate school, Garrett has worked as a computational biology scientist at Foundation Medicine. He is currently Associate Director of Cancer Genomics in the company's Research group. Foundation Medicine is a cancer diagnostics company that has developed a next-generation sequencing based cancer genomic test that analyzes routine clinical specimens for somatic alterations in relevant cancer-related genes. At Foundation Medicine Garrett has co-authored numerous conference presentations and publications including the company's analytic validation study published in Nature Biotechnology, "Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing," and work describing the diversity and importance of MET exon 14 alterations, "Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors."

Health Technology Assessment of Companion Diagnostics: A Canadian Perspective

Sohail Mulla, PhD, CADTH, Toronto, ON

Companion diagnostics are laboratory tests that aim to identify subgroups of patients who are most likely to benefit and/or least likely to experience harms from given drugs. They work by detecting specific biomarkers, the presence (or expression beyond certain levels) of which predict more favourable responses to specific drug treatments. The global market for companion diagnostics is growing, and the resulting implications for the Canadian healthcare system are significant, as there will be an increase in the number of drugs seeking public reimbursement for which there are companion diagnostics. This presentation will highlight CADTH's work to date in this area, particularly with respect to the health technology assessment of companion diagnostics.

Sohail Mulla

Dr. Sohail Mulla is a Scientific Advisor at CADTH, where he provides technical leadership in the design, development, and delivery of health technology assessments. He is also a part-time Assistant Professor in the Department of Health Research Methods, Evidence & Impact at McMaster University, where he contributes to a variety of teaching and administrative activities.

Best Practices and Lessons Learned in Integrating Biomarkers/Companion Diagnostics in Clinical Trials; A Global Central Laboratory's Perspective

Patrice Hugo, Q2 Solutions

[Abstract not available]

Patrice Hugo

Dr. Patrice Hugo is Chief Scientific Officer at Q2 Solutions, a Quintiles Quest joint venture. He currently leads global scientific strategy and is responsible for the medical affairs and scientific activities for the Central Laboratories, and Specialty Testing Centers of Excellence worldwide including the Expression Analysis Genomics, Vaccine, Biomarkers and BioAnalytical/ADME laboratories. Dr. Hugo has more than 25 years of senior scientific leadership experience with extensive management expertise in laboratory operations, biomarker discovery and validation applied to diagnostics, therapeutic targets and clinical trials. He obtained his Ph.D. in Experimental Medicine/Immunology at McGill University and completed post-doctoral fellowships in Immunology at the Walter Elisa Hall Institute in Australia, and at the Howard Hughes Medical Institute in Denver, Colorado. He then became a Principal Investigator leading the T-lymphocyte Unit at the Montreal Clinical Research Institute. He worked at PROCREA BioSciences and Caprion as Chief Scientific Officer and Executive Vice President R&D, respectively. Dr. Hugo later joined Clearstone Central Lab acquired by LabCorp as Chief Scientific Officer and became Associate Vice President and Chief Scientist at LabCorp/Covance Central Laboratories Division. Dr. Hugo has more than 75 scientific publications in internationally renowned journals. He also played an active role in a number of industry organizations, including being a member of the Board of Directors for the non-for-profit Personalized Medicine Partnership for Cancer in Quebec, and the Steering Committee for the Biomarker Factory.

Additional Panel Members for Discussion

Monette Greenway, BSc., C. Dir., Principal & Co-Founder, Precision Rx-Dx Inc.

Monette is Principal & Co-Founder of Precision Rx-Dx Inc. a strategic consultancy focused on Precision Medicine. In collaboration with her partner, Ann Humphreys and their team of senior professionals, they support pharmaceutical and biotechnology companies to build market and organization readiness for the launch of targeted therapeutics and their companion diagnostics.

Educated in Genetic Engineering and Biotechnology, Monette's career has centred on the development and successful market adoption of Life Science and diagnostic innovation. Monette has lived and worked internationally with growth mandates in the pharma biomarker development area, and hospital and community lab sectors. With over 25 years of executive experience her past roles include global President/Vice President positions with Thermo Fisher Scientific and Executive Commercial/Government relations' roles with LifeLabs Medical Laboratories, Strategic Diagnostics and Mount Sinai Services.

Raffi Tonikian, Merck

Raffi Tonikian is Associate Director Medical Affairs for Oncology Biomarkers and Breast Cancer at Merck Canada. He provides subject matter expertise for oncology biomarkers across all indications while supporting medical affairs initiatives and clinical studies. In addition, Dr. Tonikian is responsible for external collaborations and partnerships in the areas of basic and translational oncology research.

Prior to joining Merck Canada, Dr. Tonikian was Medical Advisor in Neuroscience at Novartis Pharmaceuticals Canada, where he supported medical affairs and clinical activities in the fields of Multiple Sclerosis and Neuromuscular diseases.

As a Scientist in the Departments of Protein Engineering and Translational Medicine at Biogen in Cambridge, Massachusetts, Dr. Tonikian's work focused on the screening of antibody libraries for the discovery of antibody-based therapeutics for immune and neurobiological disorders. In addition, he studied the utilization of T-cell repertoire diversity assessed using next-generation sequencing as a potential biomarker for disease activity in autoimmunity.

Raffi received his doctorate degree from the

University of Toronto in the Department of Molecular Genetics, where he used peptide phage display libraries to identify specificity profiles for signaling domains, which led to the mapping of large-scale protein interaction networks in different model organisms. During that time, he also spent two years at Genentech, Inc. as a Visiting Scientist in the Department of Protein Engineering. Dr. Tonikian performed his postdoctoral work under the auspices of Dr. Sachdev Sidhu, where he developed and utilized phage-displayed libraries to isolate antibodies against cancer related antigens. In addition, he worked on the development of antibody-DNA conjugates as biomarker detection reagents.

Thursday, May 11

SESSION 4:

Key Regulatory Issues

Chair: Fakhreddin Jamali, University of Alberta

Process Validation Lifecycle Stages (Stage 1, 2 and 3)

Naheed Sayeed-Desta, Manager, TO-Process Validation, Apotex Inc.

With the implementation of ICH Q8, Q9, Q10, Q11 and draft Q12 guidelines, regulatory bodies mandate a data driven, science and risk based decision making process utilizing data from all three stages of the PV Lifecycle- Stage 1: Process Design, Stage 2: Process Performance Qualification (PPQ) and Stage 3: Continued Process Verification (CPV).

Stage 2 PPQ can utilize practical tools to determine number of batches required for a study. This best estimate is based on statistical confidence using observed intra-batch variability and estimated inter-batch variability of similar products/processes and product label claim. Pa (Probability of Acceptance) is an innovative statistical analysis tool applicable for multi-level acceptance criteria (example Content Uniformity, Dissolution) that is fit for pharmaceuticals.

Stage 3A evaluation of substantial data gathered from Stage 2 and predetermined number of batches can be used to estimate Inherent Process Variability (IPV) and PaCS index, Stage 3B trend limits and Process Capability Quality dashboard (PCQd) for a product. These novel statistical assessments can be used to gain further understanding of new product launches prior to routine monitoring. Stage 3B is an effective quality risk management tool for mitigating risks to product quality; detecting trends and implementing preventative measures prior to failures.

The objective is to provide novel methodologies that can be used in Stage 2 and Stage 3 assessments to gain product understanding and confidence for future batches to meet the required specification. Product and process understanding of similar products and SPC methodologies are key components for successful PV Lifecycle Management. The 2011 FDA PV Guidance proposes process validation lifecycle approach.

Naheed Sayeed-Desta

Naheed Sayeed-Desta has more than 16 years of pharmaceutical industry experience and has been leading Technical Operations, Process Validation teams at Apotex Inc., the largest Canadian pharmaceutical company. In her current capacity as Manager of Process Validation, Naheed is responsible for providing strategic directions on Process Validation Stage 1, 2 and 3 life-cycle management of over 300 molecules. As a global cross site leader, Naheed champion's delivery of science and risk based approaches for solid dose products from traditional to novel manufacturing technologies and approaches across the organization.

Naheed Sayeed-Desta is a proven leader in Pharmaceutical Manufacturing Science and Technology based in Toronto. Her primary focus has been on Product Development (R&D), Tech Transfer, Continuous Improvement, Remediation Projects, Product Review, Investigations, Process Validation activities for solid oral dosage forms for regulated markets. Naheed is well known for providing pragmatic solutions for multiple manufacturing processes. As a global cross site business partner Naheed provides leadership in critical decision making. Naheed was involved in multiple high value product remediation projects for the organization. She was the lead author of AAPS PharmSciTech journal article Assessment Methodology for Process Validation Lifecycle Stage 3A. Naheed is a contributing member of PDA Post Approval Change Innovation for Availability of Medicines Program and is involved in developing PDA Technical Report on ICH Q12 tools.

A Risk-based Approach to Development and Manufacture of a New Chemical Entity using PCMM

John Groskoph, Executive Director New Products CMC, Global Chemistry Manufacturing & Controls, Pfizer Inc.
Daniel Blackwood, Research Fellow, Technology & Innovation group of Pharmaceutical Sciences- Small Molecule, Pfizer, Groton, CT

The presentation will provide an overview of the initial formulation and process development activities completed for a new chemical entity on Pfizer's PCMM (Portable Continuous Miniature and Modular) continuous direct compression line at the Pfizer Groton, CT site.

The presentation will describe the risk assessment, experimental and PAT activities used to support the development of a Solid Oral dosage form from initial laboratory scale to commercial-scale manufacturing. The use of Science of Scale (SOS) and modeling tools, to stream-line development work, as well as production-scale Design of Experiments (DoE), will be highlighted. Key lessons learned and future opportunities will also be described.

John Groskoph
John Groskoph leads the New Products CMC function at Pfizer and has over 25 years of pharmaceutical industry experience. John has worked in a variety of regulatory, quality and production assignments and brings experience in Quality & Compliance Systems, clinical trial submissions through post-approval change management. John has a particular focus on bringing new technologies and new approaches through the CMC regulatory approval process. John holds a B.S. in Electrical Engineering from Lafayette College, Pennsylvania and an M.B.A. from Columbia University, New York, USA.

Daniel O. Blackwood
Daniel is currently a Research Fellow in the Technology & Innovation group of Pharmaceutical Sciences- Small Molecule at Pfizer, based in Groton, Connecticut. Currently, he is the technical program lead for Pfizer's Portable, Continuous, Miniature, and Modular (PCM&M) development and manufacturing initiative for Oral Solid Dosage (OSD). In this role, Daniel is leading the cross-functional team of engineers and scientists in the design, fabrication, and installation/commissioning activities for PCM&M. In addition, he is a subject matter expert in drug product continuous processing technologies including gravimetric feeders, in-line powder mixers, and tablet compression equipment. Daniel received his BS in Mechanical Engineering from Cornell University in Ithaca, NY and his MS in Industrial Engineering from Columbia University in New York City.

Therapeutic Equivalence of Second Entry Products of Long Acting Bronchodilators or Inhaled Corticosteroids: The International Council for Harmonisation Guidelines

Irvin Mayers, MD, FRCPC, Professor of Medicine, Department of Medicine, University of Alberta, Edmonton, AB

Background: Generic inhalers are often perceived as inferior to their branded counterparts; however, they are safe and effective if they can meet the regulatory requirements. The approach to assess bioequivalence (BE) in oral dosage form products is not sufficient to address the complexities of inhalational products (e.g., patient-device interface); hence, more considerations are needed and caution should be applied in determining BE of inhaled compounds.
Overview: This review outlines the evaluation process for generic inhalers, explores the regulatory approaches in BE assessment, and highlights the considerations and challenges in the current *in vitro* and *in vivo* approaches (lung deposition, pharmacokinetic, and pharmacodynamic/clinical studies, as well as patient-device interface) for establishing BE of inhaled compounds.
Aims: The goal of this review is to establish uniformity in the regulatory approaches to speed drug submission process, clear misconception of generic inhalers, and have meaningful clinical endpoints such as improvement in patient's quality of life when compared to placebo and brand name drugs. As inhalational drugs become more common for other indications such as antibiotics, the technologies developed for inhaled compounds in the treatment of chronic pulmonary diseases may be extrapolated to these other agents.

Irvin Mayers
Dr. Irvin Mayers (MD, FRCPC) is a Professor of Medicine and former Pulmonary Divisional

Director, at the University of Alberta. He completed his medical training at the University of Manitoba and then his Pulmonary clinical and research training at University of Manitoba and University of Chicago. He moved to University of Saskatchewan in 1984 and then to the University of Alberta in 1994. He was divisional director of Pulmonary Medicine from 2001 t0 2011. He is past president of the Canadian Thoracic Society (CTS). Regionally, he is the Alberta Health Services Medicine site chief for the University of Alberta Hospitals. Provincially, Dr. Mayers is the Medical Advisor to Respiratory Benefits Program. Nationally, he is a member of the Canadian Drug Expert Committee. imayers@ualberta.ca

Friday, May 12

Plenary Lecture - Jason Moffat

Chair: Frank Abbott, UBC

Genome-scale CRISPR Screens and Protein Engineering for Target Discovery and Translation

Jason Moffat, Associate Professor in the Donnelly Centre at the University of Toronto

The adaptation of CRISPR/Cas9 gene editing technology to mammalian cell lines is altering the course of human functional genomics research. Forward genetic screens with pooled libraries of CRISPR guide RNAs targeting human protein-coding genes and encoded in viral vectors, enable high-resolution detection of fitness genes in human cell lines. To extend the catalog of human core and context-dependent fitness genes, we have developed high-complexity genome-scale CRISPR/Cas9 libraries (e.g. TKOv1) and applied these to fitness screens in human cell lines. Using improved methods for measuring screening quality and quantifying fitness, we are cataloguing fitness genes in different genetic backgrounds for mapping genetic interactions and target discovery. For example, genome-wide CRISPR/Cas9 screens in RNF43-mutant pancreatic ductal adenocarcinoma (PDAC) cells, which rely on WNT signaling for proliferation, revealed a unique requirement for WNT signaling circuit that engages a single WNT receptor (ie. FZD protein) encoded in the human genome. These results highlighted an underappreciated level of context-dependent specificity at the receptor level in RNF43-mutant PDAC cells. We further derived a panel of recombinant antibodies that report the expression of nine of the ten FZD proteins encoded in the human genome and confirm that functional specificity cannot be explained by protein expression patterns alone. Moreover, highly specific synthetic human anti-FZD antibodies robustly inhibited the growth of RNF43-mutant PDAC cells grown in vitro and as xenografts in vivo, providing strong orthogonal support for the functional specificity observed at the genetic level. Moreover, proliferation of of a patient –derived PDAC cell line harbouring an RNF43 variant previously associated with PDAC, was also selectively inhibited by specific anti-FZD antibodies, further demonstrating their use as a potential targeted therapy. Taken together, genome-scale forward genetic screens with pooled CRISPR/Cas9 libraries is an efficient method for identifying potential therapeutic targets.

Jason Moffat
Dr. Jason Moffat is an Associate Professor in the Donnelly Centre at the University of Toronto. He is a Canada Research Chair in Functional Genetics and Senior Fellow at the Canadian Institute for Advanced Research. The Moffat lab is interested in cancer through the lenses of genetic interactions and antibody discovery. Dr. Moffat is an expert functional genomicist who was recruited to the Donnelly Centre from the Broad Institute (MIT and Harvard, Boston) where he carried out postdoctoral work with Dr. David Sabatini. While at the Broad, Dr. Moffat helped was part of the RNAi Consortium (TRC), helping to create the first lentiviral-based short hairpin RNA (shRNA) libraries and developing methods for applying these libraries for loss-of-function, positive selection and synthetic lethal genetic screening in human and mouse cells [Moffat et al., Cell, 2006]. Dr. Moffat created the Platform for Advanced Cell Engineering (PACE) (formerly the COLT platform) has contributed to over 100 research projects and over 40 publications in the decade since its inception. The overarching goal of the platform continues to be the development of novel tools and technologies to enable the application of functional genomics screening to various research questions. The platform was integral in the recent creation of the Toronto Knockout (TKO) CRISPR gRNA library, consisting of 90,000 guide RNA sequences targeting all human coding genes [Hart et al., 2015]. The Moffat lab's primary research focus is on utilizing functional genomics to identify novel diagnostic and therapeutic targets for the treatment of cancers and develop biologics to exploit newly identified targets. Dr. Moffat has co-founded two biotechnology companies (Northern Biologics and Pionyr Immunotherapeutics [formerly Precision Immune]) to enable commercialization of these novel biologics.

Friday, May 12

SESSION 5:

What is Happening in Antibacterials?

Chair: Bastien Castagner, McGill University

To Kill a Bacterium, You Need to Think Like a Bacterium

Eric Brown, Professor and Canada Research Chair in Microbial Chemical Biology, Department of Biochemistry and Biomedical Sciences and the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Canada

Antibiotic drug resistance has reached crisis proportions, principally because modern industrial drug discovery efforts have failed to provide new antibiotics. The reasons for failure are manifold, however, a lack of understanding of the basic biology has played a large part. Where modern drug discovery emphasizes reductionist efforts on validated targets and lead chemicals, there is a profound risk of failure if the complexity of the target, indeed the system, is underestimated. Thus, my research group is working to explore largely uncharted aspects of complex biology in bacteria that underlie bacterial survival. The ultimate goal of these studies is to contribute fresh directions for new antibacterial therapies.

My research program includes on-going investigations of enigmatic antibacterial targets such wall teichoic acid synthesis in the troublesome pathogen methicillin resistant *Staphylococcus aureus*, better known as MRSA. We are likewise trying to understand the potential of biotin synthesis as a target in menacing drug-resistant Gram-negative bacteria. Further, we are developing chemical-genomic platforms to enable the discovery and characterization of new chemical probes of bacterial cell systems. These efforts typically involve screening diverse collections of synthetic chemical compounds. We pride ourselves on creative screening approaches that yield compounds with utility as tool compounds and potential as leads for new antibiotics. With success in pilot scale screens of tens of thousands of compounds, we are expanding these efforts on some of our platforms to industrial scale and screening natural product extracts also. The goal is to discover many more new chemical probes of bacteria with a better chance of identifying those with the potential for drug development.

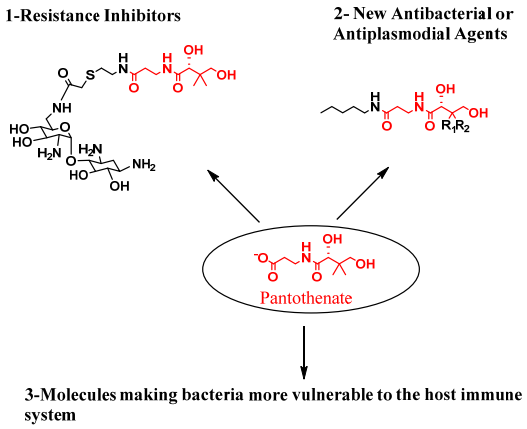
Eric Brown

Dr. Brown's research interest is the complex biology that underlies bacterial survival strategies. He and his research team aim to understand and subvert these systems in drug resistant superbugs. To this end the Brown lab research group is using tools of chemical biology and molecular genetics to probe poorly understood aspects of bacterial physiology. The overriding goal of these studies is to contribute fresh directions for new antibiotics. Dr. Brown has been the recipient of a number of awards and is a very active participant in the university and research community. He is a Fellow of the American Academy of Microbiology and has received the Canadian Society of Microbiologists Murray Award for career achievement, the Canadian Society for Molecular Biosciences Merck Frosst Prize for new investigators and a Canada Research Chair in Microbial Chemical Biology. Dr. Brown is a former department Chair and has served on advisory boards for a variety of companies as well as national and international associations, including: a term as President of the Canadian Society of Molecular Biosciences; member of the Medical Review Panel of the Gairdner Foundation; and member of the Institute of Infection and Immunity Advisory Board of the Canadian Institutes of Health Research. Currently, he is serving as one of 15 Chairs nationwide on the latter organization's College of Reviewers and on the Editorial Advisory Board of the journal *ACS Infectious Diseases*.

Using the CoA Biosynthetic Pathway in the Activation of Antimicrobial Agents

Karine Auclair, PhD, Professor, Chemistry Department, McGill University, Montreal, QC

This presentation will discuss three different strategies to tackle the problem of antimicrobial drug resistance, each taking advantage of the CoA biosynthetic pathway to activate prodrugs inside the pathogen. According to the WHO, antibiotic resistance has become such a pressing issue that humanity may regress into a post-antibiotic era where "things as common as strep throat or a child's scratched knee could once again kill". One of our targets is an antibiotic resistance-causing enzyme: aminoglycoside 6'-*N*-acetyltransferase (AAC6'). Expression of AAC6' by bacteria renders them resistant to almost all aminoglycoside antibiotics. We have designed pantothenate derivatives which in bacteria, are transformed into nanomolar inhibitors of AAC6' by the CoA biosynthetic pathway, and resensitize resistant bacteria to aminoglycoside antibiotics. Our second strategy involves the design of new pantothenate derivatives/mimics as potential new antibacterial or antiplasmodial agents. These molecules may act as CoA antimetabolites after bioactivation by CoA biosynthetic enzymes. Finally, our third target is the itaconate degradation pathway. Here again, our inhibitors are built from a pantothenate derivatives by three enzymes of the CoA biosynthetic pathway inside bacteria. Interestingly, although inhibition of itaconate degradation is innocuous under nutrient-rich conditions, it is lethal in poor media such as that within activated macrophages. This strategy may find use to resensitize some intracellular pathogens to the host immune system and treat infections with minimal selection for resistance. Overall this presentation will include medicinal chemistry with a strong emphasis on biological mechanisms.



Karine Auclair

As a graduate student (1994-1999) in the lab of Prof. J. C. Vederas at the University of Alberta (Edmonton, Canada), K. Auclair studied the biosynthesis of the cholesterol-lowering drug lovastatin (Mevacor). Amongst her thesis achievements, she reported the first purified natural Diels-Alderase. Next, she moved to the University of California at San Francisco (USA) to pursue post-doctoral studies with Prof. P. R. Ortiz de Montellano (1999-2001). Her research results contributed to the mechanistic understanding of heme oxygenases and P450 enzymes. In 2002, she started her independent career as an Assistant Professor of Chemistry at McGill University (Montreal, Canada). She was promoted Associate Professor of Chemistry with tenure in 2006 and Full Professor in 2016. She was a Visiting Scientist at Boehringer Ingelheim (Laval, Canada) in 2010. Her current research at McGill covers the areas of antibiotic resistance and P450 enzymes. Some of her key contributions include aminoglycoside resistance inhibitors active in cells, new antibacterial agents, new insights into enzyme allostery, as well as mechanistic and biocatalytic studies of P450 enzymes. Her accomplishments have been recognized by several awards including among others the Fessenden Professorship at McGIll, the Leo Yaffe Award for excellence in teaching, and the Enantioselective Synthetic Chemistry Research Award from the CSC. She has co-authored >60 peer-reviewed papers, 6 patents and 1 copyright, given >80 invited lectures, and her work has been presented during conferences at >150 occasions.

Clostridium difficile Infection: How to Trick a Toxin

Bastien Castagner, Canada Research Chair in Therapeutic Chemistry, Department of Pharmacology & Therapeutics, McGill University, Microbiome and Disease Tolerance Centre, Montreal, QC

Dr. Castagner's research focuses on the design of small-molecules and natural product analogues as novel drug candidates. He is especially interested in the chemistry and biology of inositol phosphates, which are ubiquitous intracellular molecules in eukaryotes. They play important roles in essential processes such as signalling. In addition, some bacterial toxins such as the clinically relevant Clostridium difficile toxin B (TcdB) utilize inositol hexakisphosphate as a trigger for an intracellular auto-processing step that is important in the pathogenesis. We have prepared analogues of inositol phosphate that are capable of inducing premature TcdB auto-processing that result in inactive toxin. These molecules constitute an exciting therapeutic avenue against this alarming hospital infection.

Bastien Castagner
Bastien Castagner obtained a Ph.D. in Chemistry at Columbia University in New York in 2004. His postdoctoral years were spent at ETH Zürich from 2005 - 2008, working in the area of carbohydrate chemistry with Professor Peter Seeberger. From 2009 - 2014 he was a Group Leader in the Institute of Pharmaceutical Sciences at ETH Zürich, where he was involved in drug discovery and drug delivery. He joined the Department of Pharmacology & Therapeutics at McGill as Assistant Professor in August 2014.

Selected Abstract for Oral Presentation:

Protease-mediated Suppression of DRG Neuron Excitability by Commensal Bacteria

Jessica L. Sessenwein, Gastrointestinal Disease Research Unit, Queens University
(See Abstract # 17)

Friday, May 12

SESSION 6:

Gene Therapy

Co-Chairs: Jeanne Leblond Chain, Université de Montréal, and Azita Haddadi, University of Saskatchewan

Overcoming Barriers to Nucleic Acid Delivery

Tom Anchordoquy, University of Denver, Denver, CO

It is well-established that only a small fraction (approx. 1%) of an IV-injected dose of nanoparticles actually deposits in the tumor. Therefore, the effects of 99% of the injected dose on non-target tissues becomes directly relevant to clinical development. More specifically, the systemic compatibility of nanoparticles in terms of biodegradability, liver toxicity, immune recognition, and effects on target cell viability must be considered. In addition, the low efficiency of delivery to tumors will likely require repeat administration to achieve therapeutic levels. These considerations have motivated us to design a gene delivery system that exhibits negligible toxicities and elicits minimal cytokine response upon repeated intravenous injection. To this end, we have made modifications to both the lipid and nucleic acid components of our delivery system and quantified their effects on compatibility both in vitro and in vivo. By developing a gene delivery system that elicits minimal (if any) cytokine response, we are able to enhance gene delivery to tumors upon successive injections. We feel that this general strategy needs to be considered when developing nanoparticles for drug delivery.

Tom Anchordoquy
Dr. Anchordoquy is a professor of pharmaceutical sciences at the University of Colorado Anschutz Medical Campus. Dr. Anchordoquy conducted his doctoral work on liposome stability and formulation at the University of California, Davis in the laboratory of Dr. John Crowe. He conducted postdoctoral work at the University of Colorado before joining the faculty of the School of Pharmacy in 1998. Dr. Anchordoquy's early work concentrated on the physical and chemical stability of particulate delivery systems during freezing and drying. Since joining the faculty, his drug delivery work has largely focused on developing a targeted gene delivery system (nanoparticle) that is compatible with clinical use and is capable of achieving high levels of gene expression in vivo. He has also investigated the use of exosomes for delivering drugs and distributing therapeutic cargo within specific tissues. In addition, Dr. Anchordoquy has been involved in many projects concerning the formulation of small molecule therapeutics for a wide variety of applications, e.g. diabetes, cancer, heart disease, schizophrenia. He has founded two small companies, and is the author of a dozen patent applications. He has served as an expert witness on patent disputes, as a permanent member of an NIH review panel, is on several editorial boards, and is the author of over 100 peer-reviewed publications.

Development of Gene Pill™ for Gut-Localized and Systemic Delivery of Protein Drugs

Anthony T. Cheung, PhD, enGene, Inc. Montreal, QC, Canada

The current mode of delivery for protein drugs is far from ideal, as it requires frequent needle injections or infusion, which can have a significant impact on cost and patient compliance. Oral delivery is an attractive route for protein delivery due to its simplicity of administration, especially when chronic daily treatment is required. The benefits of orally delivered protein are ample and clear but to date, there are no orally available protein drugs despite extensive research in this area over the past decades. It is extremely difficult to deliver protein drug into the body through the oral route, as the harsh environment in the gut readily destroys the protein. The size and poor permeability of most protein drugs also present formidable challenges to their passage from the gut into the circulation. In this presentation, enGene's strategy to achieve the promise of orally delivered protein therapeutics will be presented.

Instead of delivering the protein itself, enGene has developed a prototype Gene Pill™ that has the capability to deliver the gene encoding the protein drug of interest to the gut, leading to its expression and secretion into the bloodstream. The Company has demonstrated that this approach will only have a transitory action due to the natural turnover of gut mucosal cells. This unique short term action provides for a safe, easily managed treatment since the protein dose can be easily adjusted and treatment can be halted as desired. enGene's Gene Pill™ technology holds significant potential as a platform to enable oral delivery of therapeutic proteins.

Anthony Cheung

Dr. Anthony Cheung is the CEO and President of enGene, Inc. (Montreal, Canada). Prior to that, he served as the Chief Scientific Officer for the Company from 2004-2012, where he developed enGene's current platform technology for delivery of nucleotides to the gut. Dr. Cheung received his bachelor degree in Biochemistry from the University of British Columbia (Vancouver, BC) and doctorate degree in Physiology from the Tulane University School of Medicine (New Orleans, LA). Since his appointment as the CEO in 2012, Dr. Cheung has raised significant equity financing for enGene and completed two major pharma partnership transactions. Dr. Cheung has co-authored numerous book chapters, review articles and peer-reviewed journals on the topics of diabetes, gene therapy and autoimmune diseases. He has been invited to speak at many international scientific and biotechnology conferences - BIO, American Society for Gene & Cell Therapy, Diabetes Technology Meeting, Children with Diabetes - on topics related to gene therapy, drug development and bio-entrepreneurism. He also serves as Board Member and Advisor for several biotechnology companies and professional organizations including Bio-Industry Liaison Committee of the American Society for Gene & Cell Therapy.

Smart Lipid Nanoparticles for Nucleic Acids Delivery

Jeanne Leblond Chain, Ph.D., Gene Delivery Laboratory, Faculty of Pharmacy, Université de Montréal, QC

RNA interference provides a targeted approach for silencing gene expression that may prove beneficial in the treatment of diseases such as cancer and genetic disorders. To ensure effective knockdown, siRNA must be entrapped and efficiently conveyed into the cytoplasm of cells. These hydrophilic nucleic acids have to cross the lipid-rich plasmatic and/or endosomal membrane, without being degraded into lysosomes. We have developed new pH-sensitive lipids able to change conformation upon protonation at endosomal pH values, leading to the disruption of the lipid bilayer and thus to the fast release of the nucleic acids into the cytosol. The objective of this work was to design a fast-responding system at pH 5 while remaining stable at blood pH value and during storage. This was achieved by the design and synthesis of a series of switchable lipids, and their incorporation into lipid nanoparticle (LNP) composition. LNP complexed with siRNA exhibited high silencing efficiency, reaching up to 10% on HeLa cells, very similar to a commercial agent, with lower toxicity. Negative controls demonstrated that the improved efficiency was due to the conformational switch of the lipids. In vitro transfection potential was confirmed on various cells lines (HeLa, A549, Huh-7) and siRNA targets (GFP, PCSK9, survivin). In vivo applications are currently focused on liver disease, such as hypercholesterolemia, breast cancer and retinoblastoma.

Jeanne Leblond Chain

Jeanne Leblond Chain is assistant professor at the Faculty of Pharmacy since 2011. Equipped with an engineer degree in organic chemistry, she got her Ph.D. at Faculty of Pharmacy at University Paris V in France where she developed new synthetic vectors for gene therapy. She joined Pr Leroux's team in University of Montréal for postdoctoral studies in 2006. She is the director of the Gene Delivery Laboratory, which develops stimuli-responsive lipid nanoparticles for intracellular delivery of genes and drugs. In addition, she is the director of the research axis "Drug Formulation and Analysis" and has co-directed the platform of biopharmacy for 5 years. She has trained over 20

students in 5 years. She has published 18 research articles, 1 book chapter, most of them in journal with IF higher than 4.5.

Selected Abstract for Oral Presentation

Towards the Fabrication of a Bioinspired Synovial Fluid for Viscosupplementation Treatment in Osteoarthritis

Jimmy Faivre, Université de Montréal
(See Abstract # 76)
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Friday, May 12

GSK/CSPS Early Career Award Lecture

Chair: Frank Abbott, University of British Columbia

Respiratory Evaluation Sciences Program (RESP): Innovations in Analytic Approaches to Improve Efficiency in Respiratory Care

Mohsen Sadatsafavi, Assistant Professor in Outcomes Research, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC

Respiratory diseases consume a significant and escalating share of our limited health care resources. Chronic obstructive pulmonary disease (COPD) is responsible for the highest number of hospital admissions in Canada. Asthma affects around 10% of the population and is a major cause of missing school or work.

Decisions on patient care are being made at multiple levels within our health care system, from the 'bedside' (clinical care) to the 'boardroom' (policy making). Such decisions must consider the reality of resource scarcity against the needs of the population. Economic evaluation is a set of concepts and methods that enables contrasting the health and cost consequences of competing health technologies. Outcomes research studies the end-result of health services and takes into account the realities of care provision. Together, these disciplines provide the methodological framework for improving patient outcomes and the efficiency of health care delivery.

The Respiratory Evaluation Sciences Program (RESP) is an innovative and comprehensive program of respiratory health economics and outcomes research and knowledge translation, with the aim of improving patient outcomes and efficiency of health care delivery by enabling evidence-informed decision making at all levels of care. To achieve this goal, RESP employs two synergistic 'Burden of Disease' and 'Evaluation' research themes. Together, these themes complete a logical pathway by answering the key questions: 'How big is the problem?', 'What are the options to tackle the problem?', and, 'What option provides the best value for the resources it consumes?'

This seminar is a mixed-audience presentation of

1) an overview of the burden of asthma and COPD in Canada and globally,
2) the overall structure of RESP and its components,
3) its success in creating a productive and impactful asthma outcomes program, and
4) its current and planned activities in creating an innovative outcomes platform in COPD.

Mohsen Sadatsafavi

Mohsen Sadatsafavi is an academic health economist and outcomes researcher. He has a MD degree, a MHSc degree in epidemiology, and a PhD degree in Outcomes Research. In July 2012, Dr. Sadatsafavi was appointed as Assistant Professor in the Division of Respiratory Medicine, Faulty of Medicine, University of British Columbia (UBC). In July 2016, Dr. Sadatsafavi accepted the position of Assistant Professor (Tenure-Track) in Outcomes Research in Faculty of Pharmaceutical Sciences, UBC. At this position, Dr. Sadatsafavi has created, and currently is the Principal Investigator of, Respiratory Evaluation Sciences Program, a comprehensive respiratory outcomes research program (http://resp.core.ubc.ca). Dr. Sadatsafavi has published more than 80 peer-reviewed scientific papers and has obtained more than $1M in research funds from the Canadian Institutes of Health Research (CIHR), the Canadian Respiratory Research Network, the Canadian Lung Association, and the industry. For his work he has received salary support from CIHR and Michael Smith Foundation for health Research. He has taught undergraduate and graduate courses and has been supervising several trainees. He is the leader of the health economics platform of the first national network of respiratory research in Canada and is a member of the international Respiratory Effectiveness Group. Besides these, he is an avid reader, biker, and swimmer, and loves astronomy and history.

Friday, May 12

SESSION 7:

Biosimilars

Chair: Agnes Klein, Health Canada

Framework Guidelines for Biosimilars: Recent Updates, Future Considerations

Agnes Klein, Senior Medical Advisor, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON

In Canada, biosimilars are considered to be new drugs and their market authorisation is governed by two main concepts:

- Division 8 of the Drug Regulations
- Concepts and considerations that draw from the approaches taken for generic products

There is one essential difference: Health Canada (HC) will issue a Notice of Compliance for a biosimilar, but will not declare either bioequivalence or interchangeability with the reference product.

From the very beginning the Guidelines were structured as a Framework Guidance, allowing for flexibility and case by case consideration for each biosimilars, based on the studies submitted and the outcome of the review. At the same time, HC indicated that this guidance will be a living document that will be updated as both HC and the sponsors of biosimilars product will learn the best way to develop these products.

This presentation will highlight the main updates to the first version of the Guidance for Biosimilars and will discuss, briefly, some outstanding issues, including that of nomenclature of the active ingredient and the concerns that biosimilars might not be as safe and effective as the reference product to which they are compared.

Agnes Klein

Agnes V. Klein, MD, DPH, is currently the Senior Medical Advisor in the Biologics and Genetic Therapies Directorate.

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) and currently for a new E18 guideline on genomics as related to clinical trials. 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 also interested in the regulatory and clinical issues regarding subsequent entry biologics (SEBs). This interest has included authorship and co-authorship of several articles on biosimilars and a chapter in a book on biosimilars.

Recently, Dr. Klein has been involved in studies in Paediatrics and Neonatology.

Dr. Klein has made numerous presentations to professional and regulatory groups on issues surrounding drug development and regulations, 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, including biosimilars. Dr. Klein has also been involved actively with the various meetings organized through the Drug Information Association.

Dr. Klein is an active supporter for excellence in the development of medicines. As such, Dr. Klein has been closely involved in the drafting of guidelines for the development and review of Orphan Drugs as well as biosimilars.

Clinical Considerations for Authorization of Biosimilars

Jian Wang, MD, PhD, Chief, Clinical Evaluation Division – Hematology/Oncology, Centre for the Evaluation of Radiopharmaceuticals and Biotherapeutics, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON

Biosimilars are biotherapeutic products that enter the market subsequent to a previously authorized biotherapeutic, the reference biological product, to which they have been proven to be highly similar. A rigorous "totality of evidence" approach is taken for granting therapeutic indications for biosimilars based on the structural, functional, nonclinical, clinical pharmacology and clinical trial comparisons between the biosimilar and the reference.

A sensitive clinical test model should be used that is able to detect potential differences between the biosimilar and the reference.

When choosing the clinical study model, it should be clinically relevant to mechanism of action and/or involved receptor(s) of the product, such as, ligand blockade, receptor blockade, receptor down-regulation, cell depletion (via ADCC, CDC, apoptosis), and signalling induction.

Safety and immunogenicity of the biosimilar should be sufficiently characterized and there are no unique/additional safety issues expected for the indications that are not particularly studied during the comparative exercise.

Jian Wang

Dr. Jian Wang has been the Chief of Clinical Evaluation Division for the past 11 years and manages a team of clinical and medical evaluators. His division has regulatory responsibility for assessing non-clinical, PK/PD and clinical data for biological drugs for the treatment of haematological, oncological, infectious, cardiovascular and renal diseases. Radiopharmaceuticals, gene therapies and biosimilars are also regulated by his division. He actively participates in various Health Canada, ICH, WHO, PAHO, APEC and DIA working groups and expert committees.

Dr. Wang received his MD from Harbin Medical University, China and was awarded PhD in Physiology from the University of British Columbia, Canada. He joined Health Canada in 1996 with many years of scientific and clinical research experience in both academic and clinical settings.

Physicians' Evaluation of Biosimilars on Multiple Indications

Brian G. Feagan, MD, Professor of Medicine, Epidemiology and Biostatistics, Western University, London, Ontario

The advent of biologic therapy has greatly improved management of immune -mediated diseases such as Crohn's disease (CD) and ulcerative colitis (UC). The TNF antagonists were the first generation agents, however several other important classes of drugs are now available including anti-integrins and a monoclonal directed to IL-12/23 (Ustekinumab). In addition to providing benefit to patients, these new molecules have stressed payors' capacity to provide access to them.

In response to this economic pressure lower cost biosimilar products have been developed that are now approved by various international regulatory authorities. Specifically, biosimilar infliximab is approved by Health Canada for the treatment of CD and UC and substitution for innovator drug is now occurring in many insurance plans.

Patient management with biosimilars faces the challenge of interchangeability, due to multiple non-medical switching by a pharmacist. The primary concerns for patients are efficacy and safety. This situation is a provocative maneuver for formation of anti-drug antibodies. The primary reason for switching to a biosimilar is cost savings to payers and society, which can create tension between the individual's rights, expectations as a patient and the broader societal need.

There are two types of switching: (1) medically relevant switching due to lack of efficacy or because of adverse events. The challenge is establishing how to manage these patients; switching within a drug class or outside a drug class is often considered. Controlled data for current drugs is available to help decision making (1-4) but is often not available for biosimilars; (2) non-medical switching, which has nothing to do with efficacy or safety, is based upon cost savings. The lack of clinical data renders it difficult to assess the clinical and health economic consequences of this practice. (5)

With regards to anti-TNF therapy and switching to biosimilars, the EMA, FDA and Health Canada have all indicated that there is insufficient evidence to draw any conclusions

regarding the safety of non-medical switching but they also state that this issue it is not within their jurisdiction. Ultimately, policy decisions will be made on a regional level, which is not ideal. Immunogenicity is potentially the most serious consequence of multiple switching. All foreign proteins have the potential to be immunogenic. (6) The immune response, is a complex, unpredictable process (7,8) that is governed by multiple factors. (9,10) Tertiary and quaternary protein structures govern whether T cells sensitize or tolerize. This consideration has raised concerns regarding the immunogenicity of biosimilars. At the last count, there were nine biosimilar infliximab molecules under development. No high quality clinical data is available to evaluate the consequences of interchangeability of these products.

Brian Feagan, M.D., FRCPC

Brian G. Feagan completed a medical degree at the University of Western Ontario in London, Ontario, Canada. His postdoctoral training included residency in the Department of Medicine and a fellowship in Gastroenterology at the University of Western Ontario.

A Fellow of the Royal College of Physicians and Surgeons of Canada, Dr. Feagan holds membership in the Canadian and American Association of Gastroenterology, the American College of Gastroenterology, the College of Physicians and Surgeons of Ontario, Crohn's and Colitis Canada (CCC) and the European Crohn's & Colitis Organization (ECCO). He is the recipient of the 2013 Senior Achievement Award from the Crohn's & Colitis Foundation of America (CCFA) and Dean's Award of Excellence in 2013 from the University of Western Ontario. He has authored over 200 articles, book chapters and has also given over 900 invited presentations, at national and international scientific meetings.

In 1997, Dr. Feagan became Director of Robarts Clinical Trials at the Robarts Research Institute, University of Western Ontario. His research efforts focus on the design and implementation of randomized controlled trials of therapy for inflammatory bowel disease. He has been the principal investigator on numerous large-scale randomized clinical trials.

Disruption and Maturity: Commercial Implications for the Next Evolution of Biologics in Canada

Mark Omoto, General Manager, Corporate Affairs, QuintilesIMS

Biologic and specialty products have dramatically shaped the Canadian pharmaceutical market. On the Clinical front biologic products have changed the treatment paradigm in multiple therapeutic areas ranging from Oncology to Immunologic diseases' such as Rheumatoid Arthritis, Ulcerative Colitis and Psoriasis.

While biologic therapies have significantly improved the real world chronic disease outcomes and the quality of patient lives, there has also been a real world financial impact due to the higher costs associated with biologic therapies.

Over the last 5 years, sales for biologic products in Canada have gone from just over $4 Billion in 2011 to approximately $6.5 Billion in 2016. In fact biologic product sales now representative almost 25% of the $26 Billion Total Canadian Pharmaceutical market.

As the market and product pipelines continue to focus on specialty diseases and new biologic therapies, we are starting to see a new challenge emerging as biosimilars are now being introduced into what were previously brand name only therapeutic areas.

Canadian and U.S. commercial experience with biosimilars has been very limited, while Europe has had multiple biosimilar products available for over 10 years. Recent Canadian approvals for biosimilars in the Immunology, Hematology and Diabetes areas have the European commercial model to benchmark the potential impacts on product uptake and reimbursement.

Multiple stakeholders view the availability of biosimilars as a potential permanent whitewater event that will not only cause disruption to multiple therapeutic areas, but it also may be a sign of early product maturity for brand name products.

The commercial landscape for biologic products has reached a stage where we are starting to see name brand biologics being launched with similar modes of actions now having to compete with approved biosimilars. All this has created a hyper competitive pricing and market access environment, which we expect will become even more disruptive as multiple biosimilars of the same molecule become available in Canada.

If we look at how biosimilars have impacted the European commercial landscape by becoming a key component for reducing treatment costs, we can already see a similar landscape developing within the Canadian marketplace.

Mark Omoto

As General Manager, Corporate Affairs for QuintilesIMS in Canada, Mark heads thought leadership initiatives and provides consulting support services to C-Suite and Senior Executive level customers in the pharmaceuticals, biotech, and government sectors in support of QuintilesIMS's Commercial Effectiveness Services, Clinical, Technology Solutions and Data Offerings.

Prior to joining QuintilesIMS, Mark was the General Manager for Cegedim Canada, a strategic marketing and technology solutions provider to biopharmaceutical companies, healthcare organizations and agencies, which was acquired by IMS Health in 2015.

A strategic business leader with more than 25 years of experience in the pharmaceutical, information technology and consulting sectors, Mark has held increasingly senior roles as Vice President of Sales & Marketing, and President at several leading pharmaceutical, biotech, and start-up companies.

Mark brings product launch, market access and global marketing expertise across multiple therapeutic areas to his work at QuintilesIMS. He has extensive experience in the commercialization of products in therapeutic areas including; biologics, CNS, dermatology, hematology, infectious disease, immunology, multiple sclerosis, rheumatology, women's health, and vaccines.

You can contact him at mark.omoto@quintilesims.com.

Friday, May 12

SESSION 8:

Orphan Diseases

Chair: Durhane Wong-Rieger, Canadian Organization on Rare Diseases

Orphan Disease Overview: Status of Rare Disease and Orphan Drugs in Canada

Durhane Wong-Rieger, PhD, President & CEO, Canadian Organization for Rare Disorders

Rare disease and orphan drug development go hand-in-hand. The identification of (the cause of) a rare disease prompts discovery of a drug therapy and, similarly, the introduction of an orphan drug stimulates patient identification and community development. In the decade prior to the 1983 US Orphan Drug Act, there were only 10 approved new therapies for rare diseases. Today, there have been more than 500 approved new therapies. But, as the pace of orphan drug development continues unabated, we are potentially reaching a crossroads where the unintended consequences of the orphan drug legislation are raising challenges to the viability of orphan drug incentives. The label "orphan drug" has become synonymous in some environments with high price, high uncertainty, big impact on health services, and increasingly niched patient populations.

What does this mean in Canada where orphan drug regulations have been in discussion only for the past five years and not yet implemented? Is the lack of an "orphan drug" designation a hindrance or an asset? This presentation will discuss the status of rare diseases in Canada within the context of the international rare disease and orphan drug environment. The good news is Health Canada is approving drugs "designated as orphans elsewhere" in a timely and appropriate fashion, but the bad news is that developers are slow to bring clinical trials and new drugs to Canada. The good news is that we have launched Canada's Rare Disease Strategy and it is gaining traction with researchers, clinicians, health services, and even one province, but the bad news is that we have a long way to attracting necessary resources for implementation. The good news is that the governments are working collectively, for example, on panCanadian standards for newborn screening and an "Expensive Drugs for Rare Diseases Program"'; the bad news is that these initiatives are all moving very slowly.

Durhane Wong-Rieger
Durhane Wong-Rieger, PhD, is President & CEO of the Canadian Organization for Rare Disorders (the umbrella organization of patients and patient groups) and chair of the Consumer Advocare Network (a national network for patient engagement in healthcare policy and advocacy). She is also President & CEO of the Institute for Optimizing Health Outcomes (providing training and direct service on health coaching and patient self-management) and Chair of FH Canada Patient Network. Internationally, Durhane is Chair of Rare Disease International (the global alliance of rare disease patient organizations), Past-Chair of the International Alliance of Patient Organizations, member of the Editorial Board of *The Patient-Patient Centred Outcomes Research* and member of Health Technology Assessment International Patient /Citizen Involvement Interest Group. She is a certified Health Coach and licensed T-Trainer with the Stanford-based *Living A Healthy Life with Chronic Conditions*.

Exploring the Canadian Ecosystem of Rare Disease

Cate McCready, Vice-President External Affairs, BIOTECanada

Rare disease has become a prominent element of modern medicine as research and discovery adapts to the unprecedented knowledge tsumani created by the sequencing of the human genome a short 14 years ago. Resulting from this milestone, a sea of new questions and opportunities arise for public policy makers around the world as they seek to adress regulatory modernization and capacity

building, adapting to new theraputic solutions for previously untreatable and rare diseases, how to make room for these therapies within the system of care to allow patients and practicioners access to new protocols and medicines.

Canada remains an outlier within industrialized nations for not having established a regulatory framework for research and discovery for rare disease therapies. Despite this, Canada has developed a national ecosystem led by patients, physicians and biotechnology entrepreneurs creating a vibrant landscape of awareness, physician excellence, research capability and globally recognized scientific discovery dedicated to finding solutions ofr rare diseases.

As the next generation of biologic based therapeutic discovery emerges, will Canada be able to capture the potential of what the rare disease community has established? What are the issues keeping Canada apart from becoming global leaders in rare disease research? What is the biotechnology industry doing to assist policy makers in addressing barriers to care?

BIOTECanada has led an industry policy table for more than 12 years, working with Health Canada regulators to bring rare disease regulatory issues to the attention ofdepartmental policy leaders. In 2015 a policy white paper “Bridging Opportunity to Reality” to support the creation of a regulatory pathway for rare disease medicines in Canada. Work is currently continuing with Health Canada, along with HTA bodies on how rare disease medicines can be made accessible for Canadian patients. Some of this work will be explored in the presentation.

Cate McCready

With more than 20 years of practical experience in the field of communications and public affairs Cate McCready joined BIOTECanada in November of 2001 and currently serves in the role of Vice President External Affairs.

More than 600 biotechnology companies in Canada are developing technologies and platforms to increase Canadian global competitiveness by improving our natural resource management regimes, modernizing manufacturing processes, providing environmental solutions for climate change, and ensuring more effective positive heath outcomes with vaccines and therapeutics. Her role with BIOTECanada encompasses the development of national programs designed to inform and highlight the value of biotechnology innovation in Canada.

Particular focus of her responsibilities is to engage government policy makers and lead communication efforts to support association goals. Under her direction the association has created an annual National Biotechnology Week program, established a national industry strategy, and implemented the first national federal and provincial government roundtables on the bio-economy.

Her experience includes more than eight years of service within the federal government. This included legislative and communications roles for federal ministers and as a senior advisor to the Prime Minister of Canada. Prior to her federal government experience she served in corporate and non-profit roles for national organizations.

Challenges and Opportunities for Biotech Companies Developing Treatments for Rare Diseases in Canada

Michael Harvey, PhD, Executive Director, Drug Development, Clementia Pharmaceuticals Inc.

Clementia is a clinical stage biopharmaceutical company headquartered in Montreal with a U.S. subsidiary in Boston. The company is developing its lead candidate palovarotene, a novel retinoic acid receptor gamma agonist, to treat fibrodysplasia ossificans progressiva (FOP} and other diseases. FOP is an extremely rare dramatic disease in which muscle and connective tissue are replaced by bone. There are currently no approved treatments for FOP.

Dr. Clarissa Desjardins started Clementia Pharmaceuticals in 2011 after having in-licensed palovarotene from Roche. At that time palovarotene's efficacy in mouse model of FOP had just been demonstrated in an article published in Nature Medicine journal.

Since then Clementia has raised $92.5 million in financing to support the development of palovarotene for the treatment of FOP. Any investment in medical innovation encounters risk as a company navigates the challenging processes of manufacturing, developing, and commercializing a drug - especially for specialty niche product like orphan drugs. So it is important to minimize risk along the drug development process and leverage presenting opportunities.

Palovarotene received Fast Track designation from the U.S. Food and Drug Administration (FDA) and orphan designations for the treatment of FOP

from both the FDA and the European Medicines Agency (EMA). These regulatory frameworks are very valuable incentives for orphan drug developers.

Clementia has progressed rapidly with palovarotene development program and the company is currently planning for the implementation of phase 3 pivotal trials. The adaptive design of palovarotene phase 2 clinical trial gave the opportunity to adapt the protocol based on learnings. This flexibility in clinical development is key for orphan drugs for which you have to deal with lots of unknowns such as disease course and clinically meaningful endpoints.

The success of hitting various milestones in such a short period is believed to be due in large part to Clementia's ability to work hand-in-hand with the FOP community. Rare disease patient communities play a crucial role throughout orphan drug development pathway. FOP patients and families not only inspire Clementia staff on a daily basis but also contribute actively to the advancement of palovarotene program.

As for most rare diseases, local health systems show inefficiencies in handling FOP patients. These patients suffer from delayed diagnosis, misdiagnosis and poor management due to lack of FOP awareness and interest in the medical community. The development of Centers of Excellence as well as local FOP experts are viewed as potential key success factors to address these challenges.

Michael Harvey

Michael Harvey, Ph.D. is currently the Executive Director, Drug Development of Clementia Pharmaceuticals Inc., a clinical stage biopharmaceutical company developing its lead candidate palovarotene to treat the ultra-rare disease, Fibrodysplasia Ossificans Progressiva (FOP). Previously, Michael was Director of Regulatory Affairs at Thallion Pharmaceuticals, advancing its orphan drug candidates in various oncology and infectious disease indications. He has held positions in several drug development companies with rare disease programs, leveraging various regulatory frameworks available for orphan drug developers.

Rare Diseases Regulatory Pathway in Canada, A Health Canada Perspective

Fiona Frappier, PhD, Senior Policy Analyst, Office of Policy and International Collaboration, Biologics and Genetic Therapies Directorate, Health Canada

In Canada, nearly 3 million people suffer from one of approximately 7000 rare diseases for which effective treatment is lacking internationally. As new therapeutics become available, Health Canada evaluates their safety, quality and efficacy through its regulatory framework.

Health Canada continues to work with key stakeholders to encourage the development of orphan drugs and their market authorization. Meeting the needs of Canadians requires supporting the improvement of the health care system overall. This includes improving access to necessary prescription drugs and making them more affordable for Canadians.

This presentation will provide insight into the considerations and factors influencing the market authorization decisions taken with respect to Orphan Drugs in Canada. While Health Canada is authorizing a number of orphan drugs through its existing regulatory framework, it is recognized that more work can be done to increase communications about the regulatory pathways available in Canada. An Orphan Drugs Roadmap for Sponsors is in development to describe Health Canada’s regulatory process.

Fiona Frappier

Fiona Frappier, PhD, is currently a Senior Policy Analyst in the Biologics and Genetic Therapies Directorate, Health Products Food Branch. The branch is responsible for the development and enforcement the Canadian Food and Drug Act and Regulations. Fiona supports work to provide Guidance to Sponsors regarding submission requirements for clinical trial applications and new drug submissions for orphan drugs. Previous work experience includes policy development and coordination, public health system design and transformation, fostering research and stakeholder engagement. Prior to joining BGTD Fiona has worked with Health Canada and the Public Health Agency of Canada on other multi-jurisdictional policy issues such as climate change, antimicrobial resistance and genomics. Fiona completed a PhD in HIV Immunology from the University of Ottawa in 2010.

Poster Session 1

CSPS and CC-CRS

Thursday, May 11

Poster Session 1

Thursday, May 11

Clinical Sciences & Pharmacy Practice

1. Long Term REMICADE® (Infliximab) Treatment is Predictive of Retention in Stable Rheumatic Disease Patients in Canada

Philip A. Baer, Private Practice, Scarborough, ON, Majed Khraishi, Memorial University of Newfoundland, St. John's, NL, A. Marilise Marrache and Emmanuel Ewara, Janssen Inc., Toronto, ON, Canada

Purpose: To evaluate long-term retention patterns of stable rheumatic disease (RD) patients treated with REMICADE® (infliximab [IFX]).
Methods: Using QuintilesIMS database of private and public insurance claims data, our analysis included RD patients with: (1) first IFX claim between Jan 2008-May 2015; (2) no IFX claims 12 months prior to the initial claim; (3) ≥1 claim for any other drug 12 months after the initial IFX claim; and (4) ≥1 claim for any non-IFX drug 4 months after May 2015. Retention was measured at 12-month intervals and unadjusted odds ratios calculated at the 95% confidence interval within and between cohorts of RD patients. Within-group analysis compared 12 month retention by number of years on IFX. Analyses considered cohorts of patients according to age, gender, insurance type, and previous biologic experience.
Results: A total of 1,672 had ≥2 years of claims history and had been on IFX for ≥1year. Within-group comparisons showed that a patient's probability of being retained on IFX in subsequent 12-month periods increased concurrently with elapsed time on IFX. Patients on IFX for 2, 3, 4 and 5 years showed significantly higher retention in the subsequent year compared to patients on IFX for only 1 year ($P < 0.05$). Similar trends were observed for females, in the 19-64 age range, within naïve and experienced cohorts, and by those patients with private insurance coverage. Retention at 12-month intervals up to and including 5 years was significantly better at each interval for biologic-naïve vs experienced patients and public insurance vs private insurance patients in the between-group analyses.
Conclusion: Real world patients treated with IFX have excellent long-term treatment retention. Longer time on IFX appears to predict better future retention, becoming statistically significant after 2 years. The results were robust and consistent amongst various subgroups of stable Canadian rheumatology patients.
Note: Abstract was presented at the Canadian Rheumatology Association annual meeting as a poster in Ottawa, ON. February 8-11, 2017. Abstract will be published in The Journal of Rheumatology in June or July 2017.

2. Increasing Time on Treatment is Predictive of Improved Long-Term Retention for Stable REMICADE® (Infliximab) Inflammatory Bowel Disease Patients in Canada

John Marshall, McMaster University and Farncombe Family Digestive Health Research Institute, Hamilton, ON; A. Marilise Marrache and Emmanuel Ewara, Janssen Inc. Toronto, ON, Canada

Purpose: To determine the long-term retention patterns of stable Canadian IBD patients treated with REMICADE® (infliximab [IFX]).
Methods: Using QuintilesIMS Canadian private and public insurance claims data, our analysis included IBD patients with: (1) first IFX claim between Jan 2008-May 2015; (2) no IFX claims 12 months prior to the initial claim; (3) ≥1 claim for any other drug 12 months after the initial IFX claim; and (4) ≥1 claim for any non-IFX drug 4 months after May 2015. Retention was measured at 12-month intervals and unadjusted odds ratios were determined. Within-group analyses compared 12 month retention by number of years on IFX and compared subgroups of

patients according to age group, gender, prior biologic experience, region (private claims only) and insurance type.
Results: 4,360 patients had ≥2 years of claims history and had been on IFX for ≥1 year. Within-group comparisons showed that the probability of being retained on IFX in subsequent 12 month periods increased with cumulative time on IFX. Patients on IFX for 2-5 years showed significantly higher retention in the subsequent 12 months compared to patients on IFX for only 1 year ($P<0.05$). Similar trends were observed across when stratified by gender and insurance type, as well as in patients in Ontario, aged 19-64 years, and those who were biologic-naïve. Annual retention up to and including 5 years was significantly better for patients who were publicly insured, and lived in Ontario and Eastern
Canada when compared to patients who were privately insured and lived in British Columbia, respectively.
Conclusion: Real world patients treated with IFX have excellent long-term treatment retention. Previous duration of IFX treatment appears to predict better future retention, becoming statistically significant after 2 years. The results were robust and consistent amongst various subgroups of stable Canadian IBD patients.
Abstract was presented at the Canadian Digestive Diseases Week (CDDW) annual meeting as a poster in Banff, AB. March 3-6, 2017.

3. The Effect of COX-2-selective Etodolac on the Myocardial, Vascular, and Gastrointestinal Risks: Systematic Review and Meta-analysis

Zuhair Alqahtani and Fakhreddin Jamali.
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Purpose: Etodolac, an uncommonly used nonsteroidal anti-inflammatory drug (NSAID) possess high COX-2/COX-1 inhibitory properties, hence, is believed to have a safe gastrointestinal (GI) profile. The cardiovascular (CV) toxicity of etodolac is not well-established. We, therefore, carried out a systematic review and meta-analysis to assess the GI (stratified into upper and lower tract), and CV (stratified into myocardial and vascular) risks.
Methods: MEDLINE and All EBM databases were searched for observational studies or randomized controlled trials that reporting myocardial/vascular or all-cause mortality and/or GI (upper/lower bleeding, obstructions, or perforations) after etodolac use published until July 2016. We searched for English-language articles using the following keywords: NSAIDs, etodolac, randomized controlled trial, and clinical trial. Titles and abstracts of included studies were retrieved and screened independently by two reviewers to identify potentially relevant studies. Moreover, the reference lists of the retrieved articles were scanned for relevant studies. A standardized, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. The combined odds ratio values (OR; 95% CI) were calculated using the random-effect meta-analysis model.
Results: In total, 24 eligible studies on GI and CV risks of etodolac were found. The users of the drug demonstrated approximately 70% fewer severe GI risks (ulcers and/or bleeding) compared to other NSAIDs, OR 0.32 (0.18-0.56). Moreover, there were no significant CV risk as suggested by retrieved data of included case-control studies compared to other NSAIDs, OR 1.05 (0.87 -1.26).
Conclusion: A As compared to other NSAIDs, etodolac is well tolerated in terms of gastrointestinal risk and has a relatively safe cardiovascular profile. This low cost generic drug may provide a safer alternative to other NSAIDs.
Acknowledgement: Supported by King Saud University and a Self-Funded grants from University of Alberta.

4. Prescription Cannabinoid Utilization in a Canadian Province: A Population-based Study (2004-2014)

Wajd AlKabbani[1], Silvia Alessi-Severini[1], Shawn Bugden[1], Ruth Ann Marrie[2], Paul Daeninck[3], Jitender Sareen[4], James Bolton[4], Christine Leong[1].
[1]College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba; [2]Health Sciences Centre, Winnipeg, Manitoba; [3]CancerCare Manitoba, St. Boniface Hospital, Winnipeg, Manitoba; [4]PsychHealth Centre, Winnipeg, Manitoba, Canada

Purpose: Prescription cannabinoids have appeared in practice guidelines as third-line alternatives for a variety of approved and off-label indications, such as pain and spasticity. The extent of utilization from a

population perspective is unknown. This study examined prescription cannabinoid use in Manitoba over a 10-year study period.
Methods: We conducted a retrospective, cross-sectional, population-based study using the administrative healthcare databases of the Manitoba Centre for Health Policy to describe the annual prevalence and incidence of cannabinoid (nabilone, dronabinol, nabiximols) use, demographics of users, and specialty of prescribers.
Results: There was a seven-fold increase in the number of individuals who used prescription cannabinoids between 2004-2014 from 252 to 1776, 95% of whom used nabilone. The annual incidence increased from 1.2 users per 10000 to 6.2 users per 10000 ($p<0.001$) over the study period. The mean (SD) age of new users was 50.8 (14.7) years, 58% were female, and 64% resided in urban Manitoba. General practitioners were responsible for initiating 46.8% of prescriptions followed by anesthesiologists/pain specialists (25.8%).
Conclusion: The prevalent and incident use of prescription cannabinoids have increased over time. These findings provide insight into the utilization of these agents over a period where decisions regarding access to medical cannabis have undergone change. Further study is warranted to determine effectiveness and adherence to therapy.

5. Varenicline Utilization in the Canadian Province of Manitoba

Donica Janzen*, Shelley Derksen ^, and Silvia Alessi-Severini*^
*College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; ^Manitoba Centre for Health Policy, University of Manitoba, Winnipeg, Manitoba, Canada

Purpose: Varenicline (Champix) has been available on the Canadian market since 2007 with the specific indication for smoking cessation. The government-sponsored drug program of Manitoba listed varenicline in its formulary to help reduce the high smoking rates in the province, responsible for an estimated cost of over CAN$ 200 M in total health care spending per year. This study reports on the utilization of varenicline in the entire population of the province and the characteristics of the cohort of patients filling prescriptions for this agent.
Method: Administrative health databases from the Population Health Research Data Repository at the Manitoba Centre for Health Policy (MCHP) were accessed to determine incident use of varenicline in the population of Manitoba (2007-2013). The cohort of incident users was stratified by sex, age group, residence and income quintile. Co-morbid diagnoses were identified using ICD-9 and ICD-10 codes. Analyses were conducted with SAS® statistical software.
Results: 43,422 persons were started on varenicline between 2008 and 2013: incidence rates increased from 4.72 to 5.29 per 1,000 in males but decreased in females from 5.13 to 4.88; 67% were between 35 and 65 years of age and 66% lived in urban areas. More than 55% of users filled prescriptions for more than 12 weeks of continuous use. Diagnoses of cardiovascular/cerebrovascular and chronic respiratory disease affected approximately 30% and 21% of the cohort, respectively; 44% had depression.
Conclusion: Assessing the characteristics of smokers needing prescription medications can inform decision makers on how to improve success in smoking cessation.
Acknowledgments: Study funded through an unrestricted grant from Pfizer Canada. Results and conclusions are those of the authors; no official endorsement by Manitoba Health, Seniors and Healthy Living or MCHP is intended or should be inferred.

6. Regulatory Decision Making in Canada - Exploring Frontiers in Patient Involvement

Agnes V. Klein, MD, DPH[1], Stephanie Hardy, MPH[1], Robyn Lim, PhD[2], and Deborah A. Marshall[3].
[1]Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada; [2]Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada; [3]Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, O'Brien Institute for Public Health, Calgary, Alberta, Canada

Purpose: Patients can provide unique insights into practical aspects of living with their disease and its treatments. The poster will describe how Health Canada engages currently patients and is exploring the most effective ways to collect and consider patient input in the regulatory decision-making

process.
Methods: The Health Products and Food Branch (HPFB) ran a Patient Involvement Pilot Project in 2014 to explore how patient input could be gathered and used within the context of orphan drugs and add to the value of regulatory decision-making.
HPFB is assessing as well, patient involvement models used by other organizations, such as the US Food and Drug Administration, the European Medicines Agency and the Canadian Agency for Drugs and Technologies in Health to draw on lessons learned by others.
Results: Results from the Pilot Project suggest that the readiness of patients to provide input needs to be considered and the timing of when regulators receive input is important, due to strict performance targets of the review. Further exploration in the Canadian context, including (1) who is best situated to provide input; (2) at what stages in the regulatory process is it most feasible or valuable to collect, (3) is there information to enhance the regulator's understanding of patient drug experience that could be gleaned from clinical trial data, (4) what are the most appropriate formats for patient input, and (5) how should patient input be captured in decision-making process?
Conclusion: Patient involvement in regulatory decision-making is a rapidly evolving science. Additional experience in gathering and using patient input would be valuable because the Pilot Project only involved two drugs. Exploration of a system-based approach may be warranted to limit burden on patients, by collaborating with other key health system drug decision-makers, to establish a harmonised patient involvement process. Opportunities for international collaboration on this topic may also prove useful.

7. Physician Centric Health Care- Is it Time for a Paradigm Shift?

Kishor M. Wasan,[1,4], Lois Berry,[3,4] and Jawahar Kalra.[2]
[1]College of Pharmacy and Nutrition, [2]College of Medicine, [3]College of Nursing and [4]Health Sciences Council, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Purpose: The purpose of this project is not to diminish the importance of physicians in delivering quality and affordable health care, but to provide an alternative, where a team of health professionals such as pharmacists, dietitians, nurses and nurse practitioners among others are utilized to their full potential and capabilities by working in a complimentary fashion with physicians, to deliver high quality health care at affordable and potentially lower costs.
Methods: In 2010 the Ontario Provincial government under the leadership of Premier Dalton McGinity commissioned economists from the Toronto Dominion Bank to analyse the Ontario Health Care System.
Results: This group of economists from a purely economic perspective concluded that the Ontario government could save hundreds of millions of dollars in health care costs without diminishing and some cases enhancing the quality of health care delivered by utilizing all health professionals to their full potential. This report addresses the utilization of all health professionals to their full scope of practice in effective collaborative team-based models could decrease unnecessary hospitalizations and prevent inappropriate Emergency room visits by practicing preventative proactive health care interventions before they escalated out of control, thus decreasing the burden on health care institutions and freeing up physicians to deal with the more serious and acute health care problems.
Conclusions: This strategy would actually benefit physicians, freeing them up to utilize the full potential of their training and expertise to treat the serious/acute life threatening conditions while other members of the health care team dealt with non-life threatening conditions and manage chronic illness, preventing such situations from escalating to more serious conditions. Higher quality health care at an affordable price a right all should have.

Pharmacokinetics & Pharmacodynamics

8. Novel PK Model Using Tape Stripping Data: Application in Bioequivalence Assessment of Two Acyclovir Topical Cream Formulations

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Purpose: The aim of this study was to investigate the feasibility/applicability of a PK model for determination/prediction of relative bioequivalence between topical acyclovir cream formulations using tape stripping.
Methods: Data were obtained following two crossover studies using tape stripping. The first study was performed with single applications of the RLD acyclovir and concurrent application of a bioequivalent formulation. The second study (n=10) compared a test non-bioequivalent formulation with the same RLD. A PK model was developed based on the observed amount of acyclovir which penetrated the skin following the application of the RLD formulation. ADAPT 5® with population approach of the maximum likelihood expectation maximization (MLEM) algorithm was used for PK modeling. The same model was used to estimate PK parameters of the test formulations. Ratios of means and 90% confidence intervals (CIs) were calculated for the extent and the rate of penetration of the formulation through the skin. Bioequivalence was demonstrated if ratios and 90% CIs were within 80-125%.
Results: For all formulations, good correlations (indicated by an r2 $\geq$ 0.88) were shown between fitted and observed amounts of acyclovir. For the comparison between generic and RLD, the ratios and 90% CIs of F and Ka were 110% (105 to 115%) and 88% (82 to 94%) respectively. For the comparison between the test non-bioequivalent and RLD, the ratios and 90% CIs for F and Ka were 20% (15 to 25%) and 140% (100 to 180%), respectively.
Conclusion: The PK model developed for acyclovir demonstrated the ability to correctly conclude bioequivalence, or lack thereof, between the RLD and test formulations of acyclovir. The PK model should enable one to characterize the rate and extent of exposure in the skin of various topical preparations using the tape stripping technique.
Acknowledgement: This abstract has previously been presented in the American Association of Pharmaceutical Scientists (AAPS) 2016 Annual meeting on November 17.

9. Effect of Doxorubicin on Catabolism of Adenosine and Adenosine 5-'triphosphate in Systemic Blood *in vivo*

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Purpose: Previous studies have shown that metabolism of adenosine 5'-triphosphate (ATP) in systemic blood is a potential surrogate biomarker for cardiovascular toxicity. We investigate the acute effect of high dose of doxorubicin (DOX) on adenosine and ATP catabolism in systemic blood *in vivo*.
Method: Sprague Dawley (SD) rats were each given either 10 mg/kg of DOX (n = 8) or normal saline (1 mL/kg, n = 11) twice daily for 4 doses by subcutaneous (sc) injection. Blood samples were collected sequentially for up to 6 hours for measuring circulating concentrations of ATP, adenosine and their metabolites. Hemodynmic recording was obtained continuously after the last injection. Difference in response between groups was considered significant at $p < 0.05$ (t-test).
Results: Diastolic blood pressure (DBP) was significantly lower in the DOX treated rats than in the control before the final injection (87 ± 12 vs 104 ± 11 mmHg, $p < 0.05$). Blood pressure fell gradually after the last injection and the decrease was significantly greater in the DOX treated group ($p < 0.05$). Plasma concentration of adenosine was significantly lower in the DOX treated group. In contrast, plasma concentrations of uric acid and hypoxanthine as well as red blood cell (RBC) concentrations of AMP were significantly higher ($p < 0.05$).
Conclusion: Acute cardiotoxicity induced by DOX may be measured by increased breakdown of ATP to AMP in the RBC and also breakdown of adenosine to hypoxanthine and uric acid in plasma (supported

in part by Dalhousie Faculty of Health Professions Research Development Grant and Pharmacy Endowment Foundation).

10. Enterolactone Glucuronide Modulates Hepatocellular Cholesterol Trafficking

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Purpose: Cardiovascular disease (CVD) is considered one of the leading causes of death worldwide. Hypercholesterolemia is an important risk factor of CVD and lifestyle changes and drugs (Statins mainly) are used to manage cholesterol. There is an increasing interest in safer alternatives to statins and growing evidence suggest flaxseed lignans may serve as an alternative treatment for mild to moderate hypercholesterolemia or as combination therapy with statins in severe conditions. We aim to understand the underlying mechanism by which flaxseed lignans influence cholesterol metabolism in the liver.
Methods: The HepaRG cell line was used to study the influence of the mammalian lignan, enterolactone (ENL) and its metabolite, enterolactone glucuronide (ENL-Gluc), on cholesterol uptake and intracellular trafficking using fluorescing cholesterol (NBD-cholesterol). We screened for genetic modulation of several genes involved in cholesterol synthesis and trafficking via the INSIG regulation pathway in HepaRG after treatment with ENL & ENL-Gluc by qPCR and western blot.
Results: Both ENL and ENL-Gluc reduces cytosolic fluorescent cholesterol in HepaRG, which was confirmed by observing a surge in NBD-cholesterol accumulation in the endoplasmic reticulum (ER) at lower concentrations, and an increased trapping in lysosome at higher concentrations.
Conclusion: ENL and ENL-Gluc causes cholesterol retention in the ER and lysosome in HepaRG cells. Further confirmation of these changes will be available with screening for the relative genetic expression of multiple target genes that are responsible for activation of a membrane bound transcription factor, SREBP, as well as protein level measurements

11. The Impact of Diet Induced Obesity on the Microsomal Glucuronidation of Bisphenol A (BPA)

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Purpose: To examine for functional changes in glucuronidation caused by diet-induced weight gain using the suspected obesogen bisphenol A (BPA) as a test substrate for phase II metabolism.
Methods: Liver and intestine microsomes were harvested from male Sprague–Dawley rats given for 14 weeks either normal rodent chow with water (controls); normal chow and high fructose corn syrup water (HFCS); 45% high fat diet (HFD) chow with water; a combination of HFCS and HFD. BPA was incubated with the microsomal protein in incubation media (microsomal protein, UDPGA, $MgCl_2$ in phosphate buffer). After 10 min, the reaction was ended by the addition of 1M HCL and HPLC was used to assay BPA remaining. The Micheal-Menton equation was fitted to the concentration vs. consumption data. Comparisons were assessed using one way ANOVA followed by Duncan's multiple range post hoc tests.
Results:

Parameter	**BPA consumption rate**			
	Liver			
	Control	**HFD**	**HFCS**	**HFD-HFCS**
V_{max} *(nmol/mg/min)*	12.4±2.54[a]	2.37±1.49	1.69±1.70	1.08±2.65
K_m *(μM)*	367±154[a]	31.3±11.7	22.6±2.01	15.2±2.65
CL_{int} *(μL/mg/min)*	37.2±12.5[a]	70.9±19.1	74.8±1.95	70.4±5.25
	Intestine			
V_{max} *(nmol/mg/min)*	0.425±0.165	1.05±0.525	2.91±2.47	1.15±0.882
K_m *(μM)*	34.3±5.44	27.2±13.3	85.4±98.7	55.5±39.3
CL_{int} *(μL/mg/min)*	12.9±6.42[b]	39.6±11.7	47.1±21.7	20.1±3.59[c]
[a] Different from each of the high calorie groups; [b]different from HFD and HFCS; [c] different from HFCS ($p<0.05$).				

All high calorie diet groups gained weight compared to controls and had lower hepatic V_{max} and K_m but higher CL_{int}. In intestine, there was a similar increase in CL_{int} in HFD and HFCS fed groups but no significant changes in V_{max} or K_m between groups.
Conclusion: Diet-induced obesity was associated with an increase in the hepatic glucuronidation CLint of BPA. This suggested a change in the amounts or population of hepatic UGT isoforms expressed in the presence of obesity. Further assessment of which UGT isoforms might be affected by obesity is warranted.

12. Pharmacokinetic and Toxicodynamic Characterization of a Novel Doxorubicin Derivative

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Purpose: Doxorubicin (Dox-HCl) is an effective anticancer medication with poor oral bioavailability and systemic toxicities. A novel derivative was developed by conjugating doxorubicin to quercetin (DoxQ) with a potential to improve tolerability and oral bioavailability of Dox-HCl. Quercetin is a flavonoid with CYP3A4 and P-gylcoprotein (P-gp) inhibitory effects, anti-oxidant effects and significant accumulation in the lymphatics. The purpose of this study was to characterize the pharmacokinetics and safety of doxorubicin after intravenous (IV) and oral (PO) administration of DoxQ or Dox-HCl and investigate lymphatic delivery of doxorubicin after PO DoxQ administration.
Method: DoxQ and Dox-HCl were administered IV and PO (10mg/kg; n=4) to male Sprague-Dawley rats. Drug concentrations in plasma and urine were followed up to 48 hours after dosing and were quantitated by HPLC. Cumulative amounts of doxorubicin in the lymph were collected hour after PO administration in a mesenteric lymph duct cannulated rat. Cardiac troponin (cTP-I) and urine β-N-Acetylglucosaminidase (NAG) were used as markers of cardiac and renal toxicity, respectively.
Results: DoxQ intact IV showed 5 fold higher exposure with an area under the curve (AUC) of 18.6±1.98 μg*h/mL compared to 3.97±0.71 after Dox-HCl and a significant reduction in Vss (L/kg) 0.138 ± 0.015 vs 6.35 ± 1.06. Fraction excreted unchanged in urine (f_e) of DoxQ IV was ~ 5% and ~11% of Dox-HCl. In lymph, cumulative amounts of doxorubicin after oral DoxQ were twice as high as Dox-HCl. Oral DoxQ increased AUC of doxorubicin by ~1.5 fold compared to after oral Dox-HCl. Lower plasma cTP-I and urine NAG concentrations were observed after DoxQ IV than Dox-HCl.
Conclusion: DoxQ alters the pharmacokinetic disposition of doxorubicin both orally and intravenously and is in part transported through intestinal lymphatics. DoxQ increased the oral bioavailability of doxorubicin and may increase therapeutic safety compared to Dox-HCl in a rodent model.

Pharmaceutical & Analytical Chemistry

13. In-silico Physicochemical and Biopharmaceutical Characterization of MyoNovin, a Novel Skeletal Muscle Re-generating Agent

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Purpose: MyoNovin is a novel skeletal muscle-regenerating compound that was chemically developed by attaching two nitro groups to

guaifenesin with a potential to be used for treating skeletal muscle atrophies. *In-vivo*, MyoNovin triggered DNA synthesis of skeletal muscle satellite cells after oral and transdermal administration in a mouse model. However, little is known about the physicochemical properties and pharmacokinetic profile of MyoNovin. The purpose of this study is to characterize its physicochemical and biopharmaceutical properties utilizing computer software packages.
Method: Gastro-Plus, Marvin Sketch, and Virtual Computational Chemistry Laboratory Computer modelling software were utilized to predict the physicochemical properties of MyoNovin compared to its precursor guaifenesin. ADMET Predictor was used for modeling the biopharmaceutical and physicochemical parameters.
Results: MyoNovin showed lower predicted water solubility and higher LogP value compared to guaifenesin, indicating higher lipophilicity. Fed state solubility of MyoNovin in gastrointestinal fluid is predicted to be superior to fasted state solubility. MyoNovin showed 20 fold higher skin permeability (19.89 cm/s*10^7) vs guaifenesin (0.66 cm/s*10^7) and ~ 10 fold higher effective jejunal permeability (2.24 cm/s*10^4) compared to guaifenesin (0.26 cm/s*10^4).
Conclusion: Chemical structure modification of guaifenesin into MyoNovin altered its physicochemical and biopharmaceutical properties in-silico and will likely affect its pharmacokinetic profile *in-vivo*. The relatively high lipophilicity and skin permeability of MyoNovin renders it a good candidate for transdermal application, while the intestinal permeation property and fed state solubility in gastrointestinal fluid suggests better oral absorption will be achieved when administered with food. Further *in vitro* and *in vivo* studies are warranted and are in progress in our laboratory.

14. Novel & Selective FOXM1 Transcriptional Program Suppressors

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Purpose: Characterizing the FOXM1/DNA binding site and interacting residues greatly aids in understanding and designing new drug moieties capable of suppressing the transcriptional activity of the oncogenic FOXM1. Accumulating evidence suggests that targeted FOXM1 inhibition may be a promising strategy to treat human malignancies, indicating that FOXM1 inhibitors may become clinically useful drugs in the near future.
Method: We performed a series of molecular modeling and molecular dynamics simulations to determine the structural requirements needed by a drug molecule to interfere with the FOXM1/DNA binding domain. Based on our findings, we designed, synthesized and evaluated two series of compounds capable of selectively inhibiting the FOXM1 binding to the DNA.
Results: Compound 11A was able to inhibit the cell growth with GI50 of ~11.90 μM in MDA-MB-231 cell line and GI50 of ~25 μM in MCF-7 cell lines. The compound was also capable of suppressing the FOXM1/DNA interaction in EMSA (IC50: ~ 16.87 μM).Our molecular modeling studies revealed the details of interaction between the inhibitors and the FOXM1/DNA binding domain.
Conclusion: In this study, we synthesized a series of novel and potent FOXM1 inhibitors; among them, Compound 11A showed promising results. We suggest that this compound can be used as our lead for the synthesis of more potent and selective FOXM1 suppressors.

15. Acetaminophen-Related Hepatotoxicity Monitoring in Human Serum by Liquid Chromatography-Mass Spectrometry

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Purpose: We have developed a fast and sensitive assay to accurately quantify modified human serum albumin as a biomarker for acetaminophen-related hepatotoxicity. Specific assays are lacking and, currently, clinicians must wait for generic symptoms to manifest prior to determining proper treatment. Acetaminophen is metabolized into a reactive metabolite, *N*-acetyl *p*-benzoquinone imine, which is related to its hepatotoxicity and covalently binds to proteins. These modified proteins are present in very low abundance and therefore highly sensitive assays are needed.
Method: The assay uses peptic digestion, isotope dilution, solid phase extraction and selective

antibody-based enrichment of targeted peptides, coupled to liquid chromatography-multiple reaction monitoring (LC-MRM) analysis in positive electrospray ionization mode. Surrogate protein-level standards were used to facilitate external calibration, and samples were purified with phosphonate tags and TiO_2 beads, allowing subsequent peptide-based immunoprecipitation without interference by unmodified peptides. Antibody magnetic bead-based sample preparation employed robotic handling for good assay reproducibility and high throughput. Finally, absolute quantitation was performed on a triple-quadrupole mass spectrometry platform.

Results: Sample preparation and analysis was optimized to maximize throughput and sensitivity. The purification via immunoprecipitation with magnetic beads on an automated magnetic-particle processing system is currently being finalized to provide highly purified extracts, for fast LC-MRM analyses. The calibration curve is based on a surrogate modified protein standard, which after digestion yields a positional isomer to the target analyte peptide. A deuterated isotope labeled internal standard was used and corresponding transitions were incorporated into the MRM method. The final method was applied to evaluate calibration standards and quality control samples. Finally, results from clinical samples from patients exhibiting acetaminophen hepatotoxicity will be discussed.

Conclusion: This study introduces a highly sensitive biomarker assay for absolute quantitation of acetaminophen-induced modification of human serum albumin.

16. Locking in ^{89}Zirconium – Improving the Stability of Positron Emission Tomography (PET) Imaging Agents

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Purpose: Antibody-based positron emission tomography (immunoPET) is a rapidly advancing diagnostic imaging method. Zirconium-89 (^{89}Zr) is an emerging radioisotope for immunoPET. Its physical half-life matches the biological half-life of antibodies and its positron emission provides PET images of high spatial resolution. Concerns over the stability of the clinically used ^{89}Zr-chelator, desferrioxamine B (DFO), have spurred the search for a more stable ^{89}Zr-chelator. We present a new ^{89}Zr-chelate, which we characterized and assessed for its *in vitro* and *in vivo* stability compared to DFO.

Methods: We synthesized and characterized a new octadentate chelator and studied its complexation with Zr^{IV}. The ^{89}Zr-radiolabeling was optimized and the radiocomplex stability was assessed in blood serum, in EDTA challenge assays, and in a direct transchelation challenge against DFO. Finally, the *in vivo* behaviour of the ^{89}Zr-complex was assessed in a biodistribution and PET/CT imaging study in mice.

Results: Our chelator quantitatively bound ^{89}Zr within 10 min at room temperature and neutral pH using micromolar concentrations of the chelator. Mass spectrometry confirmed formation of the monometallic complex and computational studies suggested a favourable binding geometry. The radiochemical yields were comparable to those for DFO. The radiocomplex remained intact in serum over a week and performed better than DFO when exposed to excess challenge ligands in transchelation experiments. Mice excreted the radiocomplex rapidly *via* urine without signs of demetallation or organ uptake.

Conclusion: Our new chelator is a suitable ligand for the PET-radioisotope ^{89}Zr. Micromolar concentrations provide fast quantitative radiolabeling under mild conditions. Compared to DFO, our chelator provides improved complex stability as shown in challenge experiments. Furthermore, the radiocomplex was excreted rapidly without signs of demetallation *in vivo*. We are in the process of developing an immunoconjugated version of the chelator that will assess the long-term *in vivo* stability.

Biomedical Sciences

17. Protease-mediated Suppression of DRG Neuron Excitability by Commensal Bacteria

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Purpose: Gastrointestinal diseases associated with pain are accompanied by microbial dysbiosis. Although the mucosal barrier normally separates the gastrointestinal (GI) microbiota from the nervous system, the barrier is compromised during episodes of stress or inflammation, which are both associated with visceral pain. While pathogenic bacteria are known to exacerbate pain signaling, little is known about whether the normal GI commensal bacteria modulates visceral sensation. We tested the hypothesis that commensal GI bacteria can affect the excitability of dorsal root ganglion (DRG) neurons by exposing DRG neurons to media containing the secretory products of a defined community of 33 commensal GI bacteria from a healthy patient (microbial ecosystem therapeutics; MET-1).
Methods: Perforated patch clamp experiments on DRG neurons that were dissociated from male C57Bl6 mice and incubated overnight in sterile media containing supernatant from MET-1 or sterile control media.
Results: MET-1 reduced the excitability of DRG neurons in a concentration-dependent manner by significantly increasing rheobase by 30% ($p<0.05$). This was accompanied by an increase in the amplitude of voltage-gated K^+ currents. A cocktail of bacterial protease inhibitors abrogated MET-1 effects on DRG neurons. A serine protease inhibitor but not inhibitors of cysteine proteases, acid proteases, metalloproteases, or aminopeptidases abolished the effects of MET-1 on the excitability of DRG neurons. The serine protease cathepsin G recapitulated the effects of MET-1 on rheobase of DRG neurons. Inhibition of protease-activated receptor (PAR)-4, but not PAR-2, blocked the effect of MET-1 on rheobase of DRG neurons. Furthermore, *Faecalibacterium prausnitzii* recapitulated the effects of MET-1 on DRG rheobase.
Conclusions: Serine proteases derived from the commensal bacteria in MET-1 can directly impact the excitability of DRG neurons, through PAR-4. On the basis of these observations, pain signaling may be modulated by microbiota-neuronal interactions. Therapies that induce or correct microbial dysbiosis may affect visceral pain.

18. Cardioprotective Effect of 2-methoxyestradiol is Mediated through the Modulation of Proteins Involved in Myocardial Energy Metabolism and Oxidative Stress

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Purpose: 2-methoxyestradiol (2ME) is a major cytochrome P450 1B1 metabolite and has been reported to have a vasoprotective action. Although the protective effects of 2ME seems to be independent of the classical genomic estrogen receptor, the exact mechanism is yet unknown. Therefore, the overall objectives of the present study are to elucidate the potential anti-hypertrophic effect of 2ME and to explore the mechanism(s) involved.
Method: For this purpose, Sprague–Dawley rats were subjected to either sham or abdominal aortic constriction surgery (AAC) to induce cardiac hypertrophy and treated with 2ME or vehicle. Thereafter, left ventricular hypertrophy and fibrosis were measured by histopathology whereas, the large-scale analysis of proteins, proteomics, was determined using liquid chromatography tandem-mass spectrometry (LC-MS/MS).
Results: The antihypertrophic and antifibrotic effects of 2ME were evidenced by a significant decrease in the induction of cell volume and fibrosis mediated by AAC. Based on proteomics data, the protective effect of 2ME was mediated through a significant inhibition of pyruvate dehydrogenase

kinase and pyruvate carboxylase which may increase the reliance of myocardium on glucose as a source of energy at expense of fatty acid oxidation. Intriguingly, treatment with 2-ME reduced body weight in both sham and AAC rats. These data suggest that 2ME may induce its cardioprotective actions via the reduction of body weight and the induction of myocardial glucose oxidation. Furthermore, the beneficial effect of 2ME on the ACC-induced cardiac hypertrophy may be partly due to an increase in the protein expression of antioxidant enzyme, glutathione S-transferase and ferritin heavy chain.
Conclusion: Our study provides the first evidence that 2ME exerts a cardioprotective effect through the modulation of proteins involved in myocardial energy metabolism and oxidative stress.

19. Daunorubicin Induces Cardiotoxicity through a Soluble Epoxide Hydrolase-mediated Epoxyeicosatrienoic Acid Degradation Dependent Mechanism

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Purpose: Several studies have demonstrated the role of cytochrome P450 (CYP) and its associated arachidonic acid (AA) metabolites in the anthracyclines-induced cardiac toxicity. However, the ability of daunorubicin (DNR) to induce cardiotoxicty through the modulation of CYP and its associated AA metabolites has not been investigated yet. Therefore, we hypothesized that DNR induced-cardiotoxicity is mediated through the induction of cardiotoxic hydroxyeicosatetraenoic acids and/or the inhibition of cardioprotctive epoxyeicosatrienoic acids (EETs).
Method: To test our hypothesis, Sprague–Dawley rats were treated with DNR (5 mg/kg i.p.) for 24 h whereas, human ventricular cardiomyocytes, RL-14 cells were exposed to DNR in the presence and absence of 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS), soluble epoxide hydrolase (sEH) inhibitor. Thereafter, real-time PCR, Western blot analysis, and liquid chromatography-electron spray ionization mass spectroscopy were used to determine the level of gene expression, protein expression, and AA metabolites, respectively.
Results: Our results showed that DNR induced cardiotoxicity in vivo and in vitro as evidenced by the induction of hypertrophic and fibrotic markers. Moreover, the DNR-induced cardiotoxicity was associated with a dramatic decrease in the formation of cardiac EETs both in vivo and in RL-14 cells. This inhibition was associated with the induction of sEH enzyme in vivo and in vitro. Interestingly, the increase in EETs bioavailability by TUPS significantly protected against DNR induced-cardiotoxictiy. Mechanistically, the cardiotoxic effect DNR was mediated through the induction of nuclear factor-κB (NF-κB).
Conclusion: Our study provides the first evidence that DNR induces cardiotoxicity through a sEH-mediated EETs degradation-dependent mechanism.

20. Gene-Targeted Therapeutics Using Drug-LNA Bioconjugates

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Purpose: The histone lysine methyltransferase (HKMT) disruptor of telomere silencing 1-like (DOT1L) methylates histone H3 lysine 79 (H3K79). Aberrant DOT1L activity in the homeobox protein A9 (HOXA9) promoter results in its overexpression causing mixed lineage leukemia. Inhibition of DOT1L reduces overexpression of HOXA9 treating mixed lineage leukemia. However, DOT1L works at many promoters and its inhibition will alter expression of many genes. In this study a novel strategy is developed to target DOT1L inhibitors to sites of H3K79 enrichment in the HOXA9 promoter to decrease its expression. Conjugating the DOT1L inhibitor BIX1338 to locked nucleic acid (LNA) oligonucleotides complementary to unique regions in the *HOXA9* promoter enriched with H3K79 methylation will result in gene specific inhibition of DOT1L. This will reduce the overexpression of HOXA9 that leads to mixed lineage leukemia without affecting DOT1L activity elsewhere.
Method: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was used to measure the activity of DOT1L inhibitors EPZ5676 and BIX1338 *in vitro* using recombinant nucleosomes as substrates. BIX1338 was conjugated

to LNA (BIX-LNA) complimentary to the recombinant nucleosomal DNA and compared to unconjugated LNA, BIX1338 and the positive control EPZ5676 for their inhibitory effects on DOT1L methylation.
Results: We measured the *in vitro* methylation activity of DOT1L in the presence of inhibitors EPZ5676 and BIX1338 using LC-MS/MS, finding that BIX1338 was 50-fold less potent than EPZ5676. The potency of BIX1338 (IC_{50} = 2.3±1.8μM) increased 13 fold when conjugated as BIX-LNA (IC_{50} = 183±7.8nM) complimentary to the nucleosomal DNA, nearly achieving the potency of EPZ5676 (IC_{50} = 45±4.8nM).
Conclusion: Conjugating DOT1L inhibitors to LNA complimentary to DNA sequences where H3K79 methylation is found concentrates the inhibitor at the site of methylation. The result is an increase in potency as well as a sequence-specific effect.

21. Female Offspring born to Obese and Insulin Resistant Dams are not at Increased Risk of Obesity and Metabolic Dysfunction during Early Development

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Purpose: The percentage of women who are obese at the time of conception/pregnancy is increasing. Furthermore, both animal and human studies indicate that offspring born to obese dams/mothers are at increased risk for early onset obesity metabolic syndrome, potentially due to epigenetic alterations that are inherited by the developing embryo/fetus *in utero*. Our goal was to confirm in an experimental model of metabolic syndrome in the dam, whether the offspring would be at increased risk of obesity and metabolic syndrome.
Methods: Female C57BL/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 5 weeks, and then bred to a male C57BL/6J mouse fed a LFD. Pregnant dams remained on their respective diet during gestation, and at time of birth, all offspring born to HFD supplemented female dams were cross-fostered to a surrogate dam fed a LFD, whereas offspring born to LFD supplemented dams remained with the dam during nursing. Body weights and fat mass were assessed routinely in the offspring until 14 weeks-of age, as was glucose homeostasis via glucose and insulin tolerance testing. In vivo metabolism was assessed via indirect calorimetry.
Results: Dams fed a HFD for 5 weeks demonstrated robust increases in total fat mass, which was associated with marked insulin resistance. Although female offspring born to these dams were heavier at weaning, they gained weight at a reduced rate when compared to dams born to LFD supplemented dams, and thus at 14-weeks of age actually weighed less. In addition, female offspring born to dams supplemented with a HFD demonstrated no perturbations in glucose or insulin tolerance, and exhibited no change in oxygen consumption or respiratory exchange ratios.
Conclusions: Our findings suggest that factors other than increased adiposity/insulin resistance during pregnancy are responsible for the increased risk of obesity in children born to obese mothers.

22. The Effect of Lysine Demethylase 1 Inhibitors and Mocetinostat on HOXA9 Expression and Histone Modifications

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Purpose: Mixed lineage leukemia (MLL) is caused by changes in histone post-translational modifications (PTMs) within the Homeobox A9 (HOXA9) promoter increasing its expression. Therefore potential treatments for MLL include inhibitors of enzymes that add or remove PTMs in the HOXA9 promoter. Inhibitors of lysine demethylases (KDM) have been developed for this purpose as they decrease HOXA9 expression, one example being GSK2879552. Previous research has shown that the histone deacetylase (HDAC) inhibitor mocetinostat decreases expression of several KDMs including lysine specific demethylase 1 (LSD1). As a consequence mocetinostat acts like a KDM inhibitor. The aim of this study was to investigate if HOXA9 expression is influenced by mocetinostat through its mechanism of decreasing KDM expression.
Methods: We compared the KDM inhibitor GSK2879552, and the HDAC inhibitor mocetinostat measuring their effects on histone PTMs using LC-MS/MS, and HOXA9 expression using qPCR in an

MLL cell line. MOLM-13 cells were treated with increasing concentrations of both inhibitors, histones were isolated from the cells, hydrolyzed and PTMs quantified using LC-MS/MS. From the same treatment groups, the expression of HOXA9 was measured by qPCR.

Results: GSK2879552 increased histone lysine mono- and dimethylation but had little effect on trimethylation which is characteristic of decreased LSD1 activity or expression. It also potently reduced HOXA9 expression (IC_{50} = 12 nM) while increasing lysine acetylation and symmetric arginine diemthylation. Mocetinostat decreased HOXA9 expression (IC_{50} = 100 nM). We have previously shown that mocetinostat decreases expression of 7 different KDMs increasing all three types of lysine methylation in addition to the expected increase in histone lysine acetylation.

Conclusion: These results indicate that mocetinostat decreases HOXA9 expression similarly to GSK2879552. Both drugs increase histone lysine acetylation, and mono- and dimethylation which may be marker for decreasing HOXA9 expression. Therefore, mocetinostat could be a potential treatment for MLL.

23. Genetic Deletion of Soluble Epoxide Hydrolase Preserves Mitochondrial Efficiency and Cardiac Function Post-MI in Aged Mice

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Purpose: Pathophysiological responses, including cardiovascular complications, often alter with age. Cardioprotective effects of epoxyeicosatrienoic acids (EETs) toward acute myocardial ischemia-reperfusion injury have been well documented. However, biological relevance of EET-evoked cardioprotection in the ageing myocardium remains unknown. EETs are metabolized to less active metabolites by the enzyme soluble epoxide hydrolase (sEH). This study uses permanent occlusion of the left anterior descending coronary artery (LAD) in young and aged sEH null and WT mice to compare cardiac and mitochondrial function following ischemic injury.

Methods: Age-matched 16 month old (aged) and 3 month old (young) sEH null and littermate wild-type (WT) mice were subjected to permanent LAD occlusion. Echocardiography was used to assess cardiac structure and function prior-to and 7 days post-myocardial infarction with tetrazolium chloride staining to determine infarct size. Mitochondrial ultrastructure was obtained using electron microscopy. Caspase-3, 20S proteasome, aconitase and mitochondrial ETC enzymatic activities were ascertained using established protocols. Mitochondrial respiration was assessed using a Clark electrode in permeabilized cardiac fibers to obtain respiratory control ratios.

Results: Markers of cell injury, mitochondrial efficiency and overall cardiac function were preserved in aged sEH null mice, although less robustly than in their young counterparts. While aged animals of both genotypes demonstrated a similar overall age-related decline, sEH deletion conferred protection from myocardial ischemic injury regardless of age.

Conclusion: Our data demonstrate the protection originating from sEH deletion in aged mice was reduced compared to young animals, signifying unavoidable deleterious consequences of biological ageing on cardiac function.

24. The Impact of Oral Administration of Cannabinoid Oil on Gene and Protein Expression of Tumor Necrosis Factor Alpha (TNFα) and Brain Derived Neurotrophic Factor (BDNF) in an Experimental Autoimmune Encephalomyelitis (EAE) Animal Model of Multiple Sclerosis (MS)

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Purpose: Multiple sclerosis (MS) is a chronic neurological disease that targets destruction of central nervous system (CNS) myelin.
Hypothesis: Cannabinoid treatment will reduce EAE-induced production of biological targets involved in disease progression, such as TNF-α. Untreated EAE animals will display reduction of BDNF due to decreased expression of its gene activator, 5hmC, which correlates to a worsening of neurological disability scoring (NDS).
Methods: EAE Lewis rats will be used to assess changes in TNFα, BDNF, 5hmC, and 5mC expression at the protein and transcript levels. All animals will be randomly assigned to one of three experimental groups: naïve control, EAE, and EAE treatment. EAE-induction is conducted by commercially available induction kits from Hooke Laboratories. Animals will be sacrificed at 12 and 15 days post-induction (dpi) and spinal cords tissue will be collected for analysis.
Results: Significant reductions in TNFα mRNA expression is revealed in EAE-cannabinoid treated animals at 15 dpi compared to untreated EAE animals. Significant reductions in severity of NDS is revealed at day 11, 12, and 13 dpi between EAE-cannabinoid treated and EAE-untreated groups. Results also revealed a 1-day delay in onset of peak NDS in cannabinoid-treated EAE animals. Cannabinoid treatment also significantly increased BDNF protein expression on day 12 compared to EAE untreated animals. In addition, EAE-induction was responsible for significant reductions in 5hmC at day 12 & 15 irrespective of cannabinoid treatment.
Conclusion: Cannabinoid-treatment reduces TNFα mRNA expression resulting in significant time-dependent improvements in NDS along with a reduction/delay in peak onset of disease. Cannabinoid treatment also resulted in significant increases in BDNF protein expression at day 12 that contributed to the significant improvements in NDS in the EAE-cannabinoid treated animals. Immune system-induced significant reductions in 5hmC contribute to reduction of BDNF, suggesting the importance of epigenetic induced changes to DNA methylation in preventing myelin repair.

25. Ex vivo Interactions of Skeletal and Immune Cells using Contractile Collagen Microdroplets

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Purpose: Mast cells (MC) localize to fracture callus in the early inflammatory phase of bone repair. We previously showed impaired long bone healing in MC-null KitW-sh mice, also deficient in other cells that affect bone repair including osteoblasts and osteoclasts. Recent work using Cpa3Cre/+mice lacking MC reveals defects in bone healing associated with impaired vascularization and alterations in the balance between catabolic M1 and anabolic M2 macrophages in repaired tissue. The goal of this study is to use 3D co-culture to determine how MC influence vascular endothelial cells (VEC) and mesenchymal stem cells (MSC) in the bone repair micro-environment.
Methods: MC are differentiated from precursors in the suspension cells isolated from WT and Cpa3Cre/+mice bone marrow and grown in the presence of SCF and IL3. FACS analysis and RNA collection were performed at weekly intervals. VEC will be isolated from mouse aorta and MSC from bone marrow. An aqueous two-phase system of contractile collagen microdroplets will be printed with VEC or MSC and mature MC seeded in suspension around the droplet. MC will then be activated using compound 48/80 and the collagen droplets harvested at timed intervals for analyses.
Results: About 90% of cultured cells are mature MC after three weeks of culture, confirmed by PCR analysis of RNA harvested at weekly intervals. PCR analysis also showed increased mast cells genes expression in WT MC but not in MC from Cpa3Cre/+mice. In the presence of MC we anticipate VEC will form a network of vessels and MSC will differentiate into osteoblasts, evidenced by remodeling of the collagen droplet.
Conclusion: 3D collagen microdroplets mimic the in vivo micro-environment in which MC interact with VEC and MSC during bone repair. The technology provides a valuable tool to investigate

the molecular pathways by which MC influence re-vascularization and repair of cortical bone defects.
Acknowledgements: The Bone Engineering Labs are supported in part by FRQS-RSBO and the Jo Miller Orthopaedic Fund.

26. Veterinary Antimicrobial IgY and Phosvitin in Post-weaning Diarrhea Caused by Enterotoxigenic *Escherichia coli* K88 and K99

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Purpose: Enterotoxigenic *Escherichia coli* (ETEC) is one of the most economically significant swine diseases, causing fatal diarrhea during the first weeks of life. Pathogenesis of ETEC-causing diarrhea involves intestinal colonization mediated by fimbriae. A combination of IgY specific for ETEC and metal-chelating PV may show synergistic effect in inhibiting bacterial proliferation and stipulating protection against ETEC infection. This study was conducted to determine the effects of anti-ETEC IgY and PV on *in vitro* growth inhibition of ETEC strains possessing K88 and K99 fimbriae prevalent in the porcine population.
Methods: Lyophilized K88 and K99 fimbriae of ETEC were immunized to 23-week-old Single Comb White Leghorn hens. Egg yolk IgY was extracted from hyperimmunized eggs followed by PV extraction. Purified PV was hydrolyzed by enzymes alcalase (Alc), elastase (Ela), thermolysin (Thr), trypsin (Try), or savinase (Sav) under atmospheric pressure (AP, 0.1 MPa) or high hydrostatic pressure (HHP, 100 MPa) and determined for their degree of hydrolysis (DH). Different combinations of IgY, PV, and PV-hydrolysates (PVH) with the highest DH were subjected to ETEC K88 and K99 cultures to determine optimal concentrations for bacterial growth inhibition.
Results: Anti-K88 and -K99 IgY antibodies were obtained with high titres sustained over 6 to 8 weeks of the immunization period. PVH-Alc-HHP demonstrated the highest degree of hydrolysis, 38.9%. Specific IgY, PV, and PVH from alcalase-hydrolysis under high hydrostatic pressure (PVH-Alc-HHP) in combination, were used to treat ETEC K88 and K99 cultures at optimal concentrations of 100 μg/mL, 1 mg/mL and 1 mg/mL, respectively. Combined use of IgY and PVH-Alc-HHP showed the highest bactericidal effect resulting in ETEC K88 and K99 growth inhibition of 2.8 and 2.67 log CFU/mL, respectively.
Conclusion: Synergistic egg yolk IgY-PVH combinations effectively control ETEC, therefore holds much promise for the development of natural antimicrobial compounds in veterinary pharmaceutical industry.

27. Differences in Cancer Stem Cells and Biological Characteristics in Cholangiocarcinoma

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Purpose: Cholangiocarcinoma (CCA) is a primary bile duct malignancy with poor prognosis. Surgery is the only curative therapy, but 50%–95% of cases are not surgical candidates. Recent evidence suggests that cancer stem cells (CSCs) are largely responsible for the observed chemoresistance. Therefore, understanding of CSC of CCA would help to develop a successful therapy for treatment of CCA. Herein, we investigated CSC subpopulations and compared their biological characteristics from human intrahepatic and extrahepatic CCA cells.
Methods: The expression of CSC surface markers such as CD133, CD13, CD24 and CD44 were examined on human intrahepatic (i.e. HuCCT1) and extrahepatic (i.e. KMBC) CCA cell lines by using flow cytometry. CSC marker positive-expressing cells were compared to CSC marker negative-expressing cells in capacities of migration, spheroid formation, proliferation, colony formation, and differentiation *in vitro*.
Results: All the four CSC markers were expressed 90%-100% of intrahepatic CCA HuCCT1 cells, whereas they were negligible in extrahepatic CCA KMBC cells. We found that HuCCT1 cells obtained the significant high migration capacity than KMBC cells by wound healing assay. In a 72-hour suspension culturing, HuCCT1 cells were able to start forming spheroid, while KMBC cells were mostly aggregated in shape. Furthermore, both cell populations showed similar proliferation and colony forming ability. Either of cell populations could differentiate into the hepatocyte lineage.

Conclusion: The intrahepatic CCA HuCCT1 cells line is a CSC-marker-positive population, whereas extrahepatic KMBC cells are CSC markers negative. HuCCT1 cells showed higher capacities of migration and spheroid-forming compared to KMBC cells.

28. Mammalian Lignans Alter Lipid Metabolism and Influence Endoplasmic Reticulum Stress to Sensitize Cancer Cells to Clinically Relevant Anti-cancer Agents

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Purpose: Effective cancer therapies remain elusive as cancer cells are able to develop resistance to clinically relevant anti-cancer agents (ACGs). Tumor cells (TCs) with endoplasmic reticulum (ER) stress evoke an adaptive mechanism, the unfolded protein response, for survival. Transient exposure to ER stress will lead to tumor hypoxia, TC dormancy, ER associated degradation, drug resistance and promote growth and survival. Overwhelming ER stress causes the release of cytochrome *c* and apoptosis through caspase-dependent cleavage of p53 to impede tumor growth. Recent studies in our lab suggest flaxseed lignans (FLNs) alter intracellular cholesterol trafficking, which, in turn, may enhance ER stress. We hypothesize then that combination of FLNs with ACGs will increase ER stress and in turn enhance ACGs anti-tumor activity *in-vitro*.
Methods: We have concluded some preliminary experiments with several ACGs and plan to identify/confirm key targets/changes involved in energy metabolism, cholesterol synthesis, autophagy, reactive oxygen species, mitochondrial function, glucose uptake, cell survival, intracellular vesicular trafficking and apoptosis pathways using a battery of *in-vitro* assays in cancer cell lines (CCls).
Results: In several CCls FLNs modulate key targets (e.g. FASN, SREBP, ACC, mTOR, PKM2) regulating energy metabolism at mRNA/protein level. FLN reduced mitochondrial redox function and caused mitochondrial toxicity in CCls. Cytotoxicity evaluations with kinase inhibitors (e.g. Ibrutinib, Afuresertib, VS-5584) show varying combined effects. We also confirmed synergistic effects with Docetaxel and Cabazitaxel. Further, FLN and metformin with Docetaxel/Carboplatin/Doxorubicin caused a significant reduction in cell viability where individual treatments had little to no response.
Conclusion: FLNs with ACGs modulate cellular metabolic pathways and impair cancer cell survival. The findings warrant further investigations to support FLNs ability to enhance ER stress as the key mechanism involved in the disruption of cellular survival adaptations when combined with ACGs. Such combination promises improved patient longevity and quality of life.

29. The Antioxidant Activity of Recombinant Rat Fatty Acid Binding Protein 1 T94A Variant

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Purpose: Fatty acid binding protein 1 (FABP1) is found abundantly in hepatocytes making up approximately 2% of total cellular protein (0.4 – 0.8 mM). FABP1 translocates various lipophilic substances and has a role as an intracellular antioxidant. A FABP1 genetic variation in the human population was identified where the 94th amino acid, threonine, is replaced by an alanine (T94A). Considered common with a minor allele frequency of 26-38%, the purpose of the study is to determine whether the FABP1 T94A variant has a loss of function with regards to its antioxidant activity.
Methods: The FABP1 T94A was generated using site-directed mutagenesis and the plasmid was transformed into *E. coli* cells. Recombinant FABP1 T94A was expressed and purified using a GST tag system. The identity of the recombinant variant was confirmed using LC-MS. The antioxidant property of FABP1 T94A was investigated using a DCF (dichlorofluorescein) assay. TBARS (Thiobarbituric Acid Reactive Substances) assay was used to assess the protein's ability to act as an antioxidant in hydrophilic or lipophilic environments. Furthermore, the TBARS assay was used to evaluate the protein's ability as an antioxidant when bound to palmitate or α-bromo-palmitate.

Results: LC-MS showed that the recombinant FABP1 T94A variant has a molecular weight of 14939 Da, 30 Da less than the recombinant FABP1 (14969 Da) confirming the identity of the two proteins. DCF assay illustrates that both FABP1 and the T94A variant can scavenge reactive oxygen species (ROS). TBARS assay revealed that T94A is able to reduce lipid peroxides in both hydrophilic and lipophilic environments similar to that of FABP1. Binding with fatty acids did not block T94A's antioxidant activity.
Conclusion: Recombinant rat FABP1 T94A variant was successfully purified and confirmed with LC-MS. FABP1 T94A display antioxidant activity being able to scavenge ROS and can act as an antioxidant in both hydrophilic and lipophilic environments.

30. Assembling DNA Nanostructures using External Stimuli: Towards Modular Polyspecific Antibodies

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Purpose: Recently, bispecific antibodies have emerged as promising therapeutic treatments for various forms of cancer. Their appeal lies in their ability to recognize two antigens simultaneously, such as merging tumor cell recognition with the recruitment of cancer-killing T-cells. However, they can be hard to produce, requiring that the two antibodies first be incorporated into a cell for expression, then fused and purified. Current advances allow for a more modular assembly, yet still require the introduction of a mutation into the antibody sequence. An ideal system would place unmodified antibodies onto a modular scaffold, with the size and shape of the scaffold defining the number and type of antibodies. The DNA alphabet provides programmability and modularity that allows strands to self-assemble into discrete structures. Strands can easily be conjugated to many molecules. Despite these appealing features, very few biomolecules have been directly integrated into DNA nanostructures, since their assembly requires high-temperature anneals that deactivate many proteins.
Methods: We are developing a method of DNA assembly that uses a cooled microwave reactor to assemble DNA without affecting biomolecules. Assembly is being verified using gel electrophoresis and fluorophore/quencher pairs. Biomolecules such as enzymes are evaluated for continued activity following microwave exposure. Antibodies are conjugated to the individual DNA strands prior to assembly, allowing the formation of DNA nanostructures precisely displaying multiple antibodies. Final structures are tested using ELISA and flow cytometry.
Results: Microwave-induced DNA assembly has been successfully demonstrated. Under similar conditions, biomolecules such as enzymes retain their activity.
Conclusion: With promising preliminary results, current work focuses on the conjugation of antibodies to DNA, and the evaluation of the resulting assemblies.

31. Scavenger Receptor CD36 as a Target for the Treatment of Age-related Macular Degeneration

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Purpose: Pathophysiological inflammation of the dry form of age–related macular degeneration (AMD), the leading cause of central blindness, is associated with monocyte infiltration and activated macrophage accumulation at the sub-retinal level. Scavenger receptor CD36, identified as a co-receptor of the TLR2 complex mediates the inflammatory response. We propose to investigate the inhibitory effect of a novel and selective azapeptide, as a CD36 ligand in the inflammatory response in dry AMD.
Methods: For in vitro study, cultured murine peritoneal macrophages were stimulated with TLR2-specific ligands ± azapeptide. Culture supernatants were collected for proinflammatory cytokine measurement, and cells were lysed for assessment of TLR2-signaling. For in vivo study, C57BL/6 mice were submitted to photooxydative stress by blue light irradiation for 5 days ± daily treatment with azapeptide (289 nmol/kg). Mice were sacrificed and eyes collected for flatmounts. Iba1 as marker of activated macrophages in subretinal space

was quantified. Outer nuclear layer thickness (ONL) was measured as an index of photoreceptors integrity.
Results: Treatment with azapeptide reduced TLR2-elicited proinflammatory cytokines release and modulated TLR2-signaling. Iba1 was found reduced at subretinal level which correlates with the preservation of ONL.
Conclusion: Azapeptide as CD36 ligand negatively modulates the inflammatory response mediated by CD36-TLR2 heterodimer complex and appears to be a promising approach in the pharmacotherapy of dry AMD.
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32. New High-throughput Screening Approaches for Nuclear Receptor Complexes

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Purpose: Retinoid X receptors (RXRs) play a role as master regulators due to their capacity to form heterodimers with several other nuclear receptors, contributing to important aspects of biology and to an array of pathological conditions. For instance, we have shown that RXRγ-Nur77 signaling is deregulated during Parkinson's disease treatment, leading to abnormal involuntary movements (dyskinesia). While rexinoids have been developed to modulate activity of RXR-containing dimers, their lack of selectivity strongly limits their use for specific therapeutic approaches.
Method: We have previously developed a bioluminescence resonance energy transfer (BRET) assay combined with luciferase protein complementation that specifically monitors the recruitment of a co-activator motif (fused to the YFP as an energy acceptor) by dimers of RXRγ and Nur77, each receptor being fused to a *Renilla* luciferase (RLucII) fragment in such a way that their interaction reconstitutes an active energy donor.
Result: The assay was optimized for high throughput screening (HTS) format. The assay was robust with a z' of 0.714 in 384 wells/plate format. We have successfully screened 2 distinct libraries of compounds, for a total of 220,000 compounds, in both agonist and antagonist modes, with hit rates of 0.3% and 0.1%, respectively. Primary hits were confirmed and validated using a classic DR-5 gene reporter assay for Nur77 and RXRγ complex. Chemical analysis identified 12 compounds that are currently undergoing characterization in a structure-activity relationship program.
Conclusion: The ability to monitor activity of specific RXR heterodimers in HTS format offers an new avenue for the development of rexinoids with improved specificity of action on RXR partners, and therefore more targeted action on tissues expressing the desired combination of receptors.

33. Characterizing the Molecular Signaling Pathways in the Cerebral Arteries of Stroke-prone Spontaneously Hypertensive Rats (SHRsp) Before and After Stroke

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Purpose: Hemorrhagic stroke is associated with loss of cerebral blood flow autoregulation in the stroke-prone spontaneously hypertensive rat (SHRsp). To understand the signalling dysfunction involved in increased fatal risk of stroke in patients with inflammatory diseases, we use SHRsp's middle cerebral arteries (MCA). The cellular changes seen in the MCA's may be responsible for dysregulation as well as loss of autoregulation of the MCA's in Stroke.
Methods: MCA samples from SHRsp animals are collected at 9 weeks for pre-stroke and after evidence of stroke (around 15 weeks) for post-stroke. The MCAs were either isolated to measure protein levels and expression using immunofluorescence (IF). Tissues were analyzed for activation of neuro-inflammation. Vessels were analysed for total and activated inflammatory

proteins (ERK and MAPK), proteins involved in cerebrovascular contraction (PKC and MLC), changes involved in structural integrity (actin polymerization) and transient receptor potential V4 (TRPV4) activation.
Results: Preliminary results from IF indicate activated inflammatory proteins (phospho ERK & phospho MAPK) are increased after stroke with an associated decrease in expression of activated protein kinase C(phospho PKC-involved in activating contractile proteins) compared to pre-stroke SHRsps. The post stroke MCAs also indicate significant decrease in F-Actin/G-Actin Ratio and significant increase in activation of TRPV4 channel in the endothelium in comparison to pre-stroke.
Conclusion: Overall, it appears that there is an increase in active inflammation and decrease in active PKC (involved in contraction) in post-stroke. Post-strokes demonstrates significant decrease in Actin polymerisation & significant increase in activation of TRPV4 Channel, which indicate there is a decrease in vessel structural integrity and ability for the MCA to contract in response to pressure.

Drug Delivery and Pharmaceutical Technology

34. Conjugation Approaches to Develop Modified Nanoparticles for HER2 Breast Cancer Targeting

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Purpose: In this study, the effect of various formulation parameters on a targeted poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) encapsulating docetaxel (DOC) as a chemotherapy were investigated. Human epidermal growth factor receptor-2 (HER2) antibody moieties, either whole IgG (TrAb) or fragments (ScFv), were decorated on the PLGA NPs and evaluated in terms of their ability to target HER2 breast cancer cells. We observed the effects of these NPs against different cell lines. Thus, ligand modified structurally concealed PLGA NPs could be a promising delivery tool for targeting HER2 breast tumor in vitro that improves the release of chemotherapy while reducing the side effects.
Methods. A solvent evaporation technique was adapted to design NP formulations using both ester, and carboxylic acid terminated PLGA. Incorporation of ligands (TrAb or ScFv) was conducted through chemical conjugation processes. The physicochemical characterizations of formulations were executed to assess the effects of different ligands, solvents, polymers, cross-linkers (BS3 & NHS/EDC), drug loading, antibody quantification, and in vitro drug targeting.
Results. Modified PLGA NPs showed a mean diameter particle size below 400 nm with approximately neutral zeta potential. DOC encapsulation efficiency reached up to 85% for some formulations, and the amount of Anit-HER2 attachment efficiency exceeded 40%. The cellular targeting of nanoparticles was studied using two cell lines (MCF-7 and SK-BR-3), and different levels of HER2 expression were evaluated. the significant reduction in the level of HER2 expression was observed for modified NPs in HER2 overexpressed SKBR-3 cells.
Conclusion: Our data for anti-HER2 modified PLGA NPs have demonstrated a prospective potentiality to be applied as targeted chemotherapy against HER2 breast cancer.

35. Exploring the Therapeutic Potential of Copper Based Therapeutics in Platinum Insensitive Cancers

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Purpose: To investigate the potential of copper based therapeutics in the treatment of platinum insensitive cancers.
Methods: Copper binding ligands (diethyldithiocarbamate (DDC), pyrithione (Pyr), plumbagin (Plum), 8-hydroxyquinoline (8-HQ) and clioquinol (CQ)) and their respective copper complexes, as well as controls (copper, cisplatin,

carboplatin), were screened for *in vitro* cytotoxicity against 8 cancer cell lines of differing origins and platinum sensitivities. Cytotoxicity curves were generated through 3 independent experiments with the use of an INCell Analyzer to measure cell viability based on loss of plasma membrane integrity 72 hours following treatment. Based on the results, one copper complex ($Cu(DDC)_2$) was selected for further study. $Cu(DDC)_2$ was formulated into DSPC/Chol liposomes and assessed in vivo in a subcutaneous tumour model of the platinum resistant ovarian cancer cell line A2780-CP.

Results: The *in vitro* screen indicated that the cytotoxicity of the copper complexes was not influenced by the cell's sensitivity to platinum. For example, the A2780-CP cell line was 4-fold less sensitive to cisplatin then A2780-S; where the IC_{50} was 3.7 µM and 1µM, respectively. The IC_{50} of $Cu(DDC)_2$ was less than 100 nM for both cell lines. Four out of the 5 copper complexes tested exhibited IC_{50} values of less than 10 µM and warrant further study. Interestingly, 3 ligands (DDC, Pyr and 8-HQ) showed enhanced activity in the presence of copper whereas cytotoxicity of 2 of the ligands (Plum and CQ) were independent of copper complexation. In the platinum insensitive tumour model, $Cu(DDC)_2$ treatment resulted in a 50% reduction in tumour burden when compared to controls (vehicle or $CuSO_4$-liposomes) which had to be euthanized on day 18 due to tumour size.

Conclusion: The screen suggests that copper complexes are effective in the treatment of platinum insensitive cancers and are a promising class of therapeutic which should be pursued.

36. Trivalent Lanthanum (La^{3+}) Biodistribution Profiles from Intravenous and Oral Dosing of Two Lanthanum Complexes, $La(dpp)_3$ and La(XT) and their Evaluation as Treatments for Bone Resorption Disorders

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Purpose: Two such chelators, 1,2-dimethyl-3-hydroxy-4-pyridinone (Hdpp) and bis-{[bis(carboxymethyl)amino] methy}phosphinic acid (H_5XT), have previously been the subjects of extensive physical, *in vitro* and *in vivo* testing as the *tris*- and *mono*-lanthanum(III) complexes $La(dpp)_3$ and La(XT), respectively. In this study, we expand upon those studies to include 4- week intravenous (IV) and oral La^{3+} biodistribution profiles.

Methods: Rats were randomly placed in one of the following treatment groups (n=4): oral dosing of $La(dpp)_3$ and of La(XT) at 50 mg/kg/day, IV dosing of either compounds at 1 mg/kg/week or a control group. Plasma samples from each time point were analysed for creatinine, alanine transaminase (ALT) and aspartate transaminase (AST) levels. Kidney and liver histology was performed and ICP-MS was used to determine lanthanum concentrations in tissue, plasma and bone samples.

Results: Of the two compounds, La(XT) demonstrates the more favourable *in vivo* characteristics, therefore dose-dependent oral biodistribution studies were carried out with this complex. These show drug saturation above a dose of 100 mg kg^{-1} day^{-1}, so liver histology was performed in order to assess any potential toxicity. Finally, we improve upon the physical characterization of $La(dpp)_3$ to include a single crystal X-ray structure, which exhibits an 8-coordindate La^{3+} centre with two bound water molecules, and a disordered exoclatherate-type hydrogen bonded network (**Figure 1**).

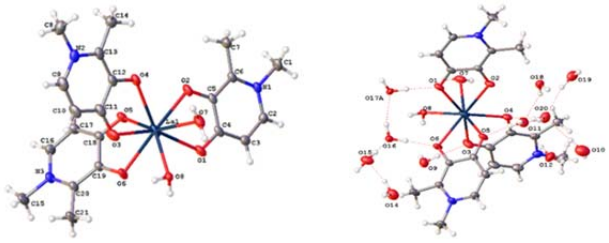

Figure 1 ORTEP diagram of the crystal structure of $[La(dpp)_3(H_2O)_2]\cdot 11.75H_2O$ showing only the water molecules bonded directly to the La^{3+} centre (above), and including the network of hydrogen-bonded water molecules (below).

Conclusions: The present study has demonstrated that lanthanum, when administered as either $La(dpp)_3$ or La(XT), accumulates in bone, the target organ, with overall uptake greater for La(XT). Biodistribution studies show that lanthanum accumulates mostly in the liver, spleen and intestine, with negligible amounts found in brain or heart tissue. Tissue levels were dose-dependent. Over the

4 weeks of chronic treatment, no significant kidney toxicity was found, and only minor abnormalities in the liver at the highest dose level. These results are encouraging to pursue La(XT) in studies of longer duration for ongoing investigations of its effect on bone.

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37. Development of Bio-conjugates Enzyme-PEG for the Treatment of Neurodegenerative Diseases

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Purpose: Glutamate, as the major excitatory neurotransmitter in the central nervous system, is involved in many aspects of normal brain function including learning, memory and behavior. During neurodegenerative diseases, the high levels of glutamate in the brain induces excitotoxicity and leads to neuronal death and loss of cognitive function. Some studies have suggested that reducing blood levels of glutamate could induce efflux from the brain to the blood, thereby leading to a decrease in cerebral glutamate concentrations. Our hypothesis is that the use of enzyme-polymer bio-conjugates could be of interest for the treatment of neurodegenerative diseases. Glutamate Oxaloacetate Transaminase (GOT), via its catalytic activity, will consume excess glutamate in the bloodstream. Conjugating GOT with biocompatible polyethylene glycol (PEG), should increase the duration of circulatory half-life. We propose, therefore, to synthesize bio-conjugates of GOT-PEG, to validate the maintenance of enzymatic activity and evaluate their therapeutic efficacy.
Methods: We have conjugated PEG on the surface of the GOT in a variety of PEG-GOT ratios. Reaction yield and efficiency was visualized via SDS PAGE and size exclusion chromatography. Degree of PEGylation was determined using NMR, prior to determining the effects of biocojugation on GOT activity.
Results: Our results demonstrate that the use of different PEG-GOT ratios allows us to control the number of PEG grafted on the surface of our enzyme, and that enzymatic activity is maintained.
Conclusion: Currently, we are working on *in vitro* and *in vivo* tests, to evaluate the effectiveness of the elimination of the excess toxic glutamate from the cerebrospinal fluid.

38. Disintegration or Dissolution as Quality Control Method for Immediate Release Tablets – How to Decide in a QbD Environment

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Purpose: Quality by Design aims to identify and use the most appropriate and meaningful methods and processes to characterize and manufacture products. Dissolution testing is a standard quality control method commonly used for oral dosage forms. Certain regulatory guidelines allow to substitute dissolution testing with disintegration testing. The current work investigated mechanisms behind disintegration and dissolution behaviour of immediate release tablets.
Method: Different manufacturing methods were used to make qualitatively and quantitatively similar tablets. This was achieved by direct compression of tablets or granulation of the same excipients before tableting. A slow eroding tablet was also made using different excipients. USP type dissolution testing of the tablets and intrinsic dissolution testing of the active pharmaceutical ingredient (API) were performed with conventional buffers and immersion media impacting disintegration. DDDPlus software was used to simulate the observed release profiles and for model fitting of the obtained data.
Results: IR tablets made by direct compression released the API fast. Model-fitting to the Kosmayer-Peppas equation showed that dissolution was diffusion controlled. However, the dissolution of the granulated and slow eroding tablets were significantly impacted by the excipients and followed zero-order release kinetics. Highly viscous media slowed intrinsic dissolution down and impacted tablet disintegration. Simulations were able

to predict dissolution behaviour except when coning was observed.

Conclusion: The study shows that for direct compressed tablets dissolution will be governed by API properties after tablet disintegration has occurred. Disintegration can be modulated by high viscosity media, which then impacts dissolution. For granulated and slow eroding tablets, the excipients and API had stronger interactions and the formulation determined dissolution behaviour.

If disintegration and dissolution can be identified as sequential but independent processes, then disintegration might be justified as QC method for IR tablets independent of the current Q values and time limits set by different guidelines.

Disclosure: This project was supported by Simulations Plus, AbbVie and the Drug Development and Innovation Centre of the University of Alberta. The Drug Development and Innovation Centre of the University of Alberta and AbbVie jointly participated in the interpretation of data, writing, reviewing, and approving the publication. Lukas Uebbing is an employee of Gutenberg University Mainz, Leandro Santoro Hernandes is a post doctoral fellow and Juliet Obianuju Njoku is a graduate student at the University of Alberta. Gregory K. Webster is an employee of AbbVie and Raimar Löbenberg is an employee of the University of Alberta.

39. The Application of Simulation Software in Early Drug Development

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Purpose: In early drug development the availability of active pharmaceutical ingredients (API) is often very limited. The current study investigated how software modeling might be used to plan and design laboratory experiments.

Method: ADMET predictor and DDDPlus software were used in this study. Ritonavir was chosen as a poorly soluble model drug. After modeling basic physicochemical descriptors of the drug molecule using (e.g. solubility, Log P, Log D and pKa), simulations in different pharmacopeial media and surfactant media were performed. The effects of pH, media composition, surfactant type and concentration were investigated.

Results: The simulations showed a strong effect of surfactant concentrations, both on the maximum amount dissolved and the dissolution rate. Media containing no surfactant (0.1 M HCl, 0.01 M HCl, SGFsp, and buffer solutions without SDS) showed slow dissolution and reached a maximum fraction dissolved of 13.13% in 60 minutes. FaSSIF and FeSSIF, which both contained low amounts of surfactants, showed faster and overall higher dissolution. When higher amounts of surfactant – in this case SDS – were used, the dissolution reached 100%. Since the required surfactant amounts would not be realistic in an *in vitro* approach, new simulations in phosphate buffer were performed using longer run times, as well as other surfactants (cetyl trimethylammonium bromide CTAB and Brij® 35) at lower concentrations.

Conclusion: Based on the simulated data, SGFsp, acetate buffer with 0.1 M SDS and acetate buffer with 0.25 M SDS were selected as media for *in vitro* testing, in order to evaluate if the simulations can predict the chosen test conditions. The test conditions included slow, medium and fast (> 80% in 15 minutes) dissolution.

The study showed how simulations can estimate pH and surfactant effects for an API. The results can be used to plan and utilize fewer laboratory experiments. This might save time to confirm dissolution conditions *in vitro*.

Disclosure: This project was supported by Simulations Plus, AbbVie and the Drug Development and Innovation Centre of the University of Alberta. The Drug Development and Innovation Centre of the University of Alberta and AbbVie jointly participated in the interpretation of data, writing, reviewing, and approving the publication. Lukas Uebbing is an employee of Gutenberg University Mainz, Leandro Santoro Hernandes and Juliet Obianuju Njoku are graduate students at the University of Alberta. Gregory K. Webster is an employee of AbbVie and Raimar Löbenberg is an employee of the University of Alberta.

40. Sodium Trimetaphosphate Crosslinked Starch Nanoparticle (SNP) Hydrogels for the Controlled Delivery of Tetramethylthiuramdi-sulfide to Influence *Glycine max* Growth

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Purpose: In recent months, there has been substantial tension between pest management and seed trade organizations in North America which has shifted the public perception away from the use of standard fungicide-based seed treatments such as thiram, captan, and iprodione. There exists an unmet economical need to design environmentally safe delivery vehicles for common agrochemicals whilst promoting soil water uptake to improve crop yields and simultaneously ameliorate concerns of potential public exposure.
Method: Hydrogels were fabricated from the base (NaOH) catalyzed esterification reaction of sodium trimetaphosphate (STMP) and solubilized starch or dispersed starch nanoparticles. A reactive extrusion process was used to fabricate large quantities of STMP cross-linked starch hydrogels. To further promote the uptake of water, the introduction of carboxymethyl groups was investigated. The physical properties of the starch hydrogels were determined as a function of base, STMP, starch concentration, and the degree of carboxymethylation. The hydrogels were loaded with the fungicide tetramethylthiuramdisulfide (thiram) to determine potential candidates for *in situ* *Glycine max* plant studies.
Results: Hydrogels prepared from soluble/dispersed starch and from reactive extrusion products yielded similar and tunable storage modulus (G') ranges from around 0.5 to >10 kPa. The reactive extrusion process has the advantage of requiring less STMP. All hydrogels were swollen to their maximum normalized weight in 20-24 h, followed by a slow degradation profile governed by the relative crosslink density; a favourable trend for the 2-3 week period when thiram activity must be regimentally controlled.
Conclusion: The ideal hydrogel formulation to improve both soil water uptake and the release of thiram will involve the optimization of crosslink density, hydrophilic content, and mechanical properties. Based on characterization and performance testing of the hydrogels, it is expected that 35 and 25 wt% colloidal and extrusion-based hydrogels will serve as the best mediators for plant health and growth.

41. Development of Highly Concentrated Thermally Stable Insulin Formulations by Experimental Investigation and Molecular Dynamic Modeling

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Purpose: Many drug delivery systems including implantable and wearable devices require thermally stable formulations of protein drugs with high concentrations due to limited device sizes and long-term use. However, development of highly concentrated formulations of such drugs is challenging due to their high susceptibility to aggregation and precipitation during storage, transportation and application. In this work we aimed to develop a highly concentrated insulin gel formulation (up to 80 mg/mL, 2160 IU/mL) with rationally selected excipients and composition by experiments and molecular dynamic modeling.
Methods: Non-ionic amphiphilic triblock copolymer (e.g. Pluronic F-127, PF-127) was selected as a gel-forming material. Resorcinol and zinc were used to further stabilize the structure of insulin. Insulin gel formulations with varying excipient compositions were prepared. The insulin gels were incubated at 37°C under shear for 30 days, and their native insulin content and secondary structure were evaluated by reversed-phase HPLC and CD spectropolarimetry, respectively. The *in vitro* release of insulin gel from microfabricated implantable devices was characterized with UV spectroscopy. The *in vivo* efficacy of insulin gel-loaded microfabricated implantable devices was evaluated in STZ-induced diabetic rats.

Results: The chemical and physical stability of insulin were found to be improved with increasing PF-127 concentration, as evidenced by reduced degradation products and preserved secondary structure. Insulin was released from implantable devices at a constant rate dependent on both membrane porosity and PF-127 concentration. Subcutaneous implantation of gel-loaded devices into diabetic rats resulted in normoglycemia for ~17 days. Molecular modeling revealed that PF-127 could reduce fibrillation of insulin by stabilizing the secondary structure of unfolded insulin *via* hydrophobic interaction with native insulin. Addition of zinc and resorcinol further prolonged the stability of the insulin formulation.
Conclusion: Highly concentrated insulin formulated in PF-127 gels exhibited enhanced stability at body temperature and maintained normoglycemia in diabetic rats for 17 days.

42. Development and Characterization of Peptide-Modified Gemini Surfactants-Based Lipoplexes for Non-Invasive Cutaneous Gene Delivery

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Purpose: Gemini surfactants have emerged as a promising class of non-viral gene delivery vectors. Their unique structure offers possibilities for structural modification, allowing for the production of compounds specifically designed to overcome delivery barriers. Although extensive research focused on the design of new derivatives, little is known about their post-transfection fate. In this work, we elucidate the structure-activity relationship of a new generation of peptide modified gemini surfactants and we correlate that to the surfactants' penetration across the skin. Our aim is to develop an effective non-invasive topical gene delivery system with a potential to treat conditions like scleroderma or melanoma.
Method: A series of 22 peptide-modified gemini surfactants was evaluated for their transgene expression and cytotoxicity in murine keratinocytes (PAM 212) at nitrogen to phosphate ratios (N/P) of 2.5, 5 and 10. Physiochemical properties of the nanoparticles were examined by measuring the particle size and surface charge using dynamic light scattering and laser Doppler velocimetry, respectively. Morphological characteristics were studied by small-angle X-ray scattering (SAXS). Skin penetration was assessed using Franz diffusion cell. Mass spectrometry (MS) was used to detect gemini surfactants in the skin tissue and Franz cell receptor compartment.
Result: The highest transfection efficiency was observed with 16-7N(GK)-16 gemini surfactant-based lipolexes at an N/P ratio of 2.5. In fact, it showed a 7-8-fold increase in gene expression compared to the first-generation unsubstituted gemini surfactants. In addition, it exhibited the highest cell viability (85%) among the tested compounds. The nanoparticles had an average particle size of 81±6 nm and surface charge of +21±1 mV. They adopted an inverted hexagonal phase which is known for its highest activity. Gemini surfactants skin penetration was correlated with the gemini surfactants chemical structure.
Conclusion: We conclude that the gemini surfactant's structure determine the physicochemical properties and transfection efficiency of the delivery system.

43. Engineering Actively Targeted Block Copolymer Micelles for Delivery of Doxorubicin to Breast Cancer Cells Overexpressing HER2 and EGFR

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Purpose: To develop and optimize a stable block copolymer micelle nanoformulation which delivers doxorubicin (DOX) to HER2-positive trastuzumab resistant metastatic breast cancer cells *in vitro* by targeting HER2 and EGFR with HTP and GE11 peptides, respectively.
Methods: A series of functionalized biocompatible poly(ethylene glycol)-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) copolymers were synthesized. Copolymer structure and composition was determined by ^{1}H-

NMR and GPC. Physico-chemical characterization of the nanoparticles included particle sizing, CMC, drug loading, stability and release kinetics. Active targeting to HER2 and EGFR will be accomplished by surface conjugation of HTP and GE11 peptides, respectively. Cytotoxicity assays were performed for free drug and non-targeted formulation.

Results: For the first time, the synthetic technique was optimized to achieve a series of copolymers of narrow polydispersity (i.e. PDI ≤ 1.05) which enabled formation of stable 30-nm micelles with a CMC of 10 μg/mL and a narrow size distribution. DOX was loaded into micelles with drug loading efficiency (53.3%), loading content (9.5% wt%) and exhibited sustained release over 72 h. Cytotoxicity assays (IC_{50}) for non-targeted formulations were consistently several fold higher than free drug. GE11 peptides displayed selective binding to EGFR.

Conclusion: The non-targeted nanoparticle formulation has been optimized and characterized to the intended specifications. *In vitro* cell studies in conjunction with release studies indicate controlled release of drug from the nanoparticle. GE11-lipid conjugates have been synthesized and are currently being tested. HTP-lipid conjugates are in late development stages. Cell binding and internalization studies are being performed to evaluate the effect of targeting and dual-targeting, *in vitro*.

44. Synthesis of Multi-stimuli Responsive Nanoassemblies via Click Chemistry for Enhanced Drug Delivery

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Purpose: Polymeric nanoparticles are of great interest as an effective drug carrier system. They are able to encapsulate anticancer therapeutics and to deliver the drugs to targeted tumor sites upon intravenous administration. A promising approach to enhanced and controlled release is stimuli-responsive degradation (SRD) that involves the incorporation of dynamic covalent linkages, which can be cleaved in response to external stimuli. Under physiological condition, the nanocarriers can be destabilized in response to appropriate stimuli in the intracellular environment. The aim of the study is to develop nanoparticles that can release drugs in response to cancer microenvironment.

Method: A novel polyester composing of both ester and sulfide linkages was synthesized by a click-type thiol-ene Michael addition reaction. Using polyethylene glycol and Brij S20 as biocompatible stabilizers, the polyester formed colloidally stable nanoparticles having hydrophobic polyester cores, which are physically-stabilized with hydrophilic stabilizers. Nanoparticles were disassembled in the presence of esterase by the cleavage of ester linkages and/or in the presence of H_2O_2 in which sulfides are converted into corresponding hydrophilic sulfones. Furthermore, encapsulation and release of the model drug, Doxorubicin (Dox), in nanoparticles was confirmed by UV/Vis spectroscopy and by fluorescence spectrometry. Cytotoxicity of empty and Dox-loaded nanoparticles were evaluated using a MTT colorimetric assay and epifluorescence microscope.

Results: DLS results indicated that particles were formed with an average diameter = 95 nm and Dox-loaded nanoparticles with the average diameter = 145 nm. The loading level of Dox was determined to be 2.7 %. The results obtained from *in vitro* cell culture experiments including cell viability and cellular uptake suggest that enzymatic and oxidation-triggered release of Doxorubicin promote the inhibition of cell proliferation after internalization into cancer cells.

Conclusion: Oxidation and enzyme-responsive polyester-based nanoparticles were successfully synthesized. Their ability of encapsulating drug and releasing the drug in response to stimuli was confirmed. *In vitro* cell culture experiment suggest that the polyester-based nanostructures provide great versatility as drug delivery nanocarriers for cancer therapy

45. Customizing Lipopolymers for Efficient siRNA Delivery to Different Leukemia Cells

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Purpose: Chemical functionalization of low molecular weight (MW) polyethylenimine (PEI) with aliphatic lipids enhances their siRNA transfer efficiency in leukemia cells owing to increased cell

membrane interaction facilitated by the hydrophobic lipid substituents. However, the response of different leukemia cell lines and primary patient cells varies depending not only on biological differences but also on several factors related to the chemistry of the lipid-substituted PEI lipopolymers. The aim of this study is to analyze the cell-type specific observations in the context of differential chemistries of the lipopolymers.
Methods: Caprylic acid (CA), palmitic acid (PA), and linoleic acid (LA) substituted polymers were synthesized by N-acylation of low MW PEI with lipid chlorides. THP-1, KG-1, KG-1a, HL-60, and K562 cell lines were utilized for cell studies. Primary cells were obtained from the peripheral blood or bone marrow of AML patients. Gene silencing and cellular uptake of particles was performed by flow cytometry.
Results: Modification with short carbon chain CA yields higher substitution compared to long chain LA at a given lipid:PEI feed ratio. LA lipopolymers resulted in higher siRNA complex uptake across different AML cell lines, K562 CML cell line, and primary AML patient cells. While LA lipopolymer showed higher gene silencing activity than CA lipopolymer in the least differentiated KG-1 AML cell line, the latter was more effective in the more differentiated THP-1 cell line. In contrast, PA lipopolymer exhibited higher silencing efficiency in K562 cells than LA lipopolymer. In primary AML patient cells, CA lipopolymer exhibited relatively more significant silencing activity than LA lipopolymer.
Conclusion: Lipid structure and composition, amount of lipid substitution, and MW of PEI backbone play a critical role in dictating the siRNA complex uptake and subsequent gene silencing efficiency of these lipopolymers in different types of leukemia cells, including primary patient cells.

46. Dual Reduction/Acidic pH-responsive Block Copolymer Micelles: Synthesis, Self Assembly and Stimuli Responsive Enhanced Release

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Purpose: Well-defined amphiphilic block copolymers and their assemblies exhibiting multi stimuli-responsive degradation (MSRD) have emerged as promising intracellular drug delivery nanocarriers for cancer therapy. We have developed a new dual stimuli reduction/acidic pH-responsive block copolymer designed with a hydrophilic poly(ethylene glycol) (PEG) block that is conjugated with a hydrophobic polymethacrylate block having pendant disulfide linkages (PHMssEt) through ketal linkage; thus PEG-ketal-PHMssEt.
Method: This block copolymer was synthesized via atom transfer radical polymerization (ATRP) in the presence of a ketal-bearing PEG macroinitiator. The macroinitiator was synthesized in three steps: First, the succinimidyl activated PEG (PEG-SC) was coupled with dimethyl ketal containing intermediate consisted of an amine and trifluoroacetamide group at each end. Second, trifluoroacetyl group was deprotected in alkaline condition. Finally, the macroinitiator was functionalized with tertiary alkyl bromine for initiation of ATRP.
Results: The copolymer self-assembles to form micellar nanostructures with ketal linkages at core/corona interfaces and glutathione-responsive disulfide linkages in cores. They feature with in situ disulfide-crosslinked cores enhancing colloidal stability during blood circulation, acidic pH-responsive sheddable coronas promoting cellular uptake after extravasation into tumors, and glutathione-responsive disulfides enhancing release of encapsulated drugs in cancerous cells. The amphiphilic block copolymers are characterized by gel permeation chromatography, ^{1}H NMR and dynamic light scattering.
Conclusion: Reduction and pH- responsive block copolymer was successfully synthesized using the ATRP method. Their aqueous micellization and release behaviours of micelles under reductive and acidic environment were assessed. Due their unique features, these polymeric micelles maybe promising candidates as programmable drug delivery systems for cancer therapy.

47. *In vivo* Near-infrared Imaging of c18-DK Peptide using Orthotopic Breast Cancer Murine Model

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Introduction: Cancer targeting peptides

significantly enhance selective delivery and therapeutic efficacy of anticancer drugs or drug carriers, decreasing side effects on the healthy cells and tissues. The cyclic c18-DK decapeptide was engineered to be stable toward proteolysis, to be specific for breast cancer and at the same time to have minimal affinity for noncancerous cells.
Methods: Female NIH-III mice were injected with $2x10^6$ MDA-MB-231-luc-D3H2LN cells in their mammary fat pad. c18-DK containing cy5.5 near-infrared dye was injected into the mice intravenously. The animals were imaged at 0.5, 2, 6 and 24 h using IVIS. Breast cancer cells were detected by bioluminescence, while labelled-peptide was tracked by near-infrared-fluorescence (680 nm excitation, 720 nm emission). Animals were euthanized (n=3) and tumor, liver, spleen and kidneys were excised and imaged at 1, 2, 4 and 24 h.
Results: Labelled c18-DK peptide distributed to several organs non-specifically within 0.5 hours, but the clearance from these organs was also rapid. After 2 h injection, the peptide was mainly observed in liver, kidneys and tumor. *Ex vivo* data showed the presence of peptide in the tumor tissue at 1 h post injection. At 6 and 24 h, the peptide was absent in the tumor, but was still observed in kidneys and liver, which are the potential sites of its elimination. The preferential accumulation of c18-DK in tumor at the 2 h post injection, in spite of its clearance from other organs, may be attributed to the existence of specific interaction between this peptide and its receptors present only in tumor tissue.
Conclusions: The results show that the c18-DK biodistribution in this model is promising. For the future step of developing a peptide-drug conjugate, the present peptide seems to be able to deliver the drug to the tumor site and be quickly removed from the body.

48. Improving Oral Bioavailability of Macromolecules by Mimicking the Cholera Toxin

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Purpose: A biomimetic approach was investigated to increase oral bioavailability of macromolecules such as proteins and heparins. 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. As a proof of concept, we synthesized bioconjugates consisting of a low permeability macromolecule (albumin) and the B subunit of cholera toxin (CT-B).
Methods: Three different chemical linkers with varying length and hydrophobicity were synthesized and purified using classical chemistry techniques. Bioconjugates of CT-B with albumin were synthesized using click chemistry and purified by size affinity spin columns. Endocytosis and permeability of conjugates were evaluated using Caco-2 cells.
Results: It was possible to synthesize three different linkers for protein-protein conjugation in adequate yield. Albumin-CT-B chimeras were produced using these linkers. The bioconjugates were analyzed for molecular weight by SDS-PAGE. Albumin-CT-B using DBCO-PEG4-NHS had the highest yield before purification. Flow cytometry results showed that Albumin (Fitc)-CT-B is significantly more internalized than Albumin (Fitc) in Caco-2 cells receiving a GM1 supplement.
Conclusion: The results of this study demonstrated the possibility of improving internalisation and permeability of macromolecules using CT-B as a carrier ligand. The mechanism of internalisation was GM-1 dependant.

49. Effect of the Synthesis Process on the Architecture and the Physicochemical Properties of PLA-PEG Nanoparticles

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Purpose: The aim of this study is to compare the nanoparticles produced by different procedures and determine the influence of the fabrication process on the architecture and the physicochemical properties of polymeric nanoparticles. The methods that we are going to compare are: Classic Nanoprecipitation, Flash Nanoprecipitation and Microfluidics. For each method, three drugs with different Log P value were used to load the nanoparticles in order to evaluate

the join effect of Log P and fabrication process on the drug encapsulation level.
Methods: A copolymer of PLA-b-PEG was used to produce nanoparticles through the methods mentioned above. We used Theophylline (Log P 0), Ketoprofen (Log P 3) and Vitamin D (Log P 7.5) to load the nanoparticles at the moment of the fabrication. The size, PdI and Zeta Potential were determined by DLS. The amount and distribution of PEG were quantified by H-RMN and the drug loading was determined by HPLC-UV.
Results: We observed that the size, the polydispersity and the architecture of the nanoparticles were strongly affected by the fabrication process. For example, Microfluidics produced smaller nanoparticles, with higher amount of PEG at the surface, but with higher polydispersity. On the other hand, the Zeta potential depended on the functional groups of the encapsulated drug.
Also, we observed that the drug loading was not significantly affected by the fabrication method and the main property that determines it was the Log P.
Conclusion: Through this comparative study, we demonstrate that the size, the polydispersity of polymeric nanoparticles, as well as their architecture, are determined by the method used for their elaboration. On the other hand, we observed that the dug loading and the Zeta potential depend mainly on the properties and the hydrophobicity of the drug.

50. Reconstitution of BCRP Membrane Transporter into Proteoliposomes to Study the Intestinal Permeability of Drugs

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Purpose: Intestinal membrane transporters play a critical role in the pharmacokinetics of orally administered drugs. *In vitro* models like the Caco-2 cell-based assay and the non-cellular PAMPA assay are used to predict the bioavailability of drugs. However, no model allows the study of isolated and specific membrane transporters. We propose to study membrane permeability with a new approach using a non-cellular lipid bilayer constituted of recombinant transporter. In this study, the two isoforms of the human BCRP/ABCG2 were expressed in the yeast *Pichia pastoris*, purified and then incorporated into liposomes.
Methods: The two isoforms of BCRP cDNA were modified by PCR and cloned into the expression vector pJ902-15 (ATUM). Transformation was carried by electroporation at 1.5 kV with the linearized vectors and electrocompetent PPS-9010 *Pichia* strain (ATUM). The clone with the highest BCRP expression was determined immunoblotting using mAb BXP-21 and inoculated in 1 L of MGY media and induced in the MMY media containing methanol. The cells were lysed using a Freezer/Mill and the microsomes were purified on an AKTÄ-FPLC system using a HisTrap HP column. Protein reconstitution into liposomes was obtained by extrusion and gel filtration and the proteoliposomes were analyzed by DLS.
Results: The two plasmids construct were successfully generated and showed strong inducible expression of BCRP in presence of methanol once transformed into the yeast *P. pastoris*. Immunoblotting of whole cell lysates shows that BCRP has an apparent weight of 65 kDa. The recombinant transporter was then purified by a Ni-affinity chromatography.
Conclusion: We have established a *P. pastoris* expression system for the production of the recombinant BCRP. To confirm that BCRP is functional, the ATPase activity will be measured. This tool will serve as a proof-of-concept for the generation of a library of recombinant transporter proteins incorporated into liposomes.

51. Development of a Suspension Vehicle for Pediatric Extemporaneous Oral Preparations

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Purpose: The off-label use of drugs is a very common practice among pharmacists. This is mainly due to the small number of available pediatric formulations, resulting in pharmacists reformulating commercialized products into extemporaneous preparations better suited for young patients. However, the suspension vehicles currently found on the market are not specifically designed for pediatric use. This could lead to unnecessary exposition to potentially harmful compounds. We therefore hypothesized that it is possible to develop a safer

suspension vehicle for children.
Methods: After reviewing the currently used methods and excipients for extemporaneous preparations, the vehicle composition was elaborated. Its physicochemical properties were then assessed against currently available vehicles. The viscosity and rheological behaviour of the solutions were analyzed using a Brookfield rheometer. Stability was assessed over a 4-month period by monitoring viscosity and pH variations, according to storage temperatures (4, 25 and 40°C). An antimicrobial effectiveness test was also conducted following USP <51> guidelines, by inoculating the test solutions with either *E. coli, P. aeruginosa, S. aureus, C. albicans* or *A. braziliensis*. The concentration (CFU/mL) was then monitored on day 0, 7, 14 and 28, using sodium benzoate 0.1% as control and propionic acid 0.5% as the tested preservative.
Results: Two HPMC-based vehicles were designed at two different pH, namely 4 and 7.5. Both vehicles presented a viscosity of 40 cP and a clear shear thinning behaviour. A slight decrease of the viscosity value with time was reported for both vehicles, but pH remained constant. Propionic acid 0.5% proved to be very effective, reducing the number of inoculated micro-organisms to 0 after just 7 days.
Conclusion: A suspension vehicle specially designed for children is currently being developed. The physicochemical and microbiological stabilities of the vehicle were assessed and found suitable for the intended usage. Perspectives include a taste evaluation by healthy volunteers (adults and children) and chemical stability studies of suspended active principles.

52. Stimuli-responsive Bonds Sensitive Towards Oxidative Stress

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Purpose: Many therapeutic agents are prone to degradation during circulation, are unable to naturally cross biological barriers, or lack selectivity for diseased tissue. Therefore, it is of great interest to couple these molecules to a carrier, which is designed to overcome these hurdles. For such drug delivery systems to be efficient a linker must be introduced between the drug and the carrier, so that the drug can be released (in its unmodified 'active' form) at the desired location. Interestingly, many types of cancer cells have increased levels of reactive-oxygen species (ROS) in vivo. ROS could therefore be an exciting new stimulus for triggering a response from "smart" drug delivery systems. Unfortunately, current ROS-responsive delivery systems, such as those based on aryl boronic esters, poly (propylene sulfide), or from cross-linked oligo (proline), respond very slowly to physiologically-relevant ROS levels (hours to days). The aim of this study is to create new "linker chemistry" that is stable in blood circulation yet rapidly degrades in areas of oxidative stress.
Method: This presentation will showcase the latest developments our group has made towards developing a ROS-responsive linker. We have evaluated stability and reactivity of a new ROS-responsive linker, and the ease with which it can be used to conjugate model and therapeutic peptides (such as antiflammin-2, an anti-inflammatory peptide) to polymeric drug delivery systems. Upon activation by ROS, the linker decomposes to release the fully unmodified ('native') peptides, which should possess their full therapeutic activity.
Conclusion: A new ROS-responsive bond that is very stable in the absence of ROS, and extremely reactive in their presence was developed. Several types of drug delivery systems most likely to benefit from this type of behavior will be discussed.

53. Development and Characterization of a Novel Crosslinked Polyester-Based Implant for Controlled Drug Release

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Purpose: There is tremendous interest in developing implantable drug delivery systems (IDDS) that improve the efficacy of the drug at the target site while sparing off-target tissues from toxicity. The purpose of this study was to develop and characterize a polyester-based implant (PBI) with pendant allyl group functionality that can be

crosslinked to varying degrees or functionalized to tailor the release profiles or improve polymer-drug compatibility, respectively.

Method: A proprietary crosslinked PBI was prepared using a syringe casting method. Five drugs of varying physicochemical properties, such as molecular weight, logP, and aqueous solubility, were loaded using a solvent swelling/evaporation technique. The control and drug-loaded implants were characterized by differential scanning calorimetry (DSC), x-ray diffraction (XRD) and scanning electron microscopy (ESEM), and *in vitro* drug release was measured in PBS with 0.5% SDS or 0.1% Tween 80. *In vitro* cytotoxicity was assessed in L929 mouse fibroblast cells.

Results: Macroscopically, the implants were smooth, non-porous, and flexible in nature. DSC and XRD confirmed the plasticizing nature of acetaminophen, curcumin, and paclitaxel on the matrices, while matrices loaded with either triamcinolone acetonide or triamcinolone hexacetonide demonstrated slight crystallinity. Sustained *in vitro* release was observed for all hydrophobic drugs. The PBIs exhibited excellent biocompatibility towards L929 mouse fibroblast cells.

Conclusion: The promising *in vitro* release profiles and the excellent biocompatibility observed highlights the potential of this IDDS as a universal platform to deliver a library of drugs for local or systemic drug therapy.

Poster Session 2

CSPS and CC-CRS

Friday, May 12

Posters - Session 2

Friday, May 12

Clinical Sciences & Pharmacy Practice

54. Developing Tools to Help Pharmacists Take Action with Asthma

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Purpose: To explore site specific application of Asthma Action Plans (AAPs) within community pharmacies.
Methods: The pilot study enrolled 10 pharmacists from 9 pharmacies at which three site visits were completed over 1-2 months. Visit 1 focused on gathering information about the pharmacy's current workflow. The research student documented the resources, staff, technology, time of patient-pharmacist interactions, as well as barriers and facilitators within practice according to the pharmacists. Visit 2 focused on educating pharmacists about AAPs, current asthma guidelines, and support tools developed by the researchers. Pharmacists were given suggestions to overcome barriers in practice expressed in Visit 1 and were asked to trial the AAPs with 5-10 patients over 2 weeks. A follow up email was sent to the pharmacists 5-7 days after Visit 2 providing additional resources and asking for an update. Visit 3 focused on learning about the pharmacist's experience with the AAPs through a semi-structured interview.
Results: Of the 10 pharmacists enrolled, 8 completed the trial and 2 completed training but did not attempt to use the AAP. Pharmacists trialed the AAPs with 3-5 patients and reported that most interactions were positive for both the pharmacist and patient. Many AAPs were also completed alongside patient care plans and prescription renewals. While pharmacists felt the use of AAPs was a door opener for a conversation, they perceived AAPs as a temporary trial and only for select patients. Pharmacists indicated they were unable to integrate AAPs into their routine workflow due to competing demands for time and cost of printing AAPs.
Conclusion: AAPs were feasible to implement, however based on our observations, routine implementation was not sustainable without attention to organizational culture.
Acknowledgement: Pawan Gill is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

55. Evaluation of Renal and Hepatic Outcomes in HIV+ Individuals following Tenofovir Disoproxil Fumarate/Emtricitabine or Tenofovir Alafenamide/Emtricitabine plus Dolutegravir Regimens

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Purpose: Coformulation of tenofovir alafenamide (TAF) and emtricitabine (F) has been approved and presented as having reduced renal effects compared to the previous formulation containing tenofovir disoproxil fumarate (TDF). Both formulations have been utilized as a backbone of HIV pharmacotherapy in conjunction with the integrase strand transfer inhibitor, dolutegravir (DTG). In this study, renal and hepatic outcomes were evaluated for each combination in the presence of DTG.
Methods: As a subsection of a larger IRB-approved study at East Tennessee State University, pretreatment and post steady-state sodium, potassium, blood urea nitrogen (BUN), serum creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) values, along with respective tenofovir regimen duration (TRD), were gathered from HIV positive adults (median age, 56

years; IQR range, 43.5-61.5 years), receiving either DTG+TAF/F (n=10, 80% male) or DTG+TDF/F (n=7, 85.7% male). Repeated measures ANCOVA, considering such covariates as age and/or TRD, or ANOVA, absence significant covariate influence, were conducted with IBM SPSS Statistics 23. Values are presented as mean±SD with significance determination at $p<0.05$.

Results: The DTG+TAF/F group had a mean TRD of 15.87±7.41 weeks; while the mean TRD in the DTG+TDF/F group was 103.59±60.91 weeks. Significant difference in ALP levels between groups (p=0.002) was seen when TRD (p=0.007) was included as a covariate. The baseline ALP was 92.80±17.33 IU/L in the DTG+TAF/F group and 87.57±24.34 IU/L in the DTG+TDF/F group; while the post-treatment values were 79.50±19.61 IU/L and 88.57±24.34 IU/L, respectively. Repeated measures ANOVA of the remaining groups, following insignificant covariate interaction, were conducted; however, no significant change (p>0.05) was detected for creatinine, sodium, potassium, BUN, AST, or ALT between groups.

Conclusion: Although remaining within the normal range, ALP was significantly decreased in the DTG+TAF/F group compared to DTG+TDF/F in the presence of the TRD covariate in this study.

56. Comparison of Renal Effects following Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Disoproxil Fumarate or Tenofovir Alafenamide Regimens in HIV-infected Patients

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Purpose: The combination of elvitegravir (E), cobicistat (C), emtricitabine (F), and tenofovir alafenamide (TAF), was recently approved as an improvement, in terms of renal outcomes, on a previous antiretroviral formulation containing tenofovir disoproxil fumarate (TDF). This study sought to evaluate changes in renal parameters following administration of either combination.

Methods: For this subset of an integrase strand transfer inhibitor study at East Tennessee State University, the records of consented HIV positive adults (median age, 50 years; IQR range, 42-55.5 years), receiving either E/C/F/TAF (n=11, 90% male) or E/C/F/TDF (n=11, 82% male), were evaluated for serum creatinine, sodium, potassium, and blood urea nitrogen (BUN) pretreatment and at steady-state. Treatment duration was also recorded. Each dependent variable was analyzed with a repeated measures ANCOVA, which considered age and/or treatment duration as covariates, or ANOVA, in the event of no covariate influence, using IBM SPSS Statistics 23. Significance was set at $p<0.05$. Values are presented as mean±SD.

Results: Subjects in the E/C/F/TAF group had received treatment for a mean of 33.05±4.60 weeks; while the E/C/F/TDF group had mean duration of 132.31±36.76 weeks. A significant effect was detected between groups (p=0.023) concerning BUN when age (p=0.016) and regimen duration (p=0.037) were included as covariates. E/C/F/TAF was found to have a baseline BUN of 12.00±3.29 mg/dL and a post-treatment value of 13.91±4.32 mg/dL; while the E/C/F/TDF group presented baseline and post-treatment values of 13.27±3.41 mg/dL and 16.45±4.13 mg/dL, respectively. Consideration of covariates did not influence the models for the remaining measures. Thus repeated measures ANOVA of the remaining groups were conducted; however, no significant change was detected for creatinine (p=0.089), sodium (p=0.478), or potassium (p=0.399).

Conclusion: This study found that BUN was increased significantly in the E/C/F/TDF group compared to E/C/F/TAF when age and treatment duration were examined as covariates in this group of subjects.

Pharmacokinetics and Pharmacodynamics

57. Improving Relative Bioavailability of Orally Dosed Aliskiren through Poly(lactic-*co*-glycolic) Acid Nanoformulation in Rats

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Purpose: Athough an effective direct renin inhibitor, aliskiren (ALS) presents with a low bioavailability coupled with high drug cost. As nanoformulation may increase drug bioavailability, our laboratory developed an ALS-loaded poly(lactic-*co*-glycolic) acid nanoparticle (ALS-NP) formulation. As such, the influence of nanoformulation on drug pharmacokinetic parameters were examined in this study. **Methods**: Following a single oral dose of ALS (n=7; 30 mg/kg) or ALS-NP (n=7; ALS dose equivalent), rats underwent pharmacokinetic sampling (0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hrs post-dose). Plasma samples were assayed using LCMS-IT-TOF (coefficient of variation of <5%). Pharmacokinetic parameters (half-life, $t_{1/2}$; maximum plasma concentration, C_{max}; time to reach C_{max}, t_{max}; the area under the plasma concentration time curve from 0 to infinity, $AUC_{0-\infty}$; apparent volume of distribution, V/F; and oral clearance, CL_{oral}) were calculated using WinNonlin and evaluated using Student's t-test with statistical significance set at $p<0.05$. All values shown as mean±SD.
Results: While $t_{1/2}$ (p=0.0517) and t_{max} (p=0.0961) were not significantly altered, C_{max} in the ALS-NP group (448.53±49.07 mg/L) was elevated compared to control (288.60±148.07 mg/L; p=0.0189). ALS-NP also presented with a 168% relative bioavailability compared to ALS with respective $AUC_{0-\infty}$ values of 2592.82±600.51 and 1538.40±678.17 hr.mg/L (p=0.0095). The V/F of ALS-NP (128.56±43.67 L/kg) was significantly reduced (p=0.0009) compared to ALS (540.33±245.57 L/kg). A significant reduction (p=0.0298) was also detected in CL_{oral} (ALS-NP, 12.26±3.59 L/hr/kg vs. ALS, 23.44±11.45 L/hr/kg)
Conclusion: This study indicates that ALS-NP can be used to improve bioavailability of the drug.

58. Enterolactone-glucuronide Upregulates INSIG-1 to Modulate Cholesterol Metabolism in Caco-2 Cells

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Purpose: To protect their cardiovascular system, consumers show increased tendency of using natural products in conjunction with prescribed medication heightens the importance of research and clinical trials in this area. Changes in cholesterol metabolism have been attributed to intake of lignans purified from several plant origins. Multiple evidence suggests that the lignan "enterolactone (ENL)" improves lipid profiles in hypercholesterolemic patients. However, the nature of this modulation is yet to be discovered. This study investigated the effect of ENL and its conjugated form ENL-Glucuronide (ENL-Gluc) on cholesterol metabolism.
Method: We measured the effect of both ENL and its conjugated form ENL-Gluc on cholesterol uptake in the Caco-2 intestinal cell line, in the presence of a cholesterol trapping molecule U-18666A. In addition, we screened for ENL and ENL-Gluc induced gene expression patterns.
Results: Treatment with ENL and ENL-Gluc reduced cholesterol uptake by 2.94 and 1.99-fold at 20 μM respectively in comparison to vehicle control of 1% DMSO. Furthermore, only ENL-Gluc. significantly upregulated Insulin Induced Gene-1 (INSIG-1) by 3.8-fold when compared to untreated control at p-*value* < 0.01. Western blot analysis confirmed gene expression results. These changes were also confirmed phenotypically by increased retention of fluorescing cholesterol (NBD-Cholesterol) in the endoplasmic reticulum as a function of increased INSIG-1 expression.
Conclusion: Glucuronide conjugation of ENL alters its pharmacological effect in regards to cholesterol metabolism, through increased expression of INSIG-1. Further investigation is necessary in more metabolically active cell lines including hepatocytes, and in assessment of downstream pathways of cholesterol synthesis.

59. Is Exposure to Cigarette Smoke Influencing NAFLD-induced Modulation of Drug Metabolism?

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Purpose: Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and type II diabetes. The prevalence of cigarette smoking and obesity is increasing globally. Playing a crucial role in the development of several diseases, cigarette smoke, along with NAFLD, can lead to the use of numerous therapeutics drugs. Little is known about the combined effects of NAFLD and cigarette smoke on drug disposition. A key player in drug metabolism is the CYP3A subfamily. We used a NAFLD mouse model generated by T0901317 (T0), a liver X-receptor agonist, to evaluate the combined impact of NAFLD and cigarette smoke on the expression of four CYP3a enzymes.
Methods: For 4 days, seven to nine-week old female C57BL/6 mice were exposed for 2 h to cigarette smoke or room air and injected *i.p.* daily with either vehicle or T0 20 mg/kg (n=5/group). Livers were collected, washed, weighted and snap frozen in liquid nitrogen. Frozen optical cutting temperature-embedded liver sections were stained with Oil red O. Total RNA was isolated and relative mRNA levels of cyp3a11, cyp3a13, cyp3a25 and cyp3a44 were assessed by qPCR.
Results: Relative hepatic mRNA levels for the 4 groups are summarized in the table below.

	Room air exposure		Cigarette smoke exposure	
	Vehicle	T0	Vehicle	T0
CYP3a11	1.00±0.03	2.05±0.09§§	0.73±0.07***	1.41±.13 **§§§
CYP3a13	1.00±0.03	0.70±0.04§§	0.72±0.07***	0.61±0.03**
CYP3a25	1.00±0.09	1.60±0.13§§	0.59±0.05*	1.03±0.0.09 §§
CYP3a44	1.00±0.17	0.39±0.06§	0.81±0.11	0.62±0.07
* *p<0.05*, ** *p<0.01*, ***p<0.001* vs vehicle exposed to room air § *p<0.05,* §§ *p<0.01*, §§§ *p<0.001* vs their respective vehicle				

Conclusion: Cigarette smoke exposure significantly decreased the mRNA levels of CYP3a11, 3a13 and 3a25. Massive fatty infiltration induced by T0 increased the expression of CYP3a11 and 3a25. Exposure to cigarette smoke appears to modulate the impact of NAFLD on drug metabolism.
Acknowledgement: Camille Thibault is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

60. Development of a Novel Validated LC-MS Assay for the Simultaneous Determination of Linagliptin and Tadalafil in Human Plasma and its Application in a Drug Interaction Study

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Purpose: To develop LC-MS assay for the simultaneous quantitation of linagliptin (LNG) and tadalafil (TDL) in human plasma, to utilize in a drug interaction study.
Method: LNG, TDL and carvedilol (internal standard) were extracted from 500 µL plasma using ethyl acetate in the presence of 0.3N NaOH. After vortexing, and centrifugation, the organic layer was transferred to clean tubes and evaporated *in vacuo*. The dried residue was reconstituted in methanol:water (50:50) and injected into C18 column connected to Single Quadrupole Mass Spectrometer with ESI. The mobile phase consisted of methanol: acidified water, with gradient elution (50:50 to 90:10 over 8 minutes) pumped at 1 mL/min. The $(M + H)^+$ ions utilized for quantification of LNG, TDL and IS were m/z,473, 390 and 407, respectively. Three male Egyptian healthy volunteers were administered LNG tablets 5 mg/day for 13 days. On day 13, they were co-administered 20 mg TDL. Serial blood sampling was performed till 96h on days 1 and 13.
Results: The components eluted within 8 min; calibration curves were linear ($r^2 \approx 0.999$) over the range of 1-5000 ng/mL LNG and 2-5000 ng/mL TDL concentrations. The CV% and mean error were <20% for both drugs. The validated lower limit of quantitation was 1 and 2 ng/mL for LNG and TDL, respectively. Plasma concentrations of both drugs were successfully measured up to 96 h. with calculated pharmacokinetics parameters for both drugs comparable to literature. Volunteers' baseline and end of study clinical investigations were in good standing and in normal ranges.

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 Pharco Pharmaceuticals, Alexandria, ARE.

61. Studying the Potential Pharmacokinetic Interaction between Linagliptin and Tadalafil in Healthy Egyptian Males: a Pilot Study

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Purpose: To study the pharmacokinetic drug interaction between linagliptin (LNG) and tadalafil (TDL) in healthy Egyptian volunteers.
Method: A Phase IV, open-label, cross-over drug interaction study between LNG and TDL was conducted at Ghabour Hospital, Alexandria, Egypt (April to September 2016). On day 1, fasting healthy male volunteers were administered a single TDL 20 mg po dose. Serial blood samples were collected till 96h post dose. After 2 months wash out period the same volunteers were administered multiple oral dosing of LNG (5mg/day) for 13 days. On day 13 a single oral dose of 20 mg TDL was co-administered. Serial blood samples were collected on days 1 and 13 till 96h post dose and daily at Cmin before taking the following LNG dose. Vital signs and drugs' side effects were measured. Non-compartmental PK methods were applied.
Results: Ten healthy male Egyptian volunteers (27 to 55 years) were recruited for the study (five non-smokers and five smokers). Volunteers showed well toleration to TDL single and LNG multiple dosing with PK parameters similar to those previously reported in Japanese and other Caucasian populations. Smoking did not alter the PK parameters of any of the drugs. Upon co-administration of both drugs, a drug interaction occurred resulting in delayed absorption peak, 2 folds decrease in Cl/f , 3 folds decrease in Vd/f, and ~2.5 folds increase in Cmax and AUC of TDL. This was accompanied by 60% increase in muscle pain in 10% of the volunteers and ~7% prolonged QTc interval.
Conclusion: There is a potential mild interaction between the two drugs, therefore it is advisable for diabetic patients on LNG to be administered lower doses of TDL. In addition to proper monitoring upon co-administration with other medications that also prolong the QTc interval.This project was supported financially by Pharco Pharmaceuticals, Alexandria, ARE.

62. Dose-Dependency of Cardiovascular Risks of NSAIDs

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Purpose: The non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat pain and inflammation. However, both arthritis and NSAIDs increase cardiovascular (CV) risks. The dose-dependency of the elevated CV risks of NSAIDs has not been well-studied. We tested the hypothesis that low but effective doses of these drugs are void of CV side effects. As the model drug, we used diclofenac because of its known high CV toxicity, and as markers of CV risks, concentrations of P450-mediated metabolites of arachidonic acid (ArA) were measured.
Methods: To identify the therapeutic dosage range of diclofenac, adult male Sprague Dawley rats were divided to 6 group (n= 4-5/group). All except one group (Healthy-0) were injected with 0.5 mL of *Mycobacterium Butyricum*/squalene in the tail base (AA). Healthy-0 rats were injected with 0.5 mL saline. When the signs and symptoms of arthritis appeared (in approximately 12 days), AA rats were treated with daily oral doses of 0, 2.5, 5, 10 or 15 mg/kg of diclofenac for 7 days (designated AA-0, AA-2.5, AA-5, AA-10, AA-15, respectively). Subsequently, all rats were euthanized and those that positively responded to diclofenac treatments were immediately dissected, their blood, heart and kidneys were harvested for ArA metabolites and diclofenac assay.
Results: Only >5 mg/kg doses controlled AA (ED50 = 4.96) in which ArA metabolites and diclofenac were measured. Only the highest dose (15 mg/kg) caused imbalances in ArA metabolic profile toward cardiotoxicity; e.g., heart 20-HETE: Healthy-0, 0.44±0.31; AA-0, 2.11±1.04; AA-5, 1.84 ±1.20; AA-15, 8.89±0.77.
Conclusion: The observation that only high diclofenac doses cause imbalances in ArA metabolites is suggestive of dose-dependency of the cardiotoxicity of NSAID. Human studies are needed to confirm the safety of low but effective doses of

NSAIDs.
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63. The Nutraceutical L-Citrulline Improves Exercise Capacity and Glucose Tolerance in Obese Mice

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Purpose: L-citrulline is an organic α-amino acid nutraceutical that has been shown to augment exercise/muscle performance in animals and humans, which may be due to elevations in mitochondrial function. It has been proposed that enhancing mitochondrial function may mitigate obesity-related insulin resistance, and thus our goal was to determine whether treatment with L-citrulline could improve glycemia in an experimental mouse model of obesity. We hypothesized that L-citrulline treatment would improve glucose homeostasis in obese mice, and this would be associated with elevations in mitochondrial function in skeletal muscle.
Methods: 10-week old C57BL/6J mice were fed either a low-fat (10% kcal from lard) or high-fat (60% kcal from lard) diet, while receiving drinking water supplemented with either vehicle or L-citrulline (100 mg/kg) for 15 weeks. Glucose homeostasis was assessed via glucose/insulin tolerance testing, while in vivo metabolism was assessed via indirect calorimetry. Mice were run on a forced exercise treadmill to assess endurance, and real-time PCR was utilized to measure gene expression.
Results: As expected, obese mice supplemented with L-citrulline exhibited an increase in exercise capacity, and this was associated with an improvement in glucose tolerance. Consistent with augmented mitochondrial function, we observed an increase in whole body oxygen consumption rates in obese mice treated with L-citrulline. In addition, mRNA expression of mitochondrial transcription factor A and nuclear respiratory factor 1 was increased in soleus muscles from L-citrulline treated obese mice. On the contrary, L-citrulline treatment worsened insulin tolerance in both lean and obese mice.
Conclusion: Taken together, L-citrulline supplementation improved both glucose tolerance and exercise capacity in obese mice, supporting the use of L-citrulline as a nutraceutical to augment performance. Nevertheless, the deterioration in insulin sensitivity following L-citrulline supplementation suggests that an L-citrulline-mediated enhancement of mitochondrial function is not necessarily beneficial towards overall glycemic control.

Pharmaceutical and Analytical Chemistry

64. Identification of Liver Protein Biomarkers of Acetaminophen Covalent Binding and Hepatotoxicity using 2D-LC-HRMS/MS

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Purpose: Acetaminophen (APAP) is a popular mild analgesic and antipyretic in the United States and most of the developed world. Although acetaminophen is safe and effective, it is the leading cause of acute liver failure. This hepatotoxicity has been related to covalent binding of APAP's reactive metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), to proteins. The aim of this *in vivo* study was to identify APAP protein targets in both rat and mouse models and compare results from both species.
Methods: Using a bottom-up proteomics approach, sample preparation involved protein extraction and digestion from rat and mouse liver samples. Liver extracts were digested with trypsin and pepsin, separately, then fractionated via strong cation exchange (SCX) chromatography prior to reversed-phase UHPLC-MS/MS. Data processing involved using ProteinPilot software to find potential

modified peptides, followed by verifying peak integration and comparing treated to control samples to remove any potential false positives.
Results: Several proteins have been identified as modified by NAPQI, including ubiquitin-conjugating enzyme, 5-hydroxyisourate hydrolase, carboxylesterase 1C, SH3 domain-containing RING finger protein 3 in mouse and two modified protein were found in both rodent species, namely carbonic anhydrase 3 and triosephosphate isomerase. These proteins are known to be involved in important biological pathways involved in cell survival during oxidative stress and thus could be linked to APAP hepatotoxicity. **Conclusion:** We have developed a highly sensitive assay for identifying covalently modified proteins, as novel biomarkers of acetaminophen exposure and toxicity. This study can also open doors to new therapeutic or protective approaches against toxicity for future studies.

65. Synthesis of potential Poly(ADP-ribose) Polymerase (PARP) Inhibitors: The Application of Flow Chemistry Techniques in Medicinal Chemistry Programs

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Purpose: Poly(ADP-ribose) Polymerase (PARP) is a family of protein which may be critical in cancer cell proliferation. To make phenanthridinone-like compounds and generate bioisosteres of known PARP inhibitors such as PJ-34, which shows anticancer properties, we plan to replace a phenyl ring with a thiophene ring system or a pyridine ring system to make phenanthridinone-like complex heterocyclic compounds. We choose to develop continuous flow photochemical methods that would give us rapid access to target compounds.
Methods: We use directly coupling reactions to synthesize amides from commercially available carboxylic acids and anilines. Amides are treated as starting materials to synthesize corresponding complex heterocyclic compounds with the thiophene or the pyridine ring system by intramolecular photochemical cyclization, using a continuous flow technique.
Results: We are able to synthesize substituted phenanthridinone-like compounds, including 16 examples of thieno[3,2-c]quinolin-4(5H)-ones and 6 examples of benzo[h]-1,6-naphthyridin-5(6H)-ones. The reactions proceed in isolated yields up to 77%, and in quantities sufficient for medicinal chemistry assay analysis.
Conclusion: As we expected, the continuous flow method provides access to complex heterocycles, benzo[*h*]-1,6-naphthyridineones and thieno[3,2-*c*]quinolinones in two steps from commercially available starting materials in good yields and with greater atom efficiency than traditional batch reactions. Expanding applications of this method makes it possible to synthesize other phenanthridinone-like complex heterocyclic compounds. The continuous flow technique also allows us to control reaction condition in real-time and shows great potential to scale up.

66. Targeting the Oncogenic FOXM1 Transcription Factor in Cancer Treatment

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Purpose: Genome-wide gene expression profiling of human cancers has consistently identified the Forkhead box M1 (FOXM1) transcription factor as one of the most commonly activated genes in cancer cells. Also, abnormal activation of the FOXM1 gene is regarded as one of the hallmarks of chemoresistant cancer cells. Accumulating evidence suggests that targeted FOXM1 inhibition could be a promising strategy to treat many types of cancer. The aim of this project is to validate the FOXM1 transcription factor as a drug target.
Methods: We recently carried out a series of molecular modeling protocols in which we have determined the binding energies of 3,323 FDA-approved drugs within the FOXM1/DNA binding domain and we have identified six promising virtual reference drugs with significant binding energies. In the lab, the MTT assay has been carried out on MDA-MB-231 breast cancer cell line to test the ability of the drugs to inhibit cancer cell proliferation, followed by Western blotting to measure the expression of the protein.
Results: Three of the drugs gave promising results including: thiostrepton (control) IC_{50} = 3.1 ± 1.2 μM, troglitazone IC_{50} = 42.4 ± 16.9 μM, and gliquidone IC_{50} > 100 μM, as they found to inhibit the expression of FOXM1 protein. Based on our

molecular modelling studies, we also found that pi-sulfur interactions contribute significantly to the binding of our hit compounds to the FOXM1/DNA binding domain.
Conclusion: We suggest that the drug troglitazone, a known anti-diabetic agent, exerts strong binding interactions in the FOXM1/DNA binding domain, making this molecule the best drug candidate in our series to inhibit the transcriptional activity of this oncogenic protein.

67. Long Term Stability Study of Doxorubicin in Two Diluents

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Purpose: The purpose of this work is to study the stability of doxorubicin in two different aqueous solutions: the first containing 0.9% sodium chloride and the second 5% dextrose. The doxorubicin solutions were stored in two different types of containers: clear polypropylene and amber polypropylene bottles at two different temperatures: 5 and 25°C.
Methods: Two doxorubicin solutions (0.04 mg/mL) were prepared with aqueous solutions containing 0.9% sodium chloride and 5% dextrose respectively. Each solution was split in two; the first half was used to fill amber polypropylene bottles and the other one clear polypropylene bottles. Each type of bottles was divided into two groups for storage at room temperature (25°C) or under refrigeration (5°C). Samples were withdrawn from the solutions just after the preparation and on days 7, 14, 30, 45, 60, 75 and 90. These samples were then analyzed by high-performance liquid chromatography (HPLC) using a reverse phase column. A particles count, a visual inspection and a pH measurement were also done at each time point. Stability was defined as follows:

- preservation of at least 90.0% of the initial concentration of doxorubicin;
- number of particles per container
  - below 600 for size above 25µm and
  - 6000 for size above 10µm;
- pH variation lower than 1 unit.

Results: After 90 days, the mean concentration of the doxorubicin is at least 90% for both diluents, both temperatures and both containers which is the recommendation for the expiration date according to the Guidance for Industry "Drug Stability Guidelines". The maximum number of particles counted respects the USP 788 "Particulate Matter in Injection". The pH variation is less than 0.5 units for all the conditions and there is no change in the organoleptic properties.
Conclusion: Doxorubicin solutions have proven stable for at least 90 days.

Biomedical Sciences

68. Increased Heart Rate in Pregnant Mice is Associated with Changes in Sinoatrial Node Calcium Homeostasis

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Purpose: Pregnancy correlates with an elevation of the heart rate (HR) and an increased incidence of cardiac arrhythmias which can compromise mother and fetus health. The HR is controlled by the automaticity of the sinoatrial node (SAN) cells. The L-type calcium current (I_{CaL}) and the calcium homeostasis are among the major mechanisms responsible for the automaticity of the heart. The main goal of the study was to examine the contributions of intracellular calcium concentrations modulation in the accelerated HR seen during pregnancy.
Method: Adult female non-pregnant (NP, 2-3 months old), pregnant (P, 18-19 gestation days) and post-partum (PP, 1-2 days post-delivery) CD1 mice were used for all experiments. On isolated SAN cells, I_{CaL} was measured using voltage clamp technique whereas the effect of pregnancy on the sarcoplasmic reticulum (SR) Ca^{2+} release was measured as the spontaneous Ca^{2+} transients. Using electrical programmed stimulation (EPS), we determined whether pregnant mice have an increased tendency to develop arrhythmias.

Results: Measured I_{CaL} density increased from -5.3 ± 0.3 pA/pF in P mice to -7.5 ± 0.5 pA/pF in NP ($p<0.01$). Results also indicate that Ca^{2+} transients frequency was significantly higher in P mice. EPS revealed that P mice are more likely to suffer from supraventricular arrhythmias compared to NP. Our findings revealed that all of the reported modifications associated with pregnancy are rapidly reversible following delivery.
Conclusion: These findings suggest that an increase of I_{CaL} and calcium transients frequency can contribute to explain the increased SAN automaticity and elevated HR seen in pregnancy. In addition, the reversible electrical remodelling showed in PP mice suggest that SAN is dynamically regulated.
Acknowledgement: Valérie Long is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

69. Impact of Non-steroidal Anti-inflammatory Drugs on Bone Repair

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Purpose: Fracture repair starts with a localized inflammatory response during which immune and vascular precursor cells are attracted to the defect and bone marrow derived mesenchymal stem cells differentiate to repair the tissue. Non-steroidal anti-inflammatory (NSAIDs) drugs administered for pain relief following fracture also modify the local inflammatory response, although their mechanism of action in this respect is not known. The goal of this research is to use a mouse model of bone repair to identify the mechanism whereby administration of NSAIDs in the early phase of repair modifies the healing process.
Methods: Bone repair in skeletally mature Bl6 mice was quantified in 2mm cortical defects drilled in the femur. Mice received either a 5 mg/kg NSAID pellet (N=9) or a placebo pellet (N=10) implanted subcutaneously for continuous delivery for 14 days PO. The quantity and quality of bone and re-vascularization of the defect were quantified using micro CT. Comparisons were made by ANOVA at the 95% confidence level.
Results: In a standardized region spanning the defect, NSAID treated mice had significantly less bone than control mice treated with placebo, with fewer trabeculae that were less well connected and more porous. In contrast to the significant reduction in bone quantity and quality there were no detectable differences in the number, volume or connectivity of blood vessels in the NSAID treated mice.
Conclusion: Systemic treatment with NSAIDs during the inflammatory phase of bone repair impairs healing by inhibiting bone formation and/or turnover rather than affecting the re-vascularization of the repair tissue. The use of NSAIDs for pain management in patients, like the elderly, who are already at risk of complications in fracture healing should be re-considered.
Acknowledgements: JRGL is supported by a CONACYT scholarship and the Bone Engineering Labs are supported in part by FRQS-RSBO and the Jo Miller Orthopaedic Fund.

70. Validation of a New Strategy to Repair Jaw Bone Voids in the Presence of Anti-Resorptive Induced Osteonecrosis

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Purpose: Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is the presence of necrotic bone following tooth extraction in cancer patients who have received high doses of bisphosphonates to inhibit bone metastases. Patients with BRONJ require surgical resection of dead bone which leaves a large defect that will not heal spontaneously. Biocompatible cements like Norian Putty are commonly used as void fillers but they have a high failure rate. The goal of this study is to use a rat model to determine if a novel, bioplastic (PCL/c) scaffold is superior to Norian Putty as a void filler.
Methods: BRONJ will be induced in rats that have received cancer doses of zoledronate and dexamethasone and undergone mandibular tooth extraction. Surgical removal of dead tissue will generate large 3mm x 2mm x 3mm defects

surrounded by healthy bone. **Group 1** will receive Norian Putty in the left defect and PCL scaffold alone in the right defect. **Group 2** will receive Norian Putty in the left and PCL/c scaffold in the right defect. After 6 weeks, Bone repair will be evaluated after 6 weeks using micro-CT, and histology to determine cellular activity.

Results: Preliminary data on the capacity of the PCL/c scaffold to expedite cartilage repair in rabbit knee joints will be presented by Dr Hoemann's group at the 2017 ORS meeting. Bone repair was impaired after tooth extraction in rats with BRONJ (Jabbour et al 2014 Oral Oncol. v50). It is anticipated the PCL/c scaffold will be superior to Norian Putty in expediting repair after surgical removal of necrotic bone.

Conclusions: There is currently no effective treatment for BRONJ, leaving patients with chronic pain and disability. A successful outcome of our work could identify PCL/c as an effective scaffold to expedite bone regeneration and effective repair of large bone voids.

Acknowledgements: The Bone Engineering Labs are supported in part by FRQS-RSBO and the Jo Miller Orthopaedic Fund.

71. Enzymology by Terahertz Chemical Microscopy

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Purpose: Enzymes are important catalysts in the healthcare and manufacturing sectors. Protein engineering is a useful technique for optimizing their structure in order to e.g., increase the selectivity/efficiency of the reactions they catalyze. Normally, this process produces libraries containing thousands and sometimes millions of variants. Unfortunately, analyze such number of mutants is not a trivial task, as the identification of active variants among millions of possibilities quickly becomes exhaustive and inefficient. To address this challenge, we propose a new tool, named the Terahertz Chemical Microscope (TCM), that monitors changes of local chemical potential associated with e.g., chemical reactions. In addition, to screen those huge libraries of mutants on the basis of catalytic activity researchers are compelled to use substrate analogue or chromo-/fluorophore-modified substrates instead of the real substrates just to be able to follow the reaction that they want to optimize. Interestingly, TCM can detect any kind of chemical change, so a variety of real substrates can be used to select the active mutant from the library.

Methods: TCM and Nuclear Magnetic Resonance (NMR) are used to study enzyme kinetics.

Results: The combination of NMR time-course data with evolution curve analysis is established with *Pseudozyma antarctica* lipase B (CalB) catalyzed hydrolysis reaction. Measuring the single point THz amplitude by TCM was also performed for kinetics study.

Conclusion: This presentation will showcase our latest developments on using the TCM as a tool for monitoring the catalytic properties of a library of CalB mutants.

72. Secretions of an Intestinal Microbial Community Increase the Excitability of Vagal Afferent Neurons

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Purpose: The intestinal microbiota has recently been shown to have pronounced effects on the central nervous system (CNS). However, it is presently unknown how intestinal microbes signal to the CNS. There are two afferent neural pathways linking the intestine and the CNS: the spinal afferent pathway and the vagal afferent pathway. We have previously found that intestinal microbes decrease the excitability of spinal afferent neurons via the actions of serine proteases on protease activated receptors. The present work was conducted to determine whether the vagal afferent pathway was similarly affected.

Methods: Perforated patch clamp electrophysiology was used to measure the excitability of the cultured vagal afferent neurons, whose cell bodies lie in the nodose ganglia. Dissociated nodose ganglion neurons were cultured overnight either in normal media or media containing supernatant from an intestinal microbial community named microbial ecosystem therapeutics (MET-1). Nodose ganglion

neuronal excitability was assayed by measuring the threshold amount of current required to elicit an action potential, the rheobase.
Results: MET-1 supernatant concentration-dependently increased the excitability of nodose ganglion neurons by decreasing the rheobase. MET-1 supernatant increased neuronal excitability without affecting resting membrane potential or input resistance. The increase in excitability elicited by MET-1 supernatant was not prevented by a serine protease inhibitor or by blocking toll-like receptor 4 signaling.
Conclusion: In contrast to spinal afferent neurons, which are inhibited by MET-1 supernatant, vagal afferent neurons are excited. The mechanisms underlying the effects of MET-1 supernatant on vagal afferent neurons also appear to be distinct from those observed in our study of spinal afferent neurons. The increased excitability of vagal afferent neurons in response to microbial secretions suggest that the vagal pathway may be the predominant neural conduit allowing microbial modulation of CNS function.

73. Characterization of Anti-Ebola Virus Glycoprotein Monoclonal IgG and Specific IgY for Initial Assessment of Double Antibody Sandwich-ELISA

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Purpose: Ebola is a major public health problem and responsible for large scale fatalities during outbreaks. The need to develop a reliable diagnostic assay has never been more important. Our work aims to develop an enzyme-linked immunosorbent assay (ELISA) for the diagnosis of the Ebola virus glycoprotein (GP) for potential use in clinics and endemic locations. The GP presents in body fluids during early stages of infection. In this study we have expressed codon optimized GP in *baculovirus*. This GP antigen has been used for immunizing chicken and obtaining IgY antibodies from egg yolks. We have cultured three anti-GP hybridomas to obtain monoclonal IgG antibodies (MAbs). We will use these MAbs and IgY in combinations to develop a hetero-sandwich ELISA for an early detection.
Methods: The recombinant GP was expressed in *baculovirus*. This recombinant antigen was used to immunize chicken to develop IgY antibodies at every two weeks. IgY was separated by water soluble method. Anti-GP hybridomas are grown for MAb production. Through a series of lab techniques, SDS-PAGE, Western Blot and ELISA, both antibodies were characterized. Preliminary double antibody sandwich (DAS)-ELISAs, based on Mab/biontinylated-MAb and Mab/IgY, were examined for initial assessment and proof of concept.
Results: Successfully the recombinant GP antigen was expressed with high yield. Anti-GP MAbs and IgY recognized the recombinant protein validating the immunogenicity of the proteins as well as affinity. The ELISA assays were able to detect nano-concentrations in both formats.
Conclusion: As a pilot study, we have successfully produced recombinant GP, IgY and MAbs for this detection system. The use of inexpensive chicken IgY antibody combined with high affinity MAbs for GP screening has been done for the first time. The Ebola GP antigen immunoassay developed could be an efficient method of screening suspected individuals during a future Ebola outbreak.

74. Melittin from *Apis Meliffera* Bee Venom Reduces Production of Inflammatory Mediators in Macrophages

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Purpose: Melittin is a natural compound found in honey bee venom that has shown to suppress inflammation by preventing the activation of NF-kB, a transcription factor that regulates expression of multiple pro-inflammatory genes. Safety assessment is critical before *in vivo* applications due to the peptide's membrane lytic activity. Yet, there is minimal report on the therapeutic window of melittin despite its current usage in acupuncture for reducing joint and muscle inflammations. The purpose of this study is to examine the therapeutic efficacy of melittin at its maximum tolerated dose (MTD) in lipopolysaccharide (LPS)-stimulated

macrophage.
Methods: The cytotoxicity of melittin (0.5–5 μg/mL) on macrophages was measured by MTT viability assay to determine the LD_{50} as well as the MTD. The effect of melittin on production of nitric oxide (NO), pro-inflammatory cytokines (TNF-α and IL-1β) and COX-2 in LPS (10 ng/mL)-stimulated macrophages was measured by Griess assay and real-time PCR (using $2^{-\Delta\Delta Ct}$ method), respectively. The student *t*-test was used to analyze the significance between groups.
Results: In macrophages, the LD_{50} and MTD of melittin was 2.5 μg/mL and 1 μg/mL, respectively. Melittin (1 μg/mL) significantly reduced the NO production in LPS-stimulated macrophage from 11.44 to 4.98 μM. Moreover, melittin (1 μg/mL) reduced LPS-stimulated production of pro-inflammatory cytokine mRNA expression by 4.12-fold and 3.01-fold for TNF-a and IL-b, respectively. Expression of COX-2 was also reduced 2.53-fold.
Conclusion: Melittin treatment at MTD neutralizes various biomarkers of inflammation in macrophage. These results help establish efficacy and toxicity profile of melittin for determining the therapeutic dose before its application in treatment of inflammatory conditions.

75. Towards the Fabrication of a Bioinspired Synovial Fluid for Viscosuplementation Treatment in Osteoarthritis

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Purpose: Synovial joints exhibit excellent lubrication and wear protection throughout individual lifetime due to the synergistic interactions between collagen from cartilage and proteoglycans plus hyaluronic acid (HA) from bathing synovial fluid. However, when cartilage defects appear, amplified by mechanical erosion, accelerated catabolic processes induce irreversible cartilage degradation, as found in Osteoarthritis (OA). The catabolism leads to the excessive production of specific collagen and proteoglycans degrading enzymes resulting in loss of lubricating and protective properties. The aim of this project is to develop an injectable intra-articular polymeric solution able to provide a long-term protection of cartilage.
Methods: We synthesized a Bottle-Brush polymer (BB) mimicking the structure of the proteoglycan aggrecan using ATRP technique. The tribological properties of BB and HA and the ability to protect surfaces were characterized using a home-made tribometer and interferometry. LDH and MTT *in vitro* cytotoxicity assays of BB solutions were performed on chondrocytes, synoviocytes, and osteoblasts from human patient with advanced OA. The stability of BB polymer was assessed by Atomic Force Microscopy and Surface Forces Apparatus. Preliminary *in vivo* studies were carried out on mechanically-induced OA on rats. After treatment, cartilage elastic modulus was evaluated using indentation mapping.
Results: BB with HA exhibited physiological friction coefficient (μ~0.02) and no surface damage up to 15 atm. No significant toxicity was observed. BB polymer was able to sustain its lubrication and wear protection even after 3 months in saline conditions. *In vivo*, BB polymer was able to slow down the irreversible loss of cartilage mechanical properties in rat knees 1 month after injection (HA treatment: loss of 80±3% of cartilage modulus; HA + BB: loss of 20±11%).
Conclusion: We developed a potential fluid for OA joint viscosupplemention able to slow down the disease course.

76. The Effect of Antioxidant-rich Newfoundland Wildberry Extracts on Neuroinflammation

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Introduction: Neuroinflammation is observed in the pathophysiology of many neurodegenerative diseases. Microglial cells, the immune cells of the brain, are heavily involved in neuroinflammation. When activated, microglial cells release many proinflammatory mediators, for example cytokines or reactive oxygen or nitrogen species. Newfoundland wildberry extracts are rich in antioxidant polyphenols. These polyphenols can scavenge the free radical species created by

microglial cell activation. This may help to decrease the damage caused by chronic inflammation.
Methods: Wild blueberries and lingonberries native to Newfoundland and Labrador were collected from different locations. Microglial cells isolated from mouse brains were cultured and treated with α-synuclein or glutamate to induce an inflammatory reaction. Mixed neuronal/glial cultures cultivated from neonatal mice were also treated in the same manner. Extracts from the collected blueberries and lingonberries were used to treat the activated microglial cultures and mixed cultures to assess their ability to decrease cellular activation and death.
Results: Blueberry fruit and leaves were able to reduce the inflammatory response of activated microglial cells as determined by the amount of cell death and alterations in cell morphology. Cell counts from microglia did not show a statistically significant protective effect of lingonberry leaf or fruit extracts against activation by glutamate or α-synuclein. Cell counts from the mixed neuronal culture did show increased cell death due to activation by α-synuclein relative to control, but blueberry fruit and leaf extracts did not mitigate the cell death.
Conclusion: Overall, extracts from blueberry fruits and leaves decreased microglia-mediated neuroinflammation. The results of the cell culture experiments were inconclusive as to whether lingonberry extracts have neuroprotective benefits against cellular activation by α-synuclein and glutamate. More experiments are necessary to assess the neuroprotective effects of these berries as well as the potential mechanisms of protection.
Acknowledgement: Catherine Grandy is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

77. Active Immunization with an Anti-Glycosaminoglycan Monoclonal Antibody for Arresting Progression and Promoting Regression of Atherosclerotic Lesions

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Purpose: The pathogenesis of atherosclerosis is associated with the early retention of low-density lipoproteins (LDL) that are trapped in the extracellular matrix of the arterial intima by interaction with glycosaminoglycan side chains of proteoglycans, a key initiating step of atherosclerosis development. The present study aimed to characterize the anti-atherogenic properties of chP3R99 mAb against sulfated glycosaminoglycan side chains which, through the induction of an idiotypic antibody network, specifically interfere with intimal retention of LDL, at different stages of atherosclerosis.
Methods: The impact of chP3R99 on the onset and progression of atherosclerosis was assessed in apoE$^{-/-}$ mice using different *s.c.* immunization schedules at beginning (preventive) and during disease development (therapeutic).
Results: Immunization with chP3R99 (50 µg) reduced atherosclerotic lesions formation in preventive setting and arrested progression of established lesions at later stages of disease. This effect was associated to the generation of anti-idiotype antibodies able to mimic glycosamino-glycan epitopes, thereby inducing anti-anti-idiotype antibodies, which recognized these polysaccharides. Preventive immunization with chP3R99 reduced infiltration of inflammatory macrophages and CD4$^+$ lymphocytes stained areas by 80% and 75% respectively, at 28 weeks of age. This effect was accompanied by a 3-fold increase in the IL-10/iNOS ratio in abdominal arteries, with a reduction in circulating levels of IL-6 by 31% ($p<0.05$) in chP3R99-treated mice. Therapeutic immunization with a higher dose of chP3R99 mAb (200 µg) promoted regression of established atherosclerotic lesions in association with generation of higher levels of anti-glycosaminoglycan autologous antibodies.
Conclusion: Targeting vascular glycosaminoglycans through an anti-idiotypic antibody cascade produced by chP3R99 impaired subendothelial retention of LDL, attenuating the maladaptive inflammatory response. chP3R99-immunization against glycosaminoglycans is a promising novel strategy to intervene the atherogenic process at different stages.
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Mechanisms of Atherosclerosis Conference, March 2017, Cancun.

78. Pharmacological Characterization of the Functional Role of Calcium-Activated Potassium Channels in Platelets

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Purpose: In arteries, stimulation of endothelial cell small (SK_{Ca}) and intermediate (IK_{Ca}) conductance calcium-activated potassium channels provides a negative-feedback mechanism to limit agonist-induced vasoconstriction. Additionally, endothelial cell K_{Ca} channels in conjunction with nitric oxide (NO) mediate vasodilation in response to agonists and physical stimuli. Platelets, like endothelial cells, possess K_{Ca} channels and generate NO via endothelial nitric oxide synthase (eNOS). NO is known to limit platelet aggregation but the role of K_{Ca} channels in platelet function and NO-generation has not been explored. Our hypothesis was that activation of K_{Ca} channels would inhibit platelet aggregation and enhance platelet NO production. Our objective was to pharmacologically characterize SK_{Ca} and IK_{Ca} channel function in platelets, and investigate their role in platelet NO production.
Methods: Platelets were isolated from the blood of healthy volunteers and aggregometry performed in the presence of SK_{Ca} (CyPPA) and IK_{Ca} (SKA-31) channel activators. Dense granule secretion was measured by ATP chemiluminesence. DAF-FM flow cytometry was used to measure NO generation.
Results: CyPPA and SKA-31 inhibited collagen-induced aggregation in a concentration dependent manner. IK_{Ca} selective channel blocker reversed the anti-aggregatory effects of 10μM SKA-31 but not CyPPA. SK_{Ca} channel-selective blocker did not reverse the effect of either CyPPA or SKA-31. CyPPA and SKA-31 inhibited NO generation back to basal resting platelet levels. CyPPA and SKA-31 demonstrated similar inhibitory effects on platelet dense granule secretion, whereas only SKA-31 significantly inhibited alpha granule secretion.
Conclusions: Activation of SK_{Ca} and IK_{Ca} channels inhibits both platelet aggregation and platelet NO generation. Furthermore, the use of selective blockers suggest that IKCa is the dominant K_{Ca} channel within platelets. These data indicate that K_{Ca} channels may provide novel targets for therapeutics to inhibit platelet aggregation.

79. Adolescent Binge Drinking Causes Long-term Impairments to: Object Memory, Anxiety Regulation, and Cerebellar Based Motor Control in a Rodent Model

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Purpose: Binge drinking among adolescents is a growing public health concern. Although binge drinking can also be harmful to adults, the adolescent population is more susceptible to aberrant neurological changes as their brains are still undergoing significant development. The goal of this project is to provide firm evidence that there are changes occurring to cerebellar physiology, an area of the brain important for motor coordination and learning, after binge drinking.
Methods: Groups of adolescent (PND 26) and periadolescent (PND 30) male rats underwent a series of behavioral tests designed to assess memory, anxiety regulation, and motor function. Subjects were exposed to either ethanol or plain air through a vapour chamber apparatus for five consecutive days (two hours per day); achieving a blood ethanol concentration equivalent to 5-6 drinks in the treatment group. Western blot experiments to investigate the role of NF-kB, PKC-gamma, and caspases are ongoing.
Results: Results from the rota-rod, cage-hang, novel object recognition, light-dark box, and elevated plus maze testing showed significant differences between the groups which persisted for up to 60 days after treatment. Both age groups displayed a similar susceptibility to effects of ethanol exposure.
Conclusion: Behavioral testing shows that there are several potential long-term problems associated with adolescent binge-drinking. Differences on anxiety tests indicate a possible failure of behavioral inhibition in the treatment group leading to riskier behavior. There also seem to be impairments to motor coordination and object memory, which involve the cerebellar and hippocampal brain regions respectively. These experiments indicate the potential dangers of binge-drinking while the brain is still developing and indicate the need for future studies in this area.

80. Chronic Inflammation and Sympathetic Nerve Mediated Contractions in Rat Isolated Caudal Artery

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Background: Sympathetic nervous system (SNS) plays a critical role in control of vascular tone. It is conceivable that the ability of SNS to maintain vascular homeostasis is altered by various pathophysiological conditions including inflammation. Chronic inflammation contributes to changes in vascular function in variety of diseases. We tested the hypothesis that sympathetic neuro-effector response is altered as consequence of inflammation**.**
Methods: Sympathetic nerve-mediated contractions induced by electrical field stimulation were studied in isolated caudal arteries of rats treated with saline and Complete Freund's adjuvant (CFA) for 21 days. Expression of alpha-adrenoceptor subtypes were also evaluated using immunofluorescence.
Results: CFA treatment yielded significantly higher plasma levels of TNFα compared to saline, while blood pressure remained unchanged between the treatments. Electrical field stimulations (1.25 - 40 Hz) resulted in frequency-dependent contractions which were abolished by tetrodotoxin. Neurogenic contractions from CFA groups were significantly greater than saline at frequencies tested. While the presence of $alpha_1$-adrenoceptor antagonist (prazosin; 0.3 μM) significantly inhibited contractions at lower frequencies of stimulation (1.25 – 5 Hz) in CFA-treated arteries compared to controls, $alpha_2$-adrenoceptor antagonist (rauwolscine; 3.0 μM) had modest effects. Inhibition of neuronal reuptake by cocaine (1.0 μM) comparably enhanced field-stimulated responses in vessels of experimental and control animals. Immunofluorescence revealed differences in expression of $alpha_1$- and $alpha_2$-adrenoceptors in the endothelium of blood vessels as well as increased expression of ionized calcium adapter binding molecule-1 in the adventitia of blood vessels of CFA vs. saline.
Conclusion: Our findings support the view that neurogenic contractions in rat caudal arteries are enhanced in animals with inflammation, and were more sensitive to inhibition by $alpha_1$-adrenoceptor antagonist. Inflammation may also lead to the re-distribution of the expression of subtypes α-adrenoceptors on the endothelial cells, and this may account for the observed altered response.
Acknowledgement: This work was supported by RDC NL and NSERC-DG and has been presented in Canadian Hypertension Society conference in October 16 2016.

81. Modeling the Human KCNQ1 Potassium Ion Channel: Applying Computational Techniques to Cardiotoxicity Studies of Drugs

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Purpose: KCNQ1 (Kv7.1) is a voltage gated potassium ion channel and one of the most important cationic channels responsible for initiating the slow delayed rectifier (I_{Ks}) current in the atrial and ventricular myocytes. Mutations in KCNQ1 have been implicated in a wide range of cardiac diseases. Thus, studying the fine structural details of this important ion channel associated with the KCNE1 beta subunit will enhance our understanding of different physiologically and pathophysiologically observed phenomena. In addition, KCNQ1/KCNE1 protein is one of the targets for drug-induced QT prolongation and cardiotoxicity.
Methods: Our methodology combined homology modeling of KCNQ1 using a paddle chimera channel as a template for the transmembrane domains. The refined and validated final model of KCNQ1 tetramer was used for docking the KCNE1 protein and further exposed to MD simulation using NAMD package. This model was employed to study the mechanism of ion conduction as well as drug binding properties.
Results: In this study, we are reporting a complete homology model for the open state of KCNQ1/KCNE1 cardiac ion channel in accordance with the most recent experimental details. The current study provides valuable insights into the structure of this ion channel; mechanisms of ion conduction. Also, we were able to model the modes of binding of channel blockers using our model.

Conclusion: The KCNQ1 ion channel model was validated through docking of small molecule drugs. These findings make us believe that with further optimizations, the model will be capable of predicting the blocking ability of new drugs and hence their cardiotoxic effects. Further studies will focus on the effect of drug binding on ion conduction and passage through the pore of the channel.

82. Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ): Potential Therapeutic Target in HIV-1 Associated Brain Inflammation and Excitotoxicity

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Purpose: Despite the implementation of combination antiretroviral therapy for the treatment of HIV-1 infection, cognitive impairments remain prevalent due to persistent viral replication and associated brain inflammation. Primary cellular targets of HIV-1 in the brain are microglia and to a lesser extent astrocytes, which in response to infection release inflammatory markers, viral proteins (i.e., envelope gp120) and exhibit impaired glutamate uptake. As ample evidence suggests that neurocognitive impairments are attributed to brain inflammation, excitotoxicity and neuronal apoptosis, we investigated the potential role of peroxisome proliferator-activated receptor gamma (PPAR-γ), a transcriptional factor related to glucose and fatty acid metabolism, as a novel target in modulating pro-inflammatory cytokines and glutamate transporter expression.
Method: Primary cultures of rat astrocytes, and mixed cultures of rat astrocytes and microglia were exposed to $gp120_{ADA}$ or vehicle (control) and treated with PPAR- γ agonists (rosiglitazone or pioglitazone). In the mixed cultures, inflammatory and oxidative stress markers (TNF-alpha, IL-1beta, iNOS) were measured using qPCR, following a 3 hour treatment incubation. In primary cultures of astrocytes, glutamate transporter (GLT-1) was quantified by qPCR and immunoblotting, following a 6 hour treatment incubation.
Results: In the mixed cultures of rat astrocytes and microglia, $gp120_{ADA}$ exposure resulted in a significant elevation of inflammatory markers (TNF-alpha, IL-1beta, iNOS) which was significantly attenuated with the treatment of rosiglitazone or pioglitazone. In primary cultures of rat astrocytes, $gp120_{ADA}$ exposure resulted in a significant decrease in GLT-1 expression, which was significantly attenuated with rosiglitazone or pioglitazone treatment.
Conclusion: Our findings suggest that PPAR-γ activation can potentially exert neuroprotective effects by downregulating the expression of pro-inflammatory cytokines and increasing the expression of glutamate transporter in the context of HIV-associated brain inflammation. Further studies are needed, as targeting PPAR-γ signaling may provide a therapeutic option for preventing and treating HIV-associated brain inflammation and excitotoxicity.
Acknowledgement: Steven Choi is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

Drug Delivery and Pharmaceutical Technology

83. Preparation and Characterisation of Albumin Microcapsules using Interfacial Cross-linking Technique

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Purpose: The purpose of the present work is to prepare and characterise Albumin microcapsules using the interfacial cross-linking technique.
Method: Preparation of Albumin microcapsules were involved three steps: (i) preparation of water/oil emulsion of Albumin in chloroform-cyclohexane mixture. (ii) Cross-linking with terephtaloyl chloride, and (iii) washing/drying of the prepared microcapsules. Physical and chemical parameters affecting size, thickness, and properties of the microcapsules were studied by changing formulation conditions such as protein concentration, pH, and reaction time. Size and

morphology of the microcapsules were assessed using Mastersizer and SEM. Chemical modifications of the membrane were studied using FT-IR, and enzymatic degradation was assessed in pepsin and trypsin media.
Results: SEM examination of the microcapsules revealed spherical particles with rough surface and polydisperse size. Process parameters such as pH, buffer species, and reaction time seem to have a slight effect on the microcapsules size, but significant effect on their shape and morphology. At high pH, the membrane appears to be less porous and thicker as seen in SEM pictures. FT-IR analysis revealed that increasing the reaction time or pH of the aqueous solution resulted in a progressive increase of a peak at 1730cm^{-1}, which is assigned to ester function group. Other pics in FT-IR spectra of microcapsules were also affected. Finally, microcapsules prepared using high pH, or high reaction time, were more resistant to enzymatic degradation for both pepsin and trypsin.
Conclusion: Albumin microcapsules were successfully prepared using interfacial cross-linking technique. FT-IR technique was found to be a powerful tool for studying the effect of different process parameters on the formation of the microcapsules membrane.

84. Influence of Physical Factors on Quality Attribute of Scored Tablets

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Purpose: Scored tablets are designed with the purpose to adjust the administration dose, to facilitate swallowing, and to reduce the medication cost. It has been reported that splitting scored tablets will generate significant high weight variation of split fragments. Caution has to be taken when attempting to split CR tablets, or tablets that contains narrow therapeutic index drugs. The purpose of this work is to investigate the effect of some physical and technological parameters that might affect the accuracy, breaking ease, and other quality attribute of scored tablet.
Method: Tablets containing 1:1 blend of MCC and lactose, as well as MCC Emcompress, were prepared by direct compression using a rotary tablet press. Scored tablets of different shapes; round, oval, square, were compressed at low, medium and high compression force. The scored tablets were then slit in different parts by hand and using a tablet splitter. The whole tablets as well as the split tablet fragments were then evaluated for their friability, hardness, thickness, weight variation, breaking force, and disintegration test.
Results: Split tablet either by hand or splitter tablet generates higher weight variability as compared to the whole tablets. Split tablet by hand has shown a lower accuracy compared to tablet splitter. Tablets with deep line score are easy to break and show a good breaking accuracy compared to unscored tablets. Tablet hardness and table shape significantly affect the breaking accuracy, while tablet size has almost no affect.
Conclusion: Formulation composition, process manufacturing and other physical characteristics have greater influence on the accuracy of split tablet.

85. Formulation and Scale-up Manufacturing of Starch Based Spray Dried Microcapsules as Drug Delivery and Taste Masking Device

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Purpose: To formulate and manufacture spray dried microcapsules at both lab and pilot scale. The encapsulation of Acetaminophen and Caffeine in Starch based microcapsules was developed to aid in the controlled release, enhance stability and mask the bitter taste.
Method: Starch 10% (w/w) was first dispersed in a phosphate buffer pH 6.8 using a low shear mixer. A second solution was prepared by dissolving Xanthan gum (0.5-1 % w/w) or a mixture of Kollidon VA64 and Cekol 150 into deionised water. Solutions were vigorously stirred using a low shear mixer. Acetaminophen and Caffeine were added to each solution with different drug loading (10, 20 and 30% w/w). Finally, the two solutions were mixed and ready for spray drying. Solutions were spray dried in a mini spray dryer, 1.25 L batch size. The process

was then scaled-up at the pilot scale with 22.5 L batch size. The resulting microcapsules at both scales were collected and characterised.
Results: Spray drying process at the lab scale was successfully optimized and perfumed**.** The process was also successfully scaled-up at the pilot scale. Scanning Electron Microscopy imaging of the spray dried powder showed a spherical microcapsules with size ranging from 1 to 10 µm. The sphericity of microcapsules was better with increasing Xanthan gum concentration. In-Vitro release kinetics at pH=6.8 showed promising results.
Conclusion: Starch based microcapsules were formulated and successfully manufactured using spray drying at both lab and pilot scale. These microcapsules represent a promising device for the drug delivery of drugs as well as for taste masking.

86. Mathematical Prediction of Hydrophilic Chemotherapeutic Elution Kinetics from a Reservoir Polyurethane Intravaginal Ring Fabricated by Fused Deposition Modeling 3D Printing

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Purpose To design and fabricate a hydrophilic reservoir intravaginal ring (IVR) releasing hydroxycholoquine (HCQ, a representative compound of water-soluble chemotherapeutics) via fused deposition modeling (FDM) 3D printing technology and to develop a mathematical prediction model for *in vitro* HCQ elution from 3D printed IVRs.
Methods A mathematical prediction model of hydrophilic compound release from a reservoir IVR was established based on the Fick's First Law. The 35% water swellable hydrophilic polyurethane, HP-60D-35 (Lubrizol), was hot-melt extruded in HAAKE™ MiniLab II Micro Compounder into 1.65 mm diameter filament, which was further utilized to feed into a lab-developed Cartesian 3D printer to FDM 3D print reservoir IVR segments with different thickness of release controlling membrane (RCM). The ratios of outer diameter to inner diameter ranged from 1.12 to 2.61. The structure of printed segments and the thickness of RCM were investigated by scanning electron microscopy (SEM) imaging. *In vitro* release studies of 8 mg HCQ were performed using 5 mL of daily replenished vaginal simulant fluid (pH 4.2).
Results The two-week in vitro HCQ release kinetics from segments with various RCM agreed with the mathematical prediction model. RCM thickness was tunable through controlling the printing perimeter to provide daily zero-order release of HCQ ranging from 23.54 ± 5.90 µg/mL/day to 261.09 ± 6.58 µg/mL/day, which were above clinically achievable blood concentrations.
Conclusion The current study demonstrated the potential utility of FDM 3D printing to rapidly fabricate complex micro-structures for tunable and predictable delivery of chemotherapeutics from IVRs in a highly-controlled manner.
Acknowledgement: Lyndon Walker is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

87. Nanoparticle Formulation and Evaluation of Novel Synthetic Immune Stimulating Agents

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Purpose: Monophosphoryl lipid A (MPLA), an adjuvant, that have shown clinical success and led to the vaccine development research to focus on preparing lipid A chemical analogues. Alberta research chemicals have synthesized novel synthetic immune stimulating agents known as ARC-004, ARC-005, and 7 acyl lipid A, adjuvants which targets specific receptors present on dendritic cells (DCs) including NOD2, TLR2 and TLR4, respectively. An FDA approved drug delivery system PLGA poly-(D, L-lactic-co-glycolide) is used to deliver the therapeutic cargoes to immune cells. The aim of this study is to compare the immune stimulation by these compounds when presented to DCs in nanoparticle (NP) versus naked form.
Method: Adjuvant-antigen loaded NPs were prepared by emulsification solvent evaporation technique. We studied the expression of CD86, CD40 markers and MHC II molecule on DCs by flow cytometry. NP size, zeta potential and poly dispersity index were measured by zetasizer. Secretion of cytokines were measured by ELISA (Enzyme Linked Immunosorbent Assay). An LC-

MS spectrometry method was established for lipid A adjuvants' quantification (loading of ARC-004 and MPLA into PLGA Np). ARC-005 and OVA antigen were measured by BCA (Bicinchoninic acid assay).
Results: NP formulation was established with desired physicochemical characteristics. A quantification method was successfully achieved by LC- MS spectrometry to determine the loading of ARC-004 in both ARC-004 NP (335 ng/mg NP) and ARC-004/OVA NP (53 ng/mg NP). Loading of ARC-005 was measured via BCA (1.12 ug/mg NP), OVA have shown 25ug and 27ug loading in OVA NP and OVA/ARC-004 NP, respectively. When adjuvant loaded NPs were compared to their corresponding naked treatments having same amount of adjuvant, higher DC stimulation were clearly observed in NP conditions through DC marker expression as well as cytokine secretion (IFN gamma, IL-6).
Conclusion: The novel adjuvants stimulated the immune system, which was further enhanced in NP formulations.

88. Identification of Complementary Therapeutic Targets to Sensitize TRAIL-induced Apoptosis in Breast Cancer Cells via RNAi Screening

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Purpose: Resistance to apoptosis is one of the hallmarks of cancer. Inducing apoptosis has become a central therapeutic strategy employed in cancer management, which bears a strong potential to eradicate cancer cells. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in variety of cancer cells without affecting most normal cells, making it a promising agent for cancer therapy. However, TRAIL therapy is clinically ineffective due to resistance induction in malignant cells.
Method: To identify novel protein targets whose silencing sensitize breast cancer cells against TRAIL, a siRNA library against 446 human apoptosis-related proteins were screened in breast cancer cells; MDA-231 in presence or absence of TRAIL. A library of small cationic lipopolymers were synthesized, screened for siRNA delivery and most effect one was used as siRNA delivery agent.
Results: Based of the inhibition of cell growth of MDA-231 cells, sixteen siRNAs were found to sensitize TRAIL-induced cell death. Among them, novel and the most promising targets BCL2L12 and SOD1 were further evaluated. Silencing both targets sensitized TRAIL-induced death in MDA-231 cells and TRAIL-resistant MCF-7 cells. Importantly, TRAIL and siRNA silencing BCL2L12 had no effect in normal cells, human umbilical vein cells (HUVEC) and human bone marrow stem cell (hBMSC).
Conclusion: siRNAs targeting BCL2L12 and SOD1 were found to be novel regulators of TRAIL-induced cell death in breast cancer cells, providing a new approach for enhancing TRAIL therapy. The combination of TRAIL and siRNA targeting BCL2L12 can be the most effective synergistic pair for breast cancer therapy without affecting non-transformed cells.

89. Investigation of the Potential Application of Rhenium in Diagnostic X-Ray Imaging

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Purpose: Although commonly used to enhance the contrast of internal body structures, iodine-based X-ray contrast agents (XCAs) are known for triggering a number of adverse reactions. This study aimed: 1) to investigate the potential use of rhenium in the development of XCAs by comparing the contrast-to-noise ratio (CNR) of rhenium with iodine and 2) to explore another application of rhenium in diagnostic X-ray imaging by coating a catheter with a rhenium-doped nanofiber scaffold.
Methods: For the first objective, a rhenium-based XCA was prepared using ammonium perrhenate. The CNR of this formulation was compared with Omnipaque™ (300 mg I per mL, GE Healthcare). The XCAs were imaged by micro-computed

tomography (μCT) from 50 to 120 kVp and planar X-ray imaging from 120 to 220 kVp. The effect of utilizing coppers attenuators on the CNR was also assessed. For the second objective, a nanofiber scaffold was produced by electrospinning a solution of a rhenium complex and 45 kDa polycaprolactone in chloroform/methanol. The scaffold's morphology was studied by scanning electron microscopy. The scaffold was then melted onto a catheter and imaged by μCT.

Results: This study demonstrated that rhenium displays a greater CNR than iodine at >120 kVp. Additionally, the CNR of rhenium improves significantly with the use >0.6 mm of copper. This material contributes to reduce the number of photons in the X-ray beam, and therefore, the dose from the X-rays. Furthermore, the catheter coated with the rhenium-doped nanofiber scaffold exhibited improved contrast in a radiographic image.

Conclusion: Rhenium exhibits a kVp-dependent superiority in CNR which can be exploited to minimize the dose to the patient in diagnostic X-ray imaging. We showed that rhenium-doped nanofiber scaffolds can be incorporated onto catheters to turn them radiopaque. This will allow for *in vivo* visualization of catheters when they are placed inside the body.

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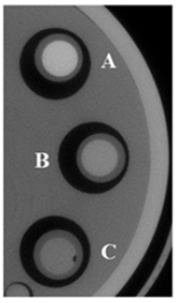
Acknowledgement: Jovan Gill is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.



X-Ray Attenuation Properties of Rhenium. Axial slice acquired through μCT showing the contrast enhancement capabilities of a rhenium-based XCA with a concentration of rhenium of (A) 200 mM, (B) 100 mM, and (C) 50 mM

90. Novel β-cyclodextrin Cationic Lipids as Efficient and Safe Delivery Systems for Poorly Soluble Chemotherapeutic Agent for the Treatment of Melanoma

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Purpose: Novel β-cyclodextrin (βCD)-modified gemini-surfactant drug delivery systems [βCDg] were developed and characterized. The βCDg was designed to combine the solubilizing capacity of the βCD and the cell-penetrating ability of the gemini-surfactant. Melphalan (Mel) was selected as a model for a poorly soluble drug. Melphalan, currently used in melanoma therapy, requires the use of an organic co-solvent for solubilization leading to poor stability and toxicity.

Methods: *In vitro* evaluations were performed in monolayer, three-dimensional spheroids and melphalan-resistant melanoma cell lines. 1D/2D ROESY-NMR methods were employed to investigate the βCDg\Mel complex. Flow injection analysis-tandem mass spectrometric (FIA-MS/MS) methods were developed and validated to assess the solubilizing capacity of the βCDg. The safety of the βCDg was evaluated by conducting acute toxicity evaluation in Sprague-Dawley rats.

Results: *In vitro* evaluations in melanoma cell lines showed that the βCDg-Mel complexes induced significantly higher cell death compared to drug-alone (2 – 3 folds decrease in melphalan IC50). βCDg did not alter the cellular death pathway triggered by melphalan and caused no intrinsic toxicity. 1D/2D ROESY-NMR results indicated that melphalan was included within the βCD inner cavity of the βCDg and form stable inclusion complex at 2:1 βCDg:Mel molar ratio. FIA-MS/MS results showed a significant increase in the solubility of Mel without the need for co-solvent (over three-fold increase in aqueous solubility of melphalan) at 2:1 molar ratio. The acute toxicity study (histopathological and hematological evaluations) indicated that βCDg was well-tolerated without significant adverse effects and no degenerative lesions were observed in all treatment groups (35,

100 and 250 mg/kg doses).
Conclusion: These findings demonstrate the applicability of the βCDg delivery system as a safe and efficient alternative to the currently approved Mel formulation. *In vivo* toxicity study will allow us to design controlled pharmacokinetic studies, and compare efficiency to the naked Mel in xenograft model.

91. Second Generation Molecular Sieving Coatings for Improved Treatment of Leukemia using Asparaginase

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Purpose: Acute Lymphoblastic Leukaemia is a disease of the bone marrow and the most common type of leukaemia in children (80% of all cases). While treatable, it remains a life-threatening condition, in part due to a component of the chemotherapy; L-asparaginase (ASNase). PEGylation of ASNase reduces immune response but correspondingly decreases enzymatic activity vital for chemotherapeutic action, so a more sophisticated polymer-protein conjugate is required – modulating polymer density over the surface of the protein to create a molecular sieving effect.
Method: Building on preliminary work showing that a molecular-sieving approach is more effective than conventional PEGylation. we have firstly improved the synthetic protocol to produce ATRP protein macroinitators with multiple initiating sites, and then secondly produced a library of protein-block copolymers, using monomers of PEG methacrylate of different weights, to produce conjugates with polymer density close to and away from the surface of the protein. This creates a volume near to the protein where substrate can be processed, affording high enzymatic activity, without compromising the protective layer of PEG that shields the enzyme from detection and attack by the immune system. Enzymatic activity and protein viability were tested *in vitro* and *in vivo*.
Results: By improving the protocol we were able to maintain protein viability and activity to higher degrees after functionalisation, maintaining enzyme activity of 90+% in conjugates, compared to unfunctionalised protein. The library of block copolymer conjugates produced shows that it is possible to tune the molecular sieving effect to optimise shielding against anti-ASNase antibodies whilst maintaining high levels of enzymatic activity.
Conclusion: These next-generation therapeutics combine the potent activity of ASNase, with little to none of the immune response side effects. This advanced molecular design is also applicable to several classes of protein-based treatment.

92. Development of Rituximab- Immuno-mixed Micelles for Active Targeting of B-cell Lymphoma

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Purpose: The aim of this study was to prepare stable mixed micelles composed of CD20 antibody, rituximab, conjugated poly(ethylene glycol)- 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (PEG-DSPE) and PEG-poly(ε-caprolactone) (PEG-PCL) and assess the effectiveness of this approach in enhancing the specific interaction of mixed micelles with target cells overexpressing CD20.
Methods: Rituximab tagged Cy5.5 NHS was coupled to NHS-PEG-DSPE Phospholipid micelles on their surface. The Cy5.5 tagged Rituximab micelles were than incubated with PEG-PCL micelles of different PCL molecular weights or PEG-poly(α-benzyl carboxylate-ε-caprolactone) (PEG-PBCL) having conjugated Cy3 at their PCL or PBCL end. The Immuno- mixed micelles were then tested for their size, polydispersity, dynamic and kinetic stability using dynamic light scattering (DLS). Flowcytometry was used to follow the association of plain versus antibody modified mixed micelles with CD20 over expressing KG-15 cells as compared with CD20 negative SUP-M2 cells using fluorescence emission wavelengths at 570 and 707 nm, for Cy3 and Cy5.5, respectively.
Results: Among different formulations under study (Table 1), mixed micelles prepared from PEG-DSPE/PEG_{114}-$PBCL_{22}$- $PPCL_4$ were found to be the most stable ones kinetically, while PEG-DSPE/ PEO_{114}-PCL_{15}-$PPCL_4$/ micelles were the least stable ones. Flowcytometry data following cells positive for cy3 showed higher association of anti-CD20 micelles with KG-15cells compared to plain micelles and CD20 negative cells (SUP-M2).
Conclusion: The results proved the successful formation of mixed rituximab micelles through incubation of two micellar populations (PEG-DSPE

and PEG-PCL or PEG-PBCL) for all formulations under study. The results also pointed to the effectiveness of mixed immune micelles modified on their surface with rituximab in enhancing their association with CD20 overexpressing cells.

Table 1.

Sample	M_n	Z diameter ± SD	PDI± SD	CMC±SD(µg/mL)
PEG_{114}-PCL_{22}-$PPCL_4$	7800	50.4±3.4	0.263±0.003	2.18±0.1
PEG_{114}-PCL_{15}-$PPCL_4$	6500	57.4±0.6	0.215±0.003	5.13±0.65
PEG_{114}-$PBCL_{22}$-$PPCL_4$	9960	71.8±0.4	0.127±0.019	2.19±0.41
NHS-PEG-DSPE	3400	23.3±2.1	0.368±0.1	45.0.7±4.8
NHS-PEG-DSPE/PEG_{114}-PCL_{22}-$PPCL_4$	-	84.4±2.9	0.399±0.017	18.24±2.5
NHS-PEG-DSPE/PEG_{114}-PCL_{15}-$PPCL_4$	-	78.2±0.6	0.268±0.003	74.9±3.7
NHS-PEG-DSPE/PEG_{114}-$PBCL_{22}$-$PPCL_4$	-	93.1±3.6	0.242±0.02	12.35±3.1

93. Copolymer Composition Influences Nanoparticle Properties and Subsequent Drug Penetration into Intact and Impaired Skin

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Purpose: Nanoparticles (NP) are used in dermopharmacy to modulate the transport of drugs into the skin. The understanding of the influence of NP physico-chemical properties on skin penetration is however a prerequisite to create efficient nanovectors. The aim of this study is therefore to determine 1) the impact of copolymer composition on the characteristics of cholecalciferol-loaded NP and 2) the impact of these NP on cholecalciferol penetration into intact and impaired skin.
Method: Poly(lactic acid)-Poly(ethylene glycol) (PLA-*b*-PEG) copolymers were synthesized by ring opening polymerization. The length of PEG was varied (no PEG, 1, 2, 5 and 10 kDa) to obtain polymers of various hydrophilicity. Cholecalciferol-loaded NP (7% $w/w_{polymer}$) of identical size were then prepared from these copolymers by flash nanoprecipitation. Manufacturing yield, particle size, zeta potential (ZP) and amount of PEG on the surface were measured as a function of polymer composition. Comparative skin penetration studies were eventually performed on intact and impaired pig skin in Franz diffusion cells.
Results: By adapting the flash nanoprecipitation process, NP with a controlled size of 100 nm were obtained regardless of polymer PEG content. The ZP and the amount of PEG on the surface varied respectively from -33 to -3mV and from 0 to 48 ethylene oxide units per nm^2 of surface when polymer PEG content increased. Noteworthy, a significant fraction of free polymer, was observed in NP suspensions made of highly pegylated copolymers. Polymer composition also influences drug penetration into both intact and impaired skin. The highest cholecalciferol absorption in skin was found after treatments with hydrophobic NP (PLA NP) or on the opposite with NP having the most hydrophilic surfaces (PLA-*b*-PEG5000 or PLA-*b*-PEG10000 NP).
Conclusion: NP characteristics and skin penetration of cholecalciferol were both found dependent on polymer composition. Further experiments are ongoing to determine the underlying mechanisms of skin penetration.

94. Delivery and Biodistribution of Traceable Polymeric Micellar Diclofenac in Healthy Rats

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Purpose: The nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with elevated risk of cardiovascular (CV) events. Previous studies have suggested that high exposure of heart and kidneys may exacerbate the risk. We hypothesized that altered biodistribution of cardiotoxic diclofenac

(DF) away from relevant organs reduces CV risks. We, therefore, encapsulated diclofenac ethyl ester (DFEE) in fluorescently-tagged polymeric micelles and studied the pharmacokinetics and biodistribution of DF in healthy Sprague Dawley rats.

Methods: DFEE was encapsulated in traceable polymeric-micelles (DFEE-TM) based on methoxypoly(ethylene oxide)-block-poly(ε-caprolactone)(PEO_{5000}-*b*-PCL_{3000})-propargyl attached to Cy5.5 from the propargyl end. The micelles were characterized for their particle size distribution, morphology, and encapsulation efficiency. Single dose DF pharmacokinetics and tissue distribution were studied following oral, intravenous, and intraperitoneal administration of DFEE-TM or free DF (n=3). Excised organs were fluorescent imaged.

Results: DFEE-TM showed spherical morphology with an average size of 45.1±0.06 nm and an entrapment efficiency of 84.7%±3.9. The 24 h DF concentration was significantly higher in blood following iv administration of DFEE-TM as compared with free DF (2.1±0.6 μg/ml vs below detection), but, significantly lower in other tissues (heart, 0.8±0.2 vs 1.4±0.2 μg/g; kidneys, 1.2±1.2 vs 4.5±1.7 μg/g; liver, 1.3±0.6 vs 2.5±0.7 μg/g; spleen, 2.8±0.4 vs 5.0±1.7 μg/g). The oral dosing of DFEE-TM resulted in reduced DF bioavailability while its intraperitoneal doses were completely bioavailable. Near-infrared fluorescence images showed micellar carrier tissue accumulations in-line with those achieved for DF using HPLC.

Conclusions: DF delivery by PEO_{5000}-*b*-PCL_{3000} micelles has high intraperitoneal bioavailability and provides improved biodistribution of DF including prolonged systemic circulation and reduced accumulation in the cardiac tissue. The micelles show strong potential for a cardiac-safe delivery of diclofenac. HA and SA were supported by the ministries of higher education of Oman and Libya, respectively. Research was supported from a self-Funded grant from University of Alberta and NSERC.

95. Biodistribution, Effectiveness and Cardiac Toxicity of Traceable Polymeric Micellar Diclofenac in Adjuvant Arthritis

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Purpose: A fluorescently-tagged polymeric micellar formulation of diclofenac (DF), a model of cardiovascular (CV) toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) was developed by encapsulating DF ethyl ester (DFEE) to test the hypothesis that a reduced heart exposure to NSAIDs improves CV safety profile. CYP 450 mediated metabolites of arachidonic acid (ArA) concentrations were measured as markers of cardiotoxicity.

Methods: DFEE was encapsulated in traceable (Cy5.5 labeled) methoxypoly(ethylene oxide)-block-poly(ε-caprolactone) (PEO_{5000}-*b*-PCL_{3000}) micelles (DFEE-TM). Biodistribution, efficacy and cardiotoxicity of DFEE-TM and free DF were compared following multiple *ip* administration to adjuvant arthritic (AA) rats of 10 mg/kg/day DF equivalent for 7 days (n=6).

Results: Both free DF and DFEE-TM resulted in a rapid reduction in the signs of AA. Moreover, histopathological assessment showed that the DFEE-TM as well as the free DF ameliorated the inflammatory cell infiltration observed in AA heart and kidney. DF was found in significantly lower concentrations in the heart following DFEE-TM administration as compared with free drug (1.5±0.6 vs 2.8±0.6 μg/g). Comparable concentrations were found in the kidneys, liver, and spleen between the two formulations. DFEE-TM yielded significantly lower cardiotoxic metabolic profile of ArA in various tissues when compared to free DF (e.g., 20 HETE in heart 0.20±0.01 vs 0.48±0.07 μg/g; in plasma, 41±6 vs 143±18 μg/L, respectively).

Conclusions: DF delivery by PEO_{5000}-*b*-PCL_{3000} micelles encapsulating DFEE provide improved biodistribution of DF in rats with AA including a reduced accumulation in the cardiac tissue. Moreover, micelles provided an effective therapy with reduced extent of the imbalance in eicosanoids of ArA that are attributed to DF, and are known to be associated with increased CV risks. HA and SA were supported by the ministries of higher education of Oman and Libya, respectively. Research was supported from a Self-Funded grant from University

of Alberta and NSERC.

96. Transdermal Delivery of Bone-Seeking Calcitonin Conjugates in a Rat Model of Bone Repair

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Purpose: Tibial stress fractures (shin splints) are common in athletes and military personnel. Currently, there are no definitive treatments other than rest and pain management, which may interfere with bone repair. In contrast, calcitonin is a peptide hormone which naturally signals osteoclast cells to stop resorbing bone matrix and indirectly signal osteoblasts to complete bone formation. There is also clinical evidence that calcitonin has analgesic effects. Unfortunately, calcitonin receptors are widely distributed in many non-skeletal tissues and the competitive uptake reduces drug availability for bone. Accordingly, we developed bisphosphonate (BP) conjugates of salmon calcitonin (sCT) and investigated their impact upon bone repair, in comparison to placebo, diclofenac and native sCT, through both subcutaneous (*s.c.*) and transdermal (*t.d.*) routes of administration.
Methods: 1 mm diameter bony defect was created surgically in tibial shin bone of rats, and bone repair studied following treatment with calcitonin analogues prepared in phosphate buffer or pluronic lecithin organogel (PLO) cream base. Pharmacokinetic evaluations of radiolabeled sCT analogues confirmed the ability of PLO gel to facilitate the transdermal delivery to superficial shin bone of either sCT or sCT-PEG-BP conjugates. We used micro-computed tomography to measure bone formation, and scans were performed at baseline, 2 and 5 weeks after daily treatments with PLO gel alone, diclofenac, sCT or sCT-PEG-BP. Histological evaluation of bone repair was also undertaken.
Results: Bone repair occurred in all treatments, including diclofenac, sCT, or sCT-PEG-BP. However, bone repair was significantly more rapid and complete after *s.c.* or *t.d.* administration of sCT-PEG-BP formulations compared to the other treatments.
Conclusion: Our results indicate the *t.d.* delivery of sCT-PEG-BP conjugates is an effective drug delivery strategy to increase bone availability of sCT and permit bone repair, and potentially serve as an alternative to NSAIDs for the analgesic therapy and augmentative treatment of shin splints.

97. Development of Polymeric Micellar DACHPt with Potential for Targeted Drug Delivery in Cancer

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Purpose: The parent compound of Oxaliplatin, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt) is a potent chemotherapeutic agent with wide spectrum of anticancer activity, lower side effects and no cross-resistance with Cisplatin in many Cisplatin-resistant cancers. However, its clinical use is restricted by its poor water solubility and some non-target side effects such as acute dysesthesias and cumulative peripheral distal neurotoxicity. Incorporation of DACHPt in Polymeric micelles may lead to changes in physiochemical properties as well as the pharmacokinetics profile of the drug, so may assist in reducing its unfavorable side effects and improve its bioavailability.
Method: Poly (ethylene oxide)-b-poly-(α-carboxylate-€-caprolactone) (PEO-b-PCCL) diblock copolymer was synthesized. Then, DACHPt was reacted with the polymer to form polymer-metal complex. The complex was dialyzed in water to prepare DACHPt loaded micelles. The average size of the micelles was measured using DLS. Complexed levels of DACHPt and platinum *in vitro* release from micelles was measured compared to the free drug using ICP-MS.
Results: High drug loading was achieved reaching 50.2% w/w (N=3) with a mean diameter size of 56 nm using PEO-b-PCCL micelles. The release profile of DACHPt from DACHPt from micelles was sustained and prolonged (only 53.6% of DACHPt was released after 120 h. n=3) compared to the free drug (96.5 % of the drug was released after 7.5 h).
Conclusion: PEO-b-PCCL diblock copolymer was used to prepare DACHPt loaded polymeric micelles. Micelles showed a high level of DACHPt loading and a slow release. Prepared micellar formulation of DACHPt has a high potential for targeted Pt delivery.
Acknowledgement: AA was funded by Umm Al-

Qura University, Mecca, Saudi Arabia. This research was supported by grants from NSERC.

98. Dual-location Dual-stimuli-responsive Polylactide (PLA) Based Triblock Copolymer and its Nanoassemblies

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Purpose: Stimuli responsive degradation (SRD) has been explored in constructing a variety of novel nanostructured materials for biomedical applications. Our research group has been working on various designs and architectures of smart block copolymers exhibiting dual stimuli-responsive degradation (DSRD) properties at dual locations. Here we report a novel polylactide-based dual acidic pH/glutathione responsive amphiphilic triblock copolymer having a ketal (K) linkage in the centre of PLA blocks and two disulfide (SS) linkages at the junctions of PLA and PEG-functionalized polymethacrylate (POEGMA) blocks, thus POEGMA-SS-PLA-K-PLA-SS-POEOMA (SS-K-TP).
Methods: PLA block has been synthesized using ROP (ring opening polymerization) of lactide monomer in the presence of disulfide bearing initiator which was further coupled with the acetal diamine using EDC coupling. The resultant polymer was then copolymerized with the PEG-functionalized polymethacrylate using direct atom transfer radical polymerization (ATRP).
Results: In aqueous solution, the resultant amphiphilic block copolymer self-assembles to form colloidally-stable micellar aggregates consisting of ketal linkages in PLA hydrophobic cores and disulfide linkages at the interfaces of PLA cores and POEGMA coronas. These micelles are responsive to two types of stimuli: acidic pH and glutathione. The ketal linkages are cleaved to corresponding alcohols and acetone in response to acidic pH (6.5-6.9) whereas disulfides are cleaved to thiols in the presence of glutathione, shedding POEGMA coronas from PLA cores.
Conclusion: The degradation kinetics thus help us improved our understanding on unfolding of micelles and cleavage efficiency of the linkages with respect to specific stimuli and thus this designed system can be employed as the effective drug delivery nanocarriers in the drug delivery systems.

99. Barium Sulfate as Tracer for *in vivo* Formulation Follow-up and its Impact on Tablet Properties

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Purpose: The challenging development of targeted formulations administrated orally is related to the properties of bioactive agents (i.e. solubility, permeability, etc.) and the gastro-intestinal (**GI**) physiology (pH, motility, etc.). Despite the extensive *in vitro* characterization during formulation development, the *in vivo* behavior could markedly differ, leading to a loss of efficacy of targeted dosage forms. Scintigraphy, the current technic offering *in vivo* traceability of oral dosage forms, has some limitations related to use of radioisotopes. The present study investigates the possibility to formulate solid dosage forms containing barium sulfate (**BS**) and follow their behavior *in vivo* using radiography.
Methods: Various polymeric matrices containing BS were evaluated in simulated gastric and intestinal fluids. Tablets were obtained by direct compression and the BS loading varied between 10% and 40%. Mechanical performance of tablets in dry (hardness measurements) and swollen form (weight and size) were followed *in vitro*. Selected formulations were administrated to beagle dogs and radiographs were taken at specific time points. Structural characterization (IR, DRX and SEM) was also performed.
Results: It was found that selected cellulose grades generate stable matrices loaded with BS. They were used as templates to evaluate targeted formulations loaded with various bioactive agents (i.e. therapeutic enzymes). SEM of tablets showed a BS accumulation as an outer layer, preventing H-association between macromolecules. Preliminary radiology results have shown that the tablets containing BS can be easily followed during their transit throughout the GI tract offering excellent contrast *in vivo* while maintaining their mechanical (Fig.1).
Conclusion: The study showed the potential use of BS as a radiographic marker for *in vivo* traceability of targeted formulations orally administrated. The

good *in vivo* - *in vitro* correlation supports the possible replacement of scintigraphy by safe and less costly BS-containing formulations.

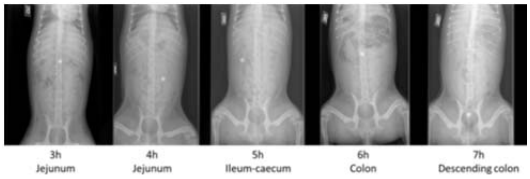

Fig.1: Ventrodorsal radiographs of a monolithic tablet carrying BS during GI transit in beagle dog (administration at 0h)

100. Tumor Microenvironment-modulating Nanoparticles for Enhancing Radiation Therapy of Castration-resistant Prostate Cancer

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Purpose: Tumor hypoxia is a poor prognostic factor in a number of malignancies such as prostate cancer (PCa). Clinically relevant hypoxic levels are detected in 30-90% PCa with oxygen concentrations below that required for effective radiotherapy (RT). Recently our laboratory has devised pharmaceutically acceptable bioreactive manganese dioxide nanoparticles (MDNPs) and demonstrated their ability to modulate tumor microenvironment by reacting with H_2O_2 and protons and producing O_2 in hypoxic tumors. The aim of this study is to investigate the effect of the MDNP treatment on enhancing radiation efficacy in castration-resistant prostate cancer models.
Methods: Polymer-lipid based MDNPs were prepared using an emulsion-ultrasonication method developed in our previous work. The effect of combination of MDNPs and RT on tumor growth delay and host survival was investigated. PCa tumor-bearing mice were treated intravenously with MDNPs or saline as a control. Four hour post injection, radiation dose was given at the site of the tumor. For fractionation-RT, animals were treated with 2 Gy radiation dose 4 h post IV administration of MDNPs (1 mM) in 5 consecutive days. Tumor growth was monitored every 2 days by measuring tumor size using a caliper.
Results: The combination of MDNP treatment with single dose 10 Gy RT improved survival rate of the prostate tumor-bearing mice for up to 53 days, which was about 3.3-fold enhancement in the mean survival rate compared to saline plus RT treatment (30 days). The combination of MDNP treatment with fractionation radiation achieved 20% curative treatment.
Conclusion: This work has demonstrated, in a preclinical prostate tumor model that the combination of MDNPs with radiation therapy is a promising treatment approach for castration-resistant prostate cancer.

101. Impact of Nanoparticle Mechanical Properties on their Diffusion across Agarose Gels

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Purpose: The diffusion of a nanoparticle across biological tissues is one of the most challenging obstacle nanomedicine is facing for its use in anti-cancer therapy or other therapeutic applications. A logical approach is to design a nanoparticle with properties to facilitate its diffusion through these complex matrices. The deformability (Young's modulus) of the nanoparticle is one of the less studied properties while recent *in vivo* studies strongly suggest that deformability may facilitate the nanoparticle escape through pores or vesicles. The objective of this study is to demonstrate that deformability of nanoparticle can help its diffusion across a dense media of agarose.
Methodology: Commercially available hard nanoparticles (polystyrene and gold-PEG nanoparticles) were compared to soft N-isopropylacrylamide microgels from a standardized synthesis. Different hydrodynamic diameters were used for hard (45 -220 nm) and soft (80 – 300 nm) nanoparticles as determined by dynamic light scattering using back scattering mode (173^o).

Diffusion of nanoparticles in agarose gels at different concentrations (0.05% - 2%) was measured using differential dynamic microscopy (DDM). The DDM setup consist of a microscope equipped with a high-speed acquisition camera.
Results: At low agarose concentrations (0.05%), we observed that the diffusion follows the Stokes-Einstein law for both hard and soft nanoparticles. At this concentration, the agarose solution is liquid and as expected, no difference in diffusion was observed in regard of their mechanical properties. However, with increasing agarose concentration, soft nanoparticles showed a different diffusion behavior, diffusing faster than the hard nanoparticles. The difference was especially marked at 0.5% agarose gel.
Conclusions: While no difference was observed in liquid solutions of agarose, there is a strong evidence that the deformability of the nanoparticle influenced their diffusion within the agarose gel. In regard with our results, mechanical property should be considered as an important parameter in the nanoparticle design.

102. Segmented Intravaginal Ring Co-delivering Hydroxychloroquine and siRNA-encapsulated Nanoparticles for Preventing HIV Infection

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Purpose: Microbicides are an excellent alternative to condoms to help reduce transmission of human immunodeficiency virus (HIV). An intravaginal ring (IVR) would be a suitable platform that can provide controlled delivery of drugs within the female genital tract with high patient acceptance. We propose to develop a segmented combination IVR whereby one-half of the IVR will be loaded with hydroxychloroquine (HCQ), an immuno-modulatory drug that can induce a quiescent state in T cells and the other half will be coated with a pH-responsive film for the rapid release of small interfering RNA (siRNA)-encapsulated nanoparticles (siRNA-NP) triggered by an increase in vaginal pH due to the presence of seminal fluid as a novel strategy for protecting against HIV infection. The siRNA will knockdown the CCR5 gene expression, a co-receptor involved in HIV-1 infection.
Methods: Solid lipid nanoparticle made of glyceryl monosterate and L-α-phosphatidylcholine was used to encapsulate siRNA using double emulsion method (siRNA-NP), mixed with a pH-sensitive polymer (Eudragit L100) and used to coat a matrix-type IVR segment, fabricated by injection molding from polyurethane. HCQ was loaded in a reservoir-type IVR segment. A release study was performed for each segment. The biocompatibility of the IVR segments was evaluated on cervicovaginal epithelial cell lines VK2/E6E7 and ECT1/E6E7 and on vaginal flora *Lactobacillus crispatus* and *jensenii*.
Results: IVR segments coated with a pH-sensitive polymer rapidly released siRNA-NP at pH8.2 but not at pH 4.2. The reservoir-type IVR segment containing HCQ continuously released drug up to 21 days with a near zero-order release profile (R^2 value =0.99). Cytotoxicity evaluation of IVR segments on vaginal cells and lactobacilli demonstrated no changes in cell viability or bacteria growth, respectively.
Conclusion: We describe an IVR system capable of controlled release of HCQ and also siRNA-NP at high pH and non-cytotoxic towards lactobacilli and vaginal/cervical epithelial cells.

103. A Novel Nanoparticle Formulation for Enhanced Delivery of Milrinone for Cardiac Applications

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Purpose: Cardiovascular diseases (CVDs) are the global causes of mortality. To effectively treat CVDs, various drugs have been evaluated. Milrinone (MRN) is a cardiac inotrope drug, widely used as MRN-Lactate for the treatment of congestive heart failure. However, it has limitations of low bioavailability and efficacy. In this study, we have demonstrated the development of a MRN-

nanoparticle formulation using human serum albumin nanoparticles (HSA-NPs) for enhanced delivery of MRN. HSA-NPs are ideal drug carriers owing to properties such as target specificity, biodegradability, non-immunogenicity and high bioavailability. The MRN-HSA-NP formulation has been prepared and optimized for complete in-vitro characterization.

Methods: The MRN-HSA-NPs were prepared by the ethanol desolvation technique. Key parameters like HSA and MRN concentration, pH of preparative solution, ethanol volume, glutaraldehyde content and reaction time, were optimized. Molecular docking studies were conducted to study the nature of binding between MRN and HSA. Further, MRN-HSA binding was confirmed by enzyme mediated drug release studies. Finally, MRN-HSA-NPs uptake and biocompatibility was evaluated using H9c2 cells.

Results: The MRN-HSA-NPs size was 154.2±5.8 nm and zeta potential was 29.5±2.9 mV. The drug encapsulation efficiency was 41.1±1.7 %. MRN was predicted to bind in the hydrophobic cavity (Sudlow's Site 1) present on the HSA molecule. MRN was released from the MRN-HSA-NPs in the presence of trypsin, pepsin, proteinase K, protease and cathepsin D as shown in Table 1. The MRN-HSA-NPs were taken up by the H9c2 cells in the presence of various nanoparticle concentrations. Also, the cell viability in the presence of MRN-HSA-NPs was significantly higher ($P<0.001$) than that due to free MRN-Lactate drug (58.8±5.7% vs 18.8±4.9%), when evaluated over 48 hours.

Conclusions: From the above studies, it may be concluded that the MRN-HSA-NP formulation is more biocompatible than the free drug MRN-Lactate and may be safely used for cardiac delivery applications.

Table 1. Drug encapsulation efficiencies at various HSA/MRN concentrations.

HSA/MRN (µM/µM)	Encapsulation efficiency (%)
1:1	86.9±13.8
1:5	23.4±4.9
1:10	30.2±5.9
1:15	41.8±1.7

104. A Novel Graphene Oxide-tetracycline Formulation for Targeting Antibiotic-resistant Bacteria

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Purpose: The overuse of antibiotics has resulted in bacterial resistance, causing a major threat to global health. The aim of this study is to design, synthesize and characterize graphene oxide (GO) nano-sheets as novel drug delivery vehicles for common antibiotics such as tetracycline (TET), to slow down the process of antibiotic resistance.

Method: GO nano-sheets were prepared by following the Modified Hummer's method. Key parameters such as oxidation time, antibiotic mixing and sonication time were optimized. GO was characterized using UV-visible spectrophotometry, Fourier transform infrared and Raman spectroscopy. The amount of bounded TET was increased by modifying sonication time (producing GO nano-flakes), mixing time and solvent used. This mixture was then used in bacterial work to quantify the minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) of two bacteria strains, *Escherichia coli* and *Staphylococcus aureus*, and comparing this against both the TET and GO in isolation. The MIC was quantified using the UV spectrum, whereas MBC was quantified using bacteria plating. The parameters considered were concentration and time intervals from the moment of bacterial exposure to the antibiotic.

Results: Sonication over 1 hour resulted in GO reduction. We found the optimum sonication time to be 20 minutes, followed by 1 hour of stirring. Characterization of TET bounded to GO (GOT) was done using UV-vis. This confirmed that the GO was not being reduced to graphene. Quantifying the amount of bounded antibiotic was made difficult by the nano-flakes. Bacterial studies were also conducted showing that GOT and TET alone were comparable in MIC and MBC, while using a lower concentration.

Conclusion: The binding of antibiotics with the use of GO has been optimized. The effect on MIC and MBC is still under experimentation.

105. Development of Radiolabeled Antibody-Targeted Gemini Nanoparticles for Melanoma

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Purpose: Malignant melanoma is the sixth most commonly diagnosed cancer and the most lethal form of skin cancer with limited treatment options. Nanotechnology is of one of the promising options to improve diagnosis and therapeutic outcome for melanoma. Gemini surfactants self-assemble into nanoparticles which can be decorated with monoclonal antibodies to selectively deliver alpha emitters (theoretically the most cytotoxic form of radiation) to tumour cells that overexpress the targeted surface antigens.
Methods: A pair of radiopharmaceuticals was selected to conjugate to the nanoparticles using the same chelating moiety: Actinium 225 as the alpha emitter and Indium 111 as radiotracer for SPECT/CT monitoring. The serum stability of the Indium 111 nanoparticles was measured by radiometry. Cellular binding and uptake in melanoma cell line were determined by flow cytometry and radiometry. Pharmacokinetic parameters were measured in nude mice.
Results: Indium 111-labeled nanoparticles show high stability in biological environment; less than 10% radionuclide dissociated in a week.
Cellular targeting studies showed that the cellular uptake of the antibody labeled nanoparticles is a specific uptake, as it could be blocked by pretreatment with free antibody. The non-targeted nanoparticles attached non-specifically to the cell surface and showed no difference from the targeted nanoparticles in binding. However, cellular internalization of the targeted nanoparticles was significantly higher than the non-targeted nanoparticles. Pharmacokinetic study in nude mice revealed a significant difference ($P < 0.05$) in the AUC, Vd and Cl_s of the antibody targeted 111In nanoparticles compared to the non-targeted nanoparticles. The half-lives were not significantly different.
Conclusion: The antibody-conjugated gemini nanoparticles are more effective in binding to the cell surface, internalizing, and penetrating the nucleus of melanoma cells than the non-targeted nanoparticles. They also show promising pharmacokinetic properties when used on nude mice models.
Acknowledgement: Kayla Wharton is the recipient of a 2017 GSK/CSPS National Undergraduate Student Research Program Award.

106. Aptamer-based Liposomes to Improve the Specificity, the Drug Loading and the Release

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Purpose: Liposome technology is limited by poor encapsulation of the drugs. In this study, we demonstrate that drug-binding aptamers can actively load drugs into liposomes (Figure 1). We designed a series of DNA aptamer sequences specific to doxorubicin, displaying multiple binding sites and various binding affinities. The impact on drug loading, drug release and therapeutic efficacy was investigated. This proof-of-concept was first demonstrated with doxorubicin and applied to tobramycin, a hydrophilic drug suffering from low encapsulation into liposomes.
Methods: Cationic liposomes (DOTAP/cholesterol/ $DSPE\text{-}PEG_{2000}$) were incubated with designed aptamers at various charge ratios to form lipoplexes. Aptamer encapsulation efficiency and stability was determined by fluorescence assay. The drug loading capacity was determined by fluorescence after incubation at various aptamer/drug ratios, for doxorubicin and tobramycin-Cy5, respectively. In *vitro* release of doxorubicin from lipoplexes was studied at pH 5 and pH 7.4. *In vitro* therapeutic efficiency was evaluated by cell viability measurements on HeLa cells.
Results: The aptamers displayed an affinity constants ranging from 68 to 380 nM for doxorubicin, and 1.15 µM for tobramycin, showing the specificity of the aptamer sequence to the drug. The binding ability of aptamers was preserved when incorporated into cationic liposomes, and resulted in a 16 and 6-fold improvement of encapsulation

efficiency of doxorubicin and tobramycin, respectively, as compared to classical liposomes (Figure 1). In addition, kinetic release profiles and cytotoxicity assay on HeLa cells demonstrated that the therapeutic efficacy of liposomal doxorubicin could be controlled by the aptamer's structure.

Conclusion: In this study, we demonstrate that drug specific aptamers can be designed to improve the loading capacity of liposomes and enhance their therapeutic efficiency.

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