### ORAL ABSTRACTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eetezadi, Sina</td>
<td>Nanomedicine, quo vadis?</td>
<td>3</td>
</tr>
<tr>
<td>Palmay, Lesley</td>
<td>Hospital-wide Roll-out of Antimicrobial Stewardship: A Stepped-Wedge Randomized Controlled Trial</td>
<td>4</td>
</tr>
<tr>
<td>Razumienko, Eva</td>
<td>Radiolabeled Antibodies as a Tool for Imaging Biomarkers in Breast Cancer</td>
<td>5</td>
</tr>
<tr>
<td>Sharma, Amy M.</td>
<td>One Drug, Two Reactive Metabolites, Two Adverse Drug Reactions: A Story of Nevirapine-Induced Idiosyncratic Toxicities</td>
<td>6</td>
</tr>
<tr>
<td>Shivnaraine, Rabindra V.</td>
<td>Allosteric Modulation of Muscarinic Cholinergic Receptor</td>
<td>7</td>
</tr>
</tbody>
</table>

### POSTER ABSTRACTS

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albaum, Jordan</td>
<td>Glucocorticoid-induced osteoporosis management among seniors</td>
<td>8</td>
</tr>
<tr>
<td>Banerjee, Nilasha</td>
<td>Estrone-3-sulphate, a potential novel ligand for targeting breast cancers</td>
<td>9</td>
</tr>
<tr>
<td>Chan, Brian Chun-Fai</td>
<td>The economic burden of chronic ulcers in Ontario</td>
<td>10</td>
</tr>
<tr>
<td>Chen, Kuan Huan Gary</td>
<td>Mechanistic study of release of ionized drugs through cationic polyacrylate membranes</td>
<td>11</td>
</tr>
<tr>
<td>Chiu, Catherine</td>
<td>SAPCD2 regulates mitotic spindle orientation in epithelial morphogenesis and asymmetric cell division</td>
<td>12</td>
</tr>
<tr>
<td>Cui, Lei</td>
<td>Radiosensitizing Effect of Gold Nanoparticles(AuNPs) under Oxia and Hypoxia</td>
<td>13</td>
</tr>
<tr>
<td>Dou, Yannan</td>
<td>Heat-activated Thermosensitive Liposomal Cisplatin (HTLC) Results in Effective Growth Delay of Cervical Carcinoma in Mice</td>
<td>14</td>
</tr>
<tr>
<td>Gholizadeh, Shervin</td>
<td>Extensive Transduction of the Central Nervous System Following A Single Intra-cerebroventricular Injection of Adeno-associated Viral Vectors in Neonatal and Juvenile Mice</td>
<td>15</td>
</tr>
<tr>
<td>Hamandi, Bassem</td>
<td>Incidence and burden of infectious-related hospitalizations among solid-organ transplant recipients: a single centre study</td>
<td>16</td>
</tr>
<tr>
<td>Ismail, Ethar</td>
<td>Generic drug pricing and generic drug substitution in private drug plans in Canada</td>
<td>17</td>
</tr>
<tr>
<td>Kabboul, Nader Nizam</td>
<td>A Mixed-Treatment Comparison (MTC) of Cardiac Rehabilitation Treatment Strategies in Patients with Coronary Heart Disease (CHD)</td>
<td>18</td>
</tr>
<tr>
<td>Kwan, Stephanie</td>
<td>The influence of drug-polymer interactions on the drug</td>
<td>19</td>
</tr>
<tr>
<td>Name</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Lam, Karen</td>
<td>release rate through polymer films</td>
<td>20</td>
</tr>
<tr>
<td>Latif, Maya</td>
<td>Regulation of cisplatin-induced programmed cell death in vivo</td>
<td>21</td>
</tr>
<tr>
<td>Li, Jason</td>
<td>Micro-porous membranes for sustained insulin delivery</td>
<td>22</td>
</tr>
<tr>
<td>Lip, Ho</td>
<td>Glyoxal and methylglyoxal: autoxidation from dihydroxyacetone and polyphenol cytoprotective antioxidant mechanisms</td>
<td>23</td>
</tr>
<tr>
<td>Lughtu-Pe, Jamie Anne</td>
<td>Controlled Release as a Strategy to Prevent Solution-Mediated Phase Transformation in Amorphous Solid Dispersions</td>
<td>24</td>
</tr>
<tr>
<td>Moscou, Kathy Dean</td>
<td>Safety and Equity: Transnational actors' influence on pharmacovigilance in low and middle-income countries: A case study of pharmacovigilance policy in Kenya and Brazil</td>
<td>25</td>
</tr>
<tr>
<td>Ngo Ndjock Mbong, Ghislaine</td>
<td>Enhanced cytotoxicity of trastuzumab modified with metal chelating polymers (MCPs) labeled to high specific activity with Indium-111 on HER2 expressing breast cancer cells.</td>
<td>26</td>
</tr>
<tr>
<td>Parikh, Chaitya</td>
<td>Testing Educational Materials about Immunizations in a Paediatric Setting</td>
<td>27</td>
</tr>
<tr>
<td>Sage, Andrew</td>
<td>Improving Lung Transplant Outcomes with Nanoscale Diagnostics</td>
<td>28</td>
</tr>
<tr>
<td>Shan, Dan</td>
<td>RGD-conjugated Nanoparticles for Targeted Anti-metastasis of Integrin αvβ3 -overexpressing Breast Cancer Cells</td>
<td>29</td>
</tr>
<tr>
<td>Shere, Mahvash</td>
<td>The Role of Social Media in Recruiting for Clinical Trials in Pregnancy</td>
<td>30</td>
</tr>
<tr>
<td>Smart, Sarah</td>
<td>Cluster Randomized Trial of the Effect of a Prenatal Education Module on Pain Management during Routine Infant Immunizations</td>
<td>31</td>
</tr>
<tr>
<td>Smy, Laura</td>
<td>The Safety of Inhaled Corticosteroid Use in Pregnancy and Childhood</td>
<td>32</td>
</tr>
<tr>
<td>Son, Ilkbae</td>
<td>Volumetric Characterization of Tri-n-acetylglucosamine binding to lysozyme</td>
<td>33</td>
</tr>
<tr>
<td>Thao, Mallory</td>
<td>Cost-Effectiveness Analysis of Screening for Lung Cancer Using Low Dose Computed Tomography: The Toronto Experience</td>
<td>34</td>
</tr>
<tr>
<td>Winterbottom, Melissa</td>
<td>Informed Consent for Chiropractic Care: The Patient’s Experience</td>
<td>35</td>
</tr>
<tr>
<td>Xuan, Ingrid</td>
<td>Modelling Autism Spectrum Disorder Through Maternal Immune Activation in Mice</td>
<td>36</td>
</tr>
<tr>
<td>Zamiri, Bita</td>
<td>The structure of the disease-associated r(GGGGCC)n repeat from the C9ORF72 gene</td>
<td>37</td>
</tr>
</tbody>
</table>
Nanomedicine, quo vadis?
Sina Eetezadi and Christine Allen

The promise of nano drug delivery systems in oncology was to enhance the therapeutic effect of anti-cancer drugs and to keep their toxicity to a minimum. Yet, to date, the achievement of significant reductions in tumor burden, recurrence and metastatic progression remains an elusive goal. The design of nanomedicines with translational potential does not only require improvements in the therapeutic index, but also a thorough consideration of the complexity of the system proposed in the light of its additional costs in comparison to standard treatment. This is particularly relevant in the current debate about the benefit of active targeting approaches using biological agents. Therefore, carefully designed, relatively simple nanoformulations with defined clinical application should be again given priority in R&D. In this talk, it is shown how high drug-loading and long-term stability of a micellar nanoformulation of doxorubicin was achieved by careful formulation design. Furthermore, it is demonstrated how the use of 3D cell culture can serve as a viable platform for the evaluation of nanomedicines in conditions which more closely reflect the in vivo tumor microenvironment and allow high-throughput in vitro efficacy assessment.
Hospital-wide Roll-out of Antimicrobial Stewardship: A Stepped-Wedge Randomized Controlled Trial

Lesley Palmay, BSc, BSc.Phm; Marion Elligsen, BSc.Phm; Sandra Walker, BSc, BSc.Phm, PharmD, FCSHP; Scott Walker, BSc.Phm, MSc.Phm, FCSHP; Thomas Einarson, BSc.Pharm, PhD; Andrew Simor, MD, FCCP; Samira Mubareka MD; Anita Rachlis, MD; Vanessa Allen, MD; Ruxandra Pinto, PhD; Nick Daneman, MD, MSc, FRCPC

The objective of this study was to determine the impact of an antimicrobial stewardship intervention in six non-intensive care services. A review of all patients reaching their 3rd or 10th day of broad-spectrum antibiotic therapy was conducted at Sunnybrook Health Sciences Centre using a stepped–wedge randomized design. The primary outcome was broad-spectrum antimicrobial utilization in the intervention period compared to the control period. Secondary outcomes included nosocomial Clostridium difficile infections, antimicrobial resistance, antimicrobial costs, length of stay and mortality. Over the two year period, 2733 antimicrobial orders were evaluated, and a change to optimize therapy was recommended in 1287 (47%). These recommendations were widely appreciated by the attending services (80% acceptance rate). Broad-spectrum antimicrobial use was reduced by 21% among those patients qualifying for the intervention (p = 0.004); however, there was no significant impact on overall broad-spectrum antimicrobial utilization (-1.2%, p =0.9). Similarly, no significant reductions in nosocomial Clostridium difficile infections or antimicrobial resistance rates were achieved. The reduced impact in the non-intensive care services appears to relate to shorter lengths of stays on these services, such that the impacts of the intervention were only realized on the post-discharge outpatient phase of treatment. Careful consideration of the targeted patient population is, therefore, warranted when planning and implementing antimicrobial stewardship interventions. Future work of the antimicrobial stewardship program may focus on intervening earlier in the course of therapy.
Radiolabeled Antibodies as a Tool for Imaging Biomarkers in Breast Cancer

Razumienko, Eva J; Dryden, Lindsay and Reilly, Raymond M.

Purpose: Our objective was to construct novel bispecific immunoconjugates (bsICs) capable of binding both HER2 and EGFR and labeled with 111In for SPECT imaging of both receptors, and for potential future imaging of HER2/EGFR heterodimerization in breast cancer.

Methods: bsICs were composed of trastuzumab Fab fragments recognizing HER2 linked to the Epidermal Growth Factor (EGF). Fab fragments were produced by digestion of trastuzumab IgG with papain and then modified with sulfo-SMCC analogues harbouring polyethyleneglycol (PEG) spacers to introduce maleimide groups for cross-linking to EGF that was thiolated with Traut’s reagent. bsICs were derivatized with DTPA for labeling with 111In. The ability to independently bind HER2 or EGFR was determined in competition assays using 111In-bsICs against unlabeled Fab or EGF on cells expressing HER2, EGFR or both receptors. The tumour and normal tissue uptake of the bsICs was examined in mice bearing BC xenografts that expressed HER2, EGFR or both receptors, and blocking with Fab and EGF performed to determine specificity for the target receptors.

Results: Conjugation of Fab to EGF was confirmed by HPLC and bsICs were labeled with 111In with high radiochemical purity. 111In-bsICs localized into tumours expressing HER2, EGFR, with the best uptake observed at 48 hrs p.i. Uptake was decreased when tumors were pretreated with excess Fab in HER2+ xenografts and EGF in EGFR+ xenografts, confirming that the probes could bind specifically in vivo.

Conclusion: 111In-bsICs composed of trastuzumab Fab and EGF bound specifically to EGFR and HER2 both in vitro and in vivo. These 111In-bsICs may be useful for imaging EGFR-HER2 heterodimerization in BC.
One Drug, Two Reactive Metabolites, Two Adverse Drug Reactions: A Story of Nevirapine-Induced Idiosyncratic Toxicities

Sharma, Amy M.¹; Uetrecht, Jack¹
1. Pharmacy, University of Toronto, Toronto, ON, Canada.

Nevirapine (NVP) treatment is associated with significant idiosyncratic immune-mediated skin rash and hepatotoxicity in humans. NVP causes a very similar rash in female Brown Norway rats, and we have shown that 12-hydroxylation of NVP is required to induce the rash. In this study we studied the further metabolism and covalent binding of NVP in the rat model and in human skin. Immune activation via IL-1β in the skin was also examined. An anti-NVP antibody was produced and used in immunoblotting studies to detect covalent binding of NVP, 12-OH-NVP, or NVP-sulfate to skin and liver proteins from rodents and humans. In vitro incubations of NVP or metabolites with hepatic microsomes or skin cytosolic fractions from humans, mice, or rats were also performed. The ability of SULT 1A1*1 to metabolize NVP was studied and cutaneous IL-1β levels were examined by ELISA. Covalent binding was observed in the epidermis of NVP or 12-OH-NVP-treated rats. Major modified bands appeared between 40K-60K. Depletion of PAPs decreased blood levels of NVP-sulfate but did not prevent rash or covalent binding in skin. Topical administration of 1-phenyl-1-hexanol (sulfotransferase inhibitor) prevented rash and covalent binding where applied, and also prevented covalent binding of 12-OH to cytosolic skin fractions and SULT 1A1*1 in vitro. IL-1β levels were significantly upregulated in skin of rats with a rash as well as skin isolates. In contrast to covalent binding in the liver, which involves direct oxidation to a quinone methide, the reactive metabolite that covalently binds in the skin is a sulfate. The sulfate responsible for the rash is formed in the skin of rats and in human skin incubations but not in mice which develop no rash. Further work is being done to confirm the role of IL-1β in NVP-induced skin rash. Funding: Canadian Institutes of Health Research.
Allosteric Modulation of Muscarinic Cholinergic Receptor

Rabindra V. Shivnaraine¹, Yuchong Li², Huiqiao Ji¹, Claudiu Gradinaru² and James W. Wells¹.
¹Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, ²Department of Physics, University of Toronto, Toronto.

G protein-coupled receptors (GPCRs), such as the M₂ muscarinic receptor, constitute the largest family of signalling proteins and are targeted by at least 50% of drugs. Despite this therapeutic importance, the mechanism by which the receptor decodes an agonist as full or partial is not understood. Varying views emerge, in part, because the oligomeric status and their role is uncertain. The M₂ muscarinic cholinergic receptor has been shown to form a complex that consists of four receptors, a tetramer. As a result of this arrangement, the receptors can interact with each other to exhibit various functional consequences. At each receptor, there are two functionally linked ligand binding sites: an orthosteric site recognised by antagonists such as N-[³H]methylscopolamine (NMS); and an allosteric site recognised by modulators such as gallamine and tacrine. The functional linkage between the sites and the interaction among four receptors allows for two general types of interaction. Interaction can occur between the two sites at one receptor (intra-molecular) and between the two sites of two adjacent receptors (inter-molecular). These two types of interaction emerge as a serpentine effect of gallamine and a biphasic effect of tacrine on the binding of NMS. To examine the nature of these interactions we have employed various biochemical, mechanistic and biophysical approaches. Using purified monomers of the receptors, we have shown that complex effects of tetramers are lost. Kinetic based mechanistic modelling that accounts for the inter- and intra-molecular interactions can mimic various aspects of the complicated effects. Biophysical studies employing Resonance Energy transfer (RET) has provided a direct measurement of both inter- and intra- molecular conformational changes of the receptor. Here, we positioned a fluorescent dye (FlAsH) within the allosteric site and a fluorophore (mCherry) at the N-terminus of the receptor. These studies illustrate the functional role of oligomers in the mechanism of action of allosteric ligands to mediate their proposed role as a novel therapeutic strategy.
Glucocorticoid-induced osteoporosis management among seniors

Albaum JM1, Lévesque LE2, Liu YY1, Cadarette SM1
1 Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada
2 Queen’s University, Kingston, Ontario, Canada

Background: Osteoporosis is a major public health concern that results in considerable fracture-related morbidity and shortened survival. Glucocorticoid (GC) therapy is the most common cause of secondary osteoporosis. Since 1996, Canadian practice guidelines have recommended that all patients starting chronic oral GC therapy (≥ 3 months) receive bone mineral density (BMD) testing and/or osteoporosis pharmacotherapy. We sought to examine trends in GC-induced osteoporosis management over time.

Methods: We identified all chronic oral GC users (≥ 2 oral GC dispensed and ≥ 450 mg prednisone equivalent over a 6-month period) aged 66 or more years in Ontario using healthcare utilization data, 1997-2011. Osteoporosis management (BMD test and/or osteoporosis pharmacotherapy) within 6 months of starting chronic oral GC therapy was examined by sex and year. Results were summarized using descriptive statistics.

Results: We identified 75,621 male (mean age=74.7, SD=6.2) and 97,966 female (mean age=75.2, SD=6.4) patients on chronic oral GC therapy between 1997 and 2011. Over eighty percent had exposure ≥ 675 mg in the 6-month window used to define chronic GC exposure. GC-induced osteoporosis management increased steadily from 7% (men) and 20% (women) in 1997, to a high of 21% (men) and 46% (women) in 2007, with little change from 2007 through to 2011.

Conclusions: Rates of GC-induced osteoporosis management improved significantly over time in both sexes yet remain low, particularly among men. This represents a missed opportunity for fracture prevention among patients requiring prolonged GC therapy.
Estrone-3-sulphate, a potential novel ligand for targeting breast cancers

Nilasha Banerjee, Naomi Miller, Christine Allen and Reina Bendayan

This study investigates the potential of estrone-3-sulphate (E3S) as a ligand for targeting Organic Anion Transporting Polypeptides (OATP), a family of membrane-associated transport proteins, for detection of hormone dependent breast cancers. E3S, a circulating inactive plasma estrogen, is an OATP substrate and a predominant source of tumour estradiol in post-menopausal patients. It has been reported that some of the OATP isoforms (e.g. OATP1A2, OATP2B1) are over expressed (up to ten times) in breast cancer tissues as compared to surrounding normal tissues. This suggests that the two-three times higher E3S concentration reported in malignant tissues, as compared to the surrounding normal tissues, could be due to higher expression and/or increased functional activity of OATPs detected in breast tumour tissues. We recently demonstrated in vitro that expression of some of the OATPs (i.e. OATP1A2, OATP1B1, OATP1B3, OATP1C1, OATP2B1, OATP3A1 and OATP4A1) were either exclusive, similar or significantly higher (p<0.001) in breast cancer cells as compared to normalized breast epithelial cells (MCF10A). Thus to assess in vivo, the potential of E3S as a ligand for targeting OATPs in breast cancers, distribution of exogenous E3S (administered intravenously) was determined in murine models of hormone-dependent (MCF-7) and independent (MDA-MB-231) breast cancers. The highest levels of tumour uptake were observed at 6h post injection (p.i) with significant difference between the level in MCF-7 (13.9 ± 3.1 %ID/g) and MDA-MB-231 (10.4 ± 1.1 %ID/g). The highest tumour-to-blood ratios (MCF-7: 7.4±1.2; MDA-MB-231: 9.1±2.1) were observed at 48 p.i., and highest tumour-to-muscle ratios (MCF-7: 10.7±1.5; MDA-MB-231: 3.8±0.7) were observed at 6h p.i. Analogous to total tumour uptake, ex vivo tumour cell uptake at 2h p.i. was 6 fold higher in MCF-7 compared to MDA-MB-231 tumour cells. Blocking studies, conducted by pre-administration of 100-fold excess E3S, resulted in significantly lower tumour uptake in both xenograft models, suggesting the involvement of an active carrier mediated process. Immunohistochemical analysis detected OATP1A2 in tumour sections from both xenografts, with significantly higher expression in the MCF-7 xenografts. Overall, the higher tumour uptake and tumour-to-muscle ratio, alongside the higher expression of OATP1A2, in the MCF-7 xenograft model suggest the potential of E3S to serve as a novel ligand for targeting hormone dependent breast cancers. To the best of our knowledge, this is the first report documenting the in vivo distribution of exogenous E3S, in models of hormone dependent and independent breast cancers.
The economic burden of chronic ulcers in Ontario

Chan BC, Mittmann N, Cadarette SM, Wodchis WP, Krahn MD

Background: The economic burden of chronic ulcers to the health care system is substantial with total costs per year estimated to upwards of $4.2 billion for pressure ulcers (PU) in New York State, $33 million for leg ulcers (LU) in Italy and $1.5 million for diabetic foot ulcer (DFU) in Sweden. There have been no studies that have analyzed the cost of chronic ulcers (PU, LU and DFU) at a population level. The primary aim of this study is to determine the direct attributable costs of chronic ulcers in the province of Ontario.

Methods: Using provincial healthcare utilization data health at ICES, a matched cohort study will be conducted. The direct attributable costs will be calculated for individuals older than 18 years of age in Ontario, in comparison to a matched non chronic ulcer cohort. The direct attributable costs of chronic ulcers will be determined from this difference. Matching will be conducted using propensity score methods. In addition to costs associated with chronic ulcers, we will determine patterns of healthcare utilization and drug utilization pre and post ulcer diagnosis.
Mechanistic study of release of ionized drugs through cationic polyacrylate membranes

Kuan Chen, Michael Chu, Dan Shan, Stephanie Kwan, and Xiao Yu Wu
Leslie Dan Faculty of Pharmacy, University of Toronto

Purpose: Eudragit RL 30 D (RL) and Eudragit RS 30 D (RS) are cationic polyacrylates widely used for pharmaceutical coating. Due to difference in contents of quaternary ammonium groups (QAGs), RL and RS have high and low permeabilities, respectively, and can blended at varying ratios to manipulate drug release rate. For non-ionic drugs, release from RL/RS is primarily diffusion controlled. However, release of ionic drugs may be controlled by ion exchange or polymer swelling due to interactions with cationic QAGs. This study is thus aimed to investigate the underlying release mechanisms of ionic drugs through RL/RS membranes by determining membrane swelling, permeability, and drug partition between polymers and medium.

Methods: Free films of varying RL:RS ratios were prepared from Eudragit RL/RS 30 D with 20% dibutyl sebacate as plasticizer. Theophylline, ibuprofen Na, and diltiazem HCl were used as non-ionic, anionic, and cationic model drugs, respectively. Swelling kinetics of membranes with respect to changes in polymer compositions and type of drugs was determined by measuring weight gain of the membranes over time. FTIR spectra of polymers saturated with drug solutions were obtained using attenuated total reflectance FTIR.

Results: Permeability of ibuprofen Na through the membranes was much lower than theophylline and diltiazem HCl, while its partition in polymer was substantially higher. Permeabilities and partition coefficients of ibuprofen Na and diltiazem HCl were significantly different from values predicted by diffusion model. For theophylline and diltiazem HCl, membrane swelling increased with increasing QAGs, however; different swelling degree and kinetics were observed for ibuprofen. Swelling in ibuprofen Na solutions was greatly inhibited as amount of QAGs in membranes increased, a strong indication that drug-polymer interaction is due to ionic complexation of the anionic ibuprofen with cationic QAGs. Swelling initially increased, but gradually decreased as ibuprofen Na diffused into the membranes, neutralized QAGs, and dehydrated the membranes. FTIR analysis showed drug-polymer interactions were not due to covalent or hydrogen bonding.

Conclusion: Drug charge has significant effects on the swelling and permeability of RL/RL membranes, and drug partition. In particular, ibuprofen Na formed ionic complexes with QAGs. Strong evidences against diffusion controlled release of ionic drugs were found. The results determined here provide greater insights into underlying release mechanisms of ionized drugs through cationic polymers.
SAPCD2 regulates mitotic spindle orientation in epithelial morphogenesis and asymmetric cell division

Catherine Chiu1*, Carine Monat-Reliat3,4*, Mélanie Robitaille1, Avais Daulat1, Peishen Zhao6, Peter Chidiac6, Michel Cayouette3,4,5, and Stéphane Angers1,2
1Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Ontario, Canada.
2Department of Biochemistry, Faculty of Medicine, University of Toronto, Ontario, Canada.
3Unité de recherche en neurobiologie cellulaire, Institut de recherches cliniques de Montréal
4Programme de biologie moléculaire, faculté de médecine, Université de Montréal
5Department of Anatomy and Cell Biology, and Division of Experimental Medicine, McGill University, Montreal
6Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada

The control of mitotic spindle orientation coordinates symmetric and asymmetric cell divisions to regulate cell fate and tissue morphogenesis. Non-canonical G-protein signalling through Galphai/LGN/NuMa is a major pathway conserved in both vertebrate and invertebrate species used to regulate mitotic spindle orientation. The molecular mechanisms underlying this pathway during mammalian cell divisions remain incompletely described.

Using tandem-affinity purification and mass spectrometry we identified a novel interactor of Galphai called SAPCD2, which also interacts with known regulators of polarity and mitotic spindle orientation including Par3 and LGN. Using a GTP exchange assay, we showed that SAPCD2 increases the binding of 35S-gamma-GTP on Galphai. Similar to LGN, we observe that SAPCD2 protein expression is cell cycle-dependent with peak amounts at mitosis. Depletion of SAPCD2 using shRNA in 3D cultures of MDCK cells resulted in defective cystogenesis and randomized spindle angle. In SAPCD2 knockout mice, we observed increased asymmetric cell divisions occurring in the developing retina at the expense of symmetric divisions. Clonal analysis revealed that these changes in cell division orientation alter the fate of terminally dividing neurons within the retina. Our results to date show that SAPCD2 is a novel interactor of Galphai and has a function to regulate mitotic spindle orientation for proper tissue morphogenesis and asymmetric cell division.
Radiosensitizing Effect of Gold Nanoparticles (AuNPs) under Oxia and Hypoxia

Lei Cui, Kenneth Tse, Payam Zahedi, Shane Harding, Gaetano Zafarana, David Jaffray, Robert G. Bristow, Christine Allen

Hypoxia exists in all solid tumors and leads to clinical radioresistance and adverse prognosis. In the present study, we hypothesized that cellular localization of gold nanoparticles (AuNPs) and hypoxia could be modifiers of AuNP-mediated breast cancer cell (MDA-MB-231) radiosensitization. We also studied the possible mechanistic effect of AuNPs on cell cycle distribution and DNA double-strand break (DSB) repair post-irradiation (IR). Using clonogenic radiation survival data, we observed that internalized AuNPs resulted in a dose enhancement factor (DEF) of 1.55±0.02 while extracellular AuNPs at 0.5 mg/mL resulted in a significantly lower DEF (1.09±0.01). Radiosensitization by AuNPs was greatest in cells under oxia, followed by chronic hypoxic cells and then acute hypoxic cells. The presence of AuNPs inhibited post-irradiation DSB repair, but did not lead to cell cycle synchronization. The relative radiosensitivity of chronic hypoxic cells is attributed to defective DSB repair (homologous recombination, HR) in these hypoxic cells due to decreased HR (RAD51)-associated protein expression. Our results support the further study of AuNPs for clinical development.
Heat-activated Thermosensitive Liposomal Cisplatin (HTLC) Results in Effective Growth Delay of Cervical Carcinoma in Mice

Yannan Dou, a Jinzi Zheng, b,c Warren D. Foltz, b,c Robert Weersink, c Naz Chaudary, d David A. Jaffray, b,c,f,g,* Christine J. Allen a,b,g,**

aLeslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada M5S 3M2, bSTTARR Innovative Center, University Health Network, Toronto, ON, Canada M5G 1L7, cTechna Institute and Radiation Medicine Program, Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada M5G 1L7, dOntario Cancer Institute, University Health Network, Toronto, ON, Canada M5G 2M9, eDepartment of Medical Biophysics, University of Toronto, Toronto, ON, Canada M5G 2M9, fDepartment of Radiation Oncology, University of Toronto, Toronto, ON, Canada M5G 2M9, gInstitute of Biomaterials & Biomedical Engineering, University of Toronto, Toronto, ON, Canada M5S 1A1

Cisplatin (CDDP) has been identified as the major chemotherapeutic agent for the treatment of cervical cancer; however, its clinical use is associated with dose limiting toxicity. Although different liposome formulations of CDDP have been developed to reduce systemic toxicity, their therapeutic advantage over the free drug has been lacking due to insufficient drug release at the tumor site. This report describes the development of a novel formulation that includes heat-activated thermosensitive liposomes containing CDDP (HTLC) with the ability to release over 95% of the loaded drug in less than 5 minutes under mild heating conditions (42°C). The physico-chemical characteristics of HTLC were assessed in terms of gel to liquid crystalline phase transition temperature, drug loading efficiency, size, and stability. The pharmacokinetics and biodistribution of HTLC were evaluated in non-tumor-bearing mice over a 24h period. In addition, the release kinetics of HTLC (co-encapsulated with gadoteridol: Gd-HP-DO3A) was monitored in real-time using magnetic resonance (MR) imaging in tumor-bearing mice with conformal heating to 42°C at the tumor volume using a laser chamber setup. MR thermometry (MRT) demonstrated that a relatively uniform temperature distribution was achieved in the tumor volume using the external laser-based heating setup. In mice bearing subcutaneously implanted ME-180 cervical tumors, the combination of HTLC and heat resulted in a 2-fold increase in tumor drug levels at 1h post-administration compared to HTLC without heating. Furthermore, the overall tumor accumulation levels for the HTLC groups (with and without heat) at 1h post-injection were significantly higher than the corresponding free CDDP group. This translated into a significant improvement in therapeutic efficacy evaluated as tumor growth delay (p<0.05) for the heated HTLC treatment group, compared to the unheated HTLC, heated or unheated free CDDP, Lipoplatin™ and saline groups. Overall findings from this study demonstrate that a heat-activated, triggered release formulation of CDDP results in a significant enhancement in the therapeutic index of this drug.
Extensive Transduction of the Central Nervous System Following A Single Intra-cerebroventricular Injection of Adeno-associated Viral Vectors in Neonatal and Juvenile Mice

Shervin Gholizadeh, Sujeenthar Tharmalingam, Margarita E. MacAldaz and David R. Hampson

Several neurodevelopmental disorders affecting the CNS are potentially treatable via viral vector-mediated gene transfer. Adeno-associated viral (AAV) vectors have been vectors of choice in recent clinical trials because of their desirable properties including safety, efficacy, and stability. Major factors affecting tropism, intensity and cell-type specificity of AAVs include encapsidation of different AAV serotypes, promoter selection, CNS milieu, and timing of administration. In this study, we evaluated the ability of single-stranded AAV pseudotype 2/9 (AAV2/9) to transduce the brain and target gene expression to specific cell types following intra-cerebroventricular injections in mice. Titer-matched AAV2/9 vectors encoding the enhanced green fluorescent protein (GFP) reporter, driven by the cytomegalovirus (CMV) promoter, or the neuron-specific synapsin-1 promoter, were injected bilaterally into the lateral ventricles of C57/BL6 mice at postnatal day 5 (neonatal) or 21 (juvenile). Brain sections were analyzed 25 days after injection using immunocytochemistry and visualized by confocal microscopy. GFP immunohistochemistry following neonatal and juvenile administration of viral vectors revealed transduction throughout the brain including the striatum, hippocampus, cerebral cortex, and cerebellum, but with different patterns of cell-specific gene expression. Transduction of astrocytes was observed with vectors carrying the CMV promoter at postnatal day 5, expanding the utility of AAVs for modeling and treating diseases involving glial cell pathology. In contrast, injection of AAV2/9-CMV-GFP vector on postnatal day 21 resulted in preferential transduction of neurons. Administration of the vector with the synapsin-1 promoter resulted in a widespread neuronal transduction. These results outline efficient methods and tools for gene delivery to the CNS by direct, early postnatal administration of AAVs. Our findings highlight the effect of promoter selection and age of administration on the intensity, distribution and cell-type specify of AAV transduction in the brain.
Incidence and burden of infectious-related hospitalizations among solid-organ transplant recipients: a single centre study

Bassem Hamandi, Paul Grootendorst, Shahid Husain and Manny Papadimitropoulos
Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada

Background: There has been a paucity of data on the healthcare resource utilization and economic burden of infectious-related (IR) complications in solid-organ transplant (SOT) recipients. The aims of this study were to describe and determine the incidence, clinical characteristics and associated length-of-stay (LOS) for SOT recipients hospitalized for IR complications.

Methods: This cohort study evaluated all SOT recipients requiring admission to a tertiary-care centre for the months of June-Sept for each of 2007, 2008, and 2011. Linear and quantile regression methods were used to estimate the effects of patient demographics, co-morbidities, transplant- and infection-related factors on LOS for IR visits.

Results: We observed 1414 readmissions in 531 SOT patients during the study period. IR complications occurred in 603/1414 (43%) readmissions in 305/531 (57%) patients, at a rate of 0.45 episodes/1000 patient-days followed. More patients were readmitted at least once within 30d of being discharged for their IR vs. non-IR admission (45% vs. 27%, p<0.002). The most frequent IR complications requiring hospitalization were: respiratory (27%), sepsis (13%), liver (12%), genitourinary (12%), and CMV-related (9%). Sepsis (17.2d and 1364d), CMV-related (17.0d and 904d), and respiratory infections (13.4d and 2092d) had the longest associated median and total LOS, respectively. We found several factors associated with increased LOS (percent increase): concurrent treatment of acute rejection (73%), infectious-disease specialist consultation (54%), culture-positive infections (39%), dialysis-dependence (30%), previous antimicrobial usage (21%), central-venous catheter use (17%), and leukopenia (15%). Quantile regression revealed that the OLS model may overstate the effect of ID consultation on the LOS at higher values of LOS.

Conclusions: Infections appear to cause significant morbidity in the transplant population, posing a significant burden on acute healthcare resource utilization. Enhanced patient risk assessment along with prompt and appropriate treatment for infectious episodes through the use of a multidisciplinary approach may decrease IR morbidity.
Generic drug pricing and generic drug substitution in private drug plans in Canada

Ethar Ismail, Paul Grootendorst

Drug insurance in Canada is provided by a patchwork of public and private drug plans. Public coverage is mainly from provincial government plans for seniors, low-income individuals and those with high drug costs relative to income. Most private coverage is provided as an employment-related benefit.

Compared to public, private plans tend to impose fewer cost controls. According to a 2011 Mercer survey, 50% of private plans do not require that lower cost generic alternatives be used when available and 80% reimburse the brand drug if the physician specifies no substitution. One possible reason for some employer-sponsored plans’ generosity is that employers find negotiations with employees over benefit design costly and not worth the potential savings.

Starting late 2006, public plans have taken measures to reduce the prices paid for generic drugs. Prices have dropped by as much as 75%. It is unclear to what extent private plans have attempted to lower prices, but there are reports that at least one attempt was unsuccessful due to pharmacies’ opposition to these moves. In 2010, however, provincial governments stepped in and mandated that pharmacies reduce the prices charged to private plans.

My research aims to investigate the nature of private drug plans by addressing the following questions:

First, have private plans been able to pay lower prices for generic drugs prior to governmental intervention in 2010? This will inform the degree of bargaining power that employer-sponsored drug plans have with pharmacies. Second, have pharmacies been compliant with the requirement to reduce generic prices for private plans? Third, if prices have fallen, have private plans taken steps to increase generic fill rate? Has the increase in potential savings from generic substitution encouraged employers to mandate generic substitution?

IMS Brogan PharmaStat data (all provinces, quarterly from 2003-2012) will be used to address these questions.
A Mixed-Treatment Comparison (MTC) of Cardiac Rehabilitation Treatment Strategies in Patients with Coronary Heart Disease (CHD)

Nader N. Kabboul 1, 2, George Tomlinson 1, 2, Murray Krahn 1, 2, Sherry Grace 1, David Alter 1, 3. 1. The University of Toronto, Ontario, Canada; 2. THETA Collaborative, Ontario, Canada; 3. Toronto Rehabilitation Institute, Ontario, Canada.

Background/Purpose: Cardiovascular disease (CVD) is the leading cause of mortality globally accounting for 7.22 million deaths from coronary heart disease (CHD) in 2004. When added to usual medical care, Cardiac Rehabilitation (CR) has been shown to reduce mortality and morbidity in CHD patients by 20-30%. Our group aimed to identify and evaluate the comparative effectiveness of different CR treatment strategies on mortality and morbidity in patients with CHD.

Methods: Extract all randomized controlled trials (RCT) from Cochrane Reviews of CR treatment strategies in CHD (exercise, psychological counseling, patient education/counseling, and smoking cessation). Identify all CR treatment strategies used in active and comparator arms of each RCT and extract the outcomes of total mortality, CV mortality, MIs, and hospitalizations. Perform Bayesian MTC on extracted data.

Results: Our group identified 117 different RCTs and 13 distinct CR treatment strategies within these RCTs (exercise, psychological counseling, patient education/counseling, smoking cessation: alone or in any combination with each other). Only the center-based exercise-based CR treatment strategies of exercise alone, exercise plus patient education, and exercise plus psychological counseling significantly (p < 0.05) reduced total mortality by 27%, 32% and 43% respectively. Exercise plus psychological counseling significantly reduced CV mortality MIs by 61% and 65% respectively. Exercise alone significantly reduced hospitalizations by 76%.

Conclusions: Previous reviews may have underestimated the true effectiveness of center-based exercise-based CR. Our study shows that previous results underestimated the impact of CR on mortality and morbidity by 13% - 35% depending on outcome evaluated.
The influence of drug-polymer interactions on the drug release rate through polymer films

S. Kwan, X. Y. Wu

Purpose: Designing a formulation that uses polymers to control the drug release rate can be an iterative, tedious design process involving several experimental attempts. The purpose of this study is to quantify drug-polymer interactions and determine its influence on the rate of drug permeation through polymer films.

Methods: Two model drugs (theophylline & valproic acid) and the ethyl acrylate and methyl methacrylate copolymer dispersion NF (Eudragit NE) polymer were selected. The drug-polymer interactions were characterized by solubility parameters calculated using the Hoptyzer-Van Krevelen group contribution method. The affinity of the drugs to the polymer was determined by the drug-polymer partition coefficient where 20-35 polymeric films were submersed in various drug solutions. Drug release tests were conducted using the side-by-side diffusion cells at 37°C and the permeability coefficients were calculated using the time lag and mass balance methods.

Results: The solubility parameter difference between valporic acid and the polymer was half as large compared to the theophylline-Eudragit pair, suggesting more interactions between valporic acid and the polymer compared to the latter. Compared to theophylline, the mean partition coefficient (n=6) for valproic acid was statistically larger. Subsequently, valproic acid was able to diffuse and permeate through the polymer at a faster rate and calculations from the mass balance and time lag methods show the mean (n=3) permeability coefficient of valproic acid to be approximately 50 to 1300 times larger.

Conclusions: In the study of the two drug-polymer pairs, the calculated solubility parameter difference reflected the interactions between the drug and the polymer and correlated well with drug partition in the polymer and drug release through the polymer films. The results demonstrate the importance of drug-polymer interactions on the drug release rate.
Preclinical pharmacokinetics, radiation dosimetry, toxicology and microSPECT/CT tumour imaging properties of $^{111}$In-BzDTPA-pertuzumab, a probe for detecting early response of breast cancer to trastuzumab (Herceptin)

Karen Lam$^1$, Conrad Chan$^1$, Susan J Done$^{2,3}$, Raymond M Reilly$^{1,4,5}$

$^1$Department of Pharmaceutical Sciences, University of Toronto, Canada; $^2$Department of Medical Biophysics and Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada; $^3$Ontario Cancer Institute and Department of Pathology, University Health Network, Toronto, Canada; $^4$Department of Medical Imaging, University of Toronto, Canada; $^5$Toronto General Research Institute, University Health Network, Toronto, Canada

Our objective was to conduct preclinical pharmacokinetic (PK), radiation dosimetry, toxicology and tumour imaging studies to support the clinical translation of $^{111}$In-isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (BzDTPA)-pertuzumab, a probe for detecting early response of breast cancer to trastuzumab (Herceptin). Methods: $^{111}$In-BzDTPA-pertuzumab (2-3 MBq; 2 µg) was administered i.v. to groups of 4 female non-tumour bearing BALB/c mice. Tissues and blood samples for biodistribution and PK analyses were obtained up to 166 h post-injection (p.i.) and measured in a $\gamma$-counter. Radiation absorbed doses in humans were projected using OLINDA software from mouse organ mean residence times. Acute toxicity was studied in 3 groups of 10 BALB/c mice administered saline (control), unlabelled BzDTPA-pertuzumab, or $^{111}$In-BzDTPA-pertuzumab at 23 times the planned human dose (MBq/kg). Toxicity was assessed by monitoring body mass, by hematology and clinical biochemistry parameters, and by tissue histopathology. MicroSPECT/CT imaging was performed at 72 h p.i. of $^{111}$In-BzDTPA-pertuzumab in athymic mice with s.c. HER2-positive MDA-MB-361 tumours. Results: The highest concentrations of radioactivity were found in the blood and lungs. $^{111}$In-BzDTPA-pertuzumab displayed biphasic elimination with a $t_{1/2\alpha}$ of 3.8 h and a $t_{1/2\beta}$ of 228.2 h. The projected whole-body radiation dose in humans was 0.05 mSv/MBq. The projected doses to the liver and kidneys were 0.23 and 0.33 mSv/MBq, respectively. The acute toxicity study revealed no significant differences between mice administered $^{111}$In-BzDTPA-pertuzumab or unlabelled BzDTPA-pertuzumab compared to saline control mice with respect to complete blood counts, serum creatinine and alanine aminotransferase levels. No significant reductions in body mass and no morphologic changes were observed in any of the groups. MicroSPECT/CT images demonstrated high tumour uptake of $^{111}$In-BzDTPA-pertuzumab (34.5 ± 9.2 %ID/g). Conclusion: The results of these preclinical studies support the advancement of $^{111}$In-BzDTPA-pertuzumab to a Phase I/II clinical trial in breast cancer patients. A Clinical Trial Application has been approved by Health Canada.
Cisplatin \[ \text{cis-PtCl}_2(\text{NH}_3)_2 \] represents the canonical member of a group of platinum-based chemotherapeutic agents widely used either alone or in combination with other agents to treat a range of malignancies including sarcomas, lymphomas, germ cell tumors and several carcinoma subtypes. Use of platinum-containing compounds faces considerable clinical limitation currently due to their induction of cellular death in proliferating tissues as well as related neuro-, oto- and nephrotoxicity. The mechanism of toxicity has long been thought to be directly tied to nucleophilic attack of the drug on DNA purine bases, ultimately forming inter- and intra-strand crosslinks initiating cell death. However recent experiments in our laboratory into the mechanism of cisplatin toxicity have caused us to question the current dogma. In addition, the cellular mechanisms of cisplatin-induced toxicity remain unclear as previous investigations have provided evidence for the occurrence of several forms of programmed cell death (apoptosis, autophagy and necroptosis) in specific cellular contexts.

To examine the mechanism of cisplatin toxicity in greater detail we utilized the developing murine embryo (E10.5-15.5) to model rapid cellular proliferation in a variety of solid organs. Treatment with cisplatin \textit{in vivo} at levels equivalent to or below that used during human clinical exposure resulted in widespread tissue injury. In wild-type animals injury was characterized by formation of TUNEL-positive DNA double-stranded breaks, phosphorylation of P53 and gamma H2AX, activation of caspase-3 and caspase-7 activity, and cleavage of the caspase-3/7 dependent target PARP. Analysis of cellular morphology in affected populations by electron microscopy revealed the formation of apoptotic bodies. These findings indicate that under normal circumstance, cisplatin causes cell death through the induction of apoptosis in a wide variety of tissues. To further define the mechanistic nature of the cell death induced by cisplatin, levels of comparative cellular injury following cisplatin treatment were determined \textit{in vivo} in mice carrying genetic modification of the apoptotic pathway. Strikingly, animals homozygous for a null mutation of caspase-3 alone exhibited complete inhibition of cisplatin-induced cell death for a wide variety of tissues 24 hours following treatment. This effect was seen despite the presence of caspase-7 activity in both wild-type and caspase-3 null mice following cisplatin treatment, identifying a novel functional variance between these two key executioner caspases. In addition to dramatically reducing the rate of programmed cell death, we demonstrate that loss of caspase-3 induces an alternative, normally quiescent pathway of programmed cell death exhibiting features of necroptosis. These findings have important implications toward the maintenance of both normal and malignant cells in a wide variety of tissue following cisplatin treatment.
Micro-porous membranes for sustained insulin delivery

Jason Li, Michael Chu, Claudia Gordijo, Azhar Z. Abbasi and Shirley Wu
Faculty of Pharmaceutical Sciences, University of Toronto, Toronto, Canada

A simple membrane-reservoir system was developed for the sustained release of insulin. The system consists of silicone reservoir capped with a micro-porous silicone membrane and filled with an insulin gel. The effect of membrane hydrophobicity and porosity on insulin release kinetics was examined. Membrane hydrophobicity was tuned using various membrane materials and surface treatments (EVAC, untreated PDMS, PDMS + 2kDa PEG, PDMS + 20kDa PEG), and was quantified using contact angle measurements. Insulin release from the devices was conducted at 37°C under mixing conditions. Released insulin was quantified using UV-Vis spectroscopy over a 30-day period. CD spectroscopy was used to confirm the structural integrity of released insulin.

Material hydrophobicity was found to be a key parameter in determining insulin permeation through the porous membranes. Insulin permeability was significantly elevated through hydrophilic membranes (PDMS treated with PEG) as compared to hydrophobic ones (EVAC and untreated PDMS). Therefore, insulin delivery through a porous membrane is maximized when hydrophilic materials or surface treatments are employed for membrane construction.

Insulin release rates from the devices were also dependent on membrane porosity, with higher insulin release rates corresponding to more porous membranes. Linear insulin release profiles were observed for all membrane geometries; however those with higher porosity exhibited faster reservoir depletion. Thus insulin release rates may be tuned for future in vivo studies by altering membrane porosity.
Fructose, a dietary monosaccharide, is increasingly integrated into the Western diet with the addition of sweetener and high fructose corn syrup. Fructose and its metabolites, glycoaldehyde, glyceraldehyde and hydroxypyruvate had been shown to produce methylglyoxal and glyoxal, two reactive dicarbonyls related to diabetic complications. In this study, fructose did not cause protein carbonylation and instead protected against apparent carbonylation by Fenton's reagent; fructose did not form significant levels of dicarbonyl compounds over a period of six days under standard conditions (37 °C, pH 7.4). In contrast, dihydroxyacetone, a fructose metabolite, caused protein carbonylation and autoxidized to form dicarbonyls. Under oxidative conditions (Fenton’s reaction), the protein carbonylation and autoxidation were even more prominent. Natural polyphenols were tested for their ability to protect against glyoxal- and methylglyoxal-induced cytotoxicity, reactive oxygen species formation and mitochondrial membrane potential disturbance. The polyphenols investigated were gallic acid, methyl gallate, ethyl gallate, propyl gallate, rutin and curcumin. The polyphenols were assayed using primary and GSH-depleted hepatocytes. The polyphenols were also investigated for their rescuing ability and were found to provide greater hepatoprotection when toxins were pre-incubated for 30 min before adding the polyphenols. However, rutin was less protective when rescuing hepatocytes, perhaps because rutin metabolites may scavenge reactive oxygen species more effectively than rutin itself. The longer the alkyl group attached to the gallate compound, the more cytoprotective the polyphenol was. However, the gallates with longer alkyl groups were less able to scavenge reactive oxygen species, and to maintain the mitochondrial membrane potential. The clinical relevance of natural polyphenols against fructose metabolites and reactive carbonyl species had been demonstrated in this study.
Controlled Release as a Strategy to Prevent Solution-Mediated Phase Transformation in Amorphous Solid Dispersions

Jamie Lugtu-Pe, Alireza Ghaffari, David Dubins, Xiao Yu Wu

Purpose: While conventional solid dispersion technology is a promising strategy for solubility enhancement, the challenge of solution mediated phase transformation (i.e. recrystallization) has yet to be overcome in order for this approach to be applicable to a wide range of drug candidates. One concept to minimize recrystallization involves tailoring release rate so that concentration within the GIT is maintained well below a certain threshold concentration, such that the rate of dissolution matches rate of absorption and onset of recrystallization can be avoided. This work is thus aimed to design and characterize a Controlled Release Solid Dispersion (CRSD) system capable of maximizing dissolution of poorly water-soluble drugs.

Methods: Ketoprofen and celecoxib were used as model BCS Class II drugs with water solubility of 0.5 mg/mL and 3.3 µg/mL, respectively. Polymer carriers investigated were: polyvinylpyrrolidone (PVP K30), polyvinylacetate (PVAc), and Kollidon SR®. Film-casting and spray drying were used to obtain amorphous solid dispersions of drug in various polymers at varying ratios. Amorphicity/crystallinity was examined by DSC. Solubility enhancement and dissolution were evaluated under varying degrees of sink conditions.

Results: DSC thermograms of SD formulations exhibited a loss in endothermic peak while the melting peak of the drug remained in the physical mixtures (PMs), confirming higher amorphicity of SDs. The extent of dissolution was up to 3-fold greater for all SDs than the corresponding PMs of the same compositions. However, the relative release rates were dependent on drug candidate. Ketoprofen SD exhibited slower dissolution rates compared to the PMs while celecoxib SDs were relatively faster than the PMs. Formation of a tacky complex during dissolution was observed in the case of ketoprofen SDs, but not in the case of celecoxib SDs.

Conclusions: Amorphous dispersion of the studied drugs in various polymers was formed as determined by DSC. Drug dissolution rate and extent depended on the drug properties for a given polymer. Further investigation is needed to elucidate effects of drug properties on SD formation and dissolution profile.
Safety and Equity: Transnational Actors’ Influence on Pharmacovigilance in Low and Middle-Income Countries

A Case Study of Pharmacovigilance Policy in Kenya and Brazil

Kathy Moscou, RPh, MPH, PhD Candidate

Postmarket drug safety is a growing priority for low and middle-income (LMI) countries particularly where the rapid scale up of medicines for AIDS, TB and malaria has grown substantially and access to essential medicines to treat non-communicable chronic diseases is expanding. In the past 5 years, activities to detect, assess, understand and prevent adverse effects and drug-related problems (pharmacovigilance) have increased in LMI countries with the support of transnational actor initiatives such as the 2009 opening of the World Health Organization drug-safety monitoring centre-Africa in Ghana. Knowledge of adverse effects and drug-related problems, however, has not kept pace with increased availability of medicines. Pharmacovigilance in LMI countries is hindered by poverty, and an overburdened, under-resourced health care system. Limited resources are allocated to perceived higher priority public health areas. Pharmacovigilance policy choices and resource allocation have implications for medicine safety, incidence of adverse drug reactions and population health. Transnational actors influence pharmacovigilance policy choices.

This research addresses a gap in the literature by investigating the relationship between state and transnational actors on pharmacovigilance policy choices in Kenya and Brazil. The interaction of the global pharmaceutical industry, civil society and supranational harmonization networks on the strength or weakness of national pharmacovigilance policies has not been examined. Transnational actors’ influence on pharmacovigilance in Kenya and Brazil will be investigated using a case study methodology. A conceptual framework has been developed to guide the comparative analysis.

This research contributes to the literature on pharmacovigilance, health policy, and political economy. It builds on existing theories pertaining to mechanisms for transnational actors’ influence and examines the impact of uptake of cross-purpose policy ideas. The research will assist policy makers in analyzing the impact of strategic interactions between state and non-state actors on the pharmacovigilance regime, and hence contribute to policy choices that improve drug safety.
Enhanced cytotoxicity of trastuzumab modified with metal chelating polymers (MCPs) labeled to high specific activity with Indium-111 on HER2 expressing breast cancer cells

1Ghislaine Ngo Ndjock Mbong, 2Yijie Lu, 1Conrad Chan, 2Mitchell A. Winnik, and 1Raymond M. Reilly
1Department of Pharmaceutical Sciences, 2Department of Chemistry, University of Toronto, Toronto, Ontario, Canada.

Purpose: We have been studying Auger electron radioimmunotherapy of human epidermal growth factor 2 (HER2) positive breast cancer (BC) using trastuzumab (Herceptin®) derivatized with diethylenetriaminepentaacetic acid (DTPA) chelators for labeling with Indium-111 (111In) and modified with nuclear localizing sequence (NLS) peptides [111In-NLS-DTPA-trastuzumab]. 111In-NLS-DTPA-trastuzumab targets HER2+ human BC cells and its nuclear importation caused DNA double strand breaks by the Auger electrons emitted by 111In. The survival of high expression (1×10^6 receptors/cells) HER2+ BC cells exposed to the radioimmunoconjugate (RIC) was less than 10%. However, intermediate (5×10^5 receptors/cells) and low expression (5×10^4 receptors/cells) HER2+ BC cells were insensitive with a survival of 50% and 90% respectively. A proposed explanation for this insensitivity was that the achieved specific radioactivity (SA) (amount of radioactivity per mass) was lower (1 MBq/µg) than the estimated SA (11.6 MBq/µg). In order to increase the potency of 111In-labeled-trastuzumab, we conjugated trastuzumab to a metal chelating polymer (MCP) harboring multiple DTPA for complexing 111In: 111In-MCP-trastuzumab.

Methods: The MCP with a polyglutamide backbone, 29 DTPA groups, and a hydrazide reactive group was linked to sodium meta periodate oxidized carbohydrates on the Fc domain of trastuzumab. HER2 immunoreactivity, internalization and nuclear importation of the immunoconjugate were evaluated in cellular binding and fractionation studies on HER2+ SK-BR-3 cells. The specific activity was assessed by 111In-labeling decreasing masses of the immunoconjugate with the same radioactivity (9.25 MBq). The effect of 111In-MCP-trastuzumab labeled to high SA (5.5MBq/µg) on the clonogenic survival (CS) of SK-Br-3 cells was compared to low SA 111In-labeled trastuzumab conjugates.

Results: The immunoconjugate did bind to HER2 with high affinity (K_D=10.7±1.5nM); was internalized and imported into the nucleus of HER2+ BC cells. It was labeled to a high SA (8.9MBq/µg) using a low mass (1µg). This represents a SA that is 8 fold higher than previously achieved. High SA 111In-MCP-trastuzumab was 4-5 fold more cytotoxic than low SA RICs to SK-Br-3 cells.

Conclusion: We constructed a new radioimmunoconjugate 111In-MCP-trastuzumab. This RIC can be labeled with up to 8-fold higher SA than 111In-NLS-DTPA-trastuzumab. This was further translated into an increase cytotoxicity potency for high HER2 BC cells.
Testing Educational Materials about Immunizations in a Paediatric Setting

Chaitya Parikh, BSc\(^1\), Sarah Smart, BSc\(^1\), Vibhuti Shah MD\(^2\), Woojin Yoon, MSc\(^2\),
Michael Sgro, MD\(^3\) and Anna Taddio, PhD\(^1\)
\(^1\)University of Toronto, Toronto, Canada; \(^2\)Mt. Sinai Hospital, Toronto, Canada; \(^3\)St. Michael's Hospital, Toronto, Canada

Background: Immunization procedures are a common source of iatrogenic pain for infants. In a recently developed clinical practice guideline, led by Taddio et al., evidence-based recommendations/strategies for management of immunization pain were made. Parent-friendly educational tools outlining these pharmacological, physical, and psychological pain-relieving strategies for infants <12 months old were made in the form of a one-page information factsheet and short informational video. Pilot-testing with newborn parents from Mount Sinai Hospital revealed substantial knowledge gain of the pain-relieving strategies from these tools; however whether parents would actually utilize these strategies remained untested. Parents suggested that providing both the factsheet and video together beforehand at their community clinic would be the most useful.

Objective: To assess whether providing an educational material on infant immunization pain management strategies vs. control (general information about immunization) to parents visiting their community paediatric clinic, leads to increased utilization of the number of pain-relieving strategies.

Methods: Parents were recruited during their infant’s 2-month or 4-month well baby visit to receive either the intervention educational material or the control general information about immunization. Infants and parents were then observed during their immunization procedure where their use of pain management strategies was assessed.

Results: As an interim analysis of this study is not planned complete results cannot be reported at this time. Currently 85 parents have been recruited from an expected sample size of 160. We expect that the delivery of written and video educational materials will lead to a statistically significant increase in utilization of infant pain management strategies by parents. We expect that the application of these strategies will result in a lower infant pain response in the intervention group as compared to the control group.
Improving Lung Transplant Outcomes with Nanoscale Diagnostics

Andrew T. Sage\textsuperscript{1}, Xiao-Hui Bai\textsuperscript{2}, Laili Mahmoudian\textsuperscript{1}, Mingyao Liu\textsuperscript{2}, Shaf Keshavjee\textsuperscript{2}, and Shana O. Kelley\textsuperscript{1}
\textsuperscript{1}Department of Pharmaceutical Science, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada. \textsuperscript{2}Latner Thoracic Surgery Research Laboratories, University Health Network Toronto General Research Institute, Toronto, Ontario, Canada

Introduction: Over the last few decades, lung transplantation (LTx) has become a well-established therapy for patients suffering end-stage lung disease. Despite the many innovations that have occurred in recent years, the outcomes following LTx still lag behind those of other organ transplant procedures due to a lack of predictive genes. Recently, several biomarkers for LTx have been identified; however, traditional methods for determining genetic profiles of lung tissue are impractical for use in the transplant setting. By developing diagnostics based on electrochemical reporters and nanostructured microelectrodes (NMEs), rapid detection of these biomarkers can be achieved. Herein, we demonstrate the development of an ultra-rapid diagnostic tool that is capable of profiling donor lungs in less than 30 minutes.

Methods: NMEs were electrodeposited on glass microchips and functionalized with peptide nucleic acid (PNA) probes specific to previously identified LTx biomarkers. The binding of the complementary targets was monitored using a redox-active Ru\textsuperscript{3+/4+} reporter system.

Results: The novel LTx diagnostic platform was studied and validated using DNA oligonucleotides (100 nM) as well as total RNA (0.75 to 25 ng/µl) purified from human lung tissue. We then profiled lysed and unpurified biopsies from donor lungs (n = 18) that were collected prior to transplantation for the expression levels of IL-6, IL-10, ELGN1, and ATP11B. The genomic profiles of donor lung samples from lungs that were rejected for transplantation were compared with those that were successfully transplanted and no incidence of primary graft dysfunction (PGD) was reported in the patients. Our NME-based assay successfully identified a significant (p<0.05) difference between genomic expression levels of the LTx biomarkers in the injured and healthy lungs.

Conclusion: This novel diagnostic tool represents a significant advance in the field of transplantation and will be of great benefit to clinicians, surgeons, and patients.
RGD-conjugated Nanoparticles for Targeted Anti-metastasis of Integrin \(\alpha_v\beta_3\) -overexpressing Breast Cancer Cells

Dan Shan\(^1\), Ping Cai\(^1\), Preethy Prasad\(^1\), Jason Li\(^1\), Franky Liu\(^1\), Andrew Mike Rauth\(^2\), Xiao Yu Wu\(^1\)

\(^1\)Leslie Dan Faculty of Pharmacy, University of Toronto, \(^2\)Ontario Cancer Institute, Toronto, Canada

Background: The use of active targeting nanoparticles as a delivery system for both diagnosis and treatment in cancer therapy has been explored extensively. \(\alpha_v\beta_3\) integrin receptor, part of a major family of cell adhesion receptors, is overexpressed by many human invasive breast carcinomas and has been associated with widespread metastasis in highly aggressive metastatic breast cancer. RGD-conjugated solid lipid nanoparticles (RGD-SLN) for targeting of integrin \(\alpha_v\beta_3\) -overexpressing breast cancer cells have been synthesized and optimized for anti-metastasis effect.

Methods: RGD-conjugated solid lipid nanoparticles (RGD-SLN) were synthesized with a fatty acid lipid core and a poly(ethylene glycol) corona and decorated with varying concentrations of RGD peptides (0\%, 0.5\%, 1\%, 5\% and 10\% mol/mol ratio). Nanoparticle binding to \(\alpha_v\beta_3\) integrin receptor and \textit{in vitro} cellular uptake on \(\alpha_v\beta_3\) positive MDA-MB-231 cells were investigated by fluorescence microscopy. The influence of RGD-SLN on cell adhesion and invasion via binding to \(\alpha_v\beta_3\) integrin receptor was examined using standard adhesion and transwell invasion assays. Whole animal biodistribution, tumor uptake and intratumoral distribution of quantum dot-loaded nanoparticles were investigated to identify optimized RGD concentrations.

Results: RGD-SLN showed specific binding for \(\alpha_v\beta_3\) integrin receptors and formed clusters at the focal adhesion of cell membrane for receptor mediated cell uptake. RGD ligand concentration was optimized to reduce liver trapping. 1\% mol of RGD on the SLN surface was found to have most tumor retention and low liver uptake among all formulations in orthotopic human breast tumor. \textit{In vitro} treatment with RGD-SLN has reduced tumor cell adhesion and invasion thereby reducing cell metastasis.

Conclusions: We have optimized the RGD-SLN formulation to maximize tumor accumulation and minimize liver uptake. The RGD-SLN formulation has the potential to prevent metastasis through interference with cell adhesion and invasion.
The Role of Social Media in Recruiting for Clinical Trials in Pregnancy

Mahvash Shere\textsuperscript{1,2}, Gideon Koren\textsuperscript{1,2}
\textsuperscript{1}University of Toronto, \textsuperscript{2}The Hospital for Sick Children

Background: Recruitment of women in the periconceptional period to clinical studies using traditional advertising through medical establishments is difficult and slow. Given the widespread use of the internet as a source for medical information and research, we analyze the impact of social media as the primary recruitment tool in the second phase of an ongoing randomized, open-label clinical trial among pregnant women. This study aims to assess the effectiveness of social media as a recruitment tool through the comparison of diverse recruitment techniques in two different phases of the trial.

Methods: Recruitment in Phase 1 of the study consisted solely of traditional healthcare-based sources such as referrals from clinics, healthcare professionals, and hospital postings. This was compared to Phase 2 of the study where traditional recruitment was continued and expanded, while social media was used as a supplementary source. Social media platforms included sponsors’ websites, social networking websites, local online classifieds as well as discussion forums. Yearly recruitment and recruitment rates in the two arms were compared using the Mann Whitney U test. The contributions of each recruitment source to overall recruitment were analyzed, and the impact of potential confounders on recruitment rate was evaluated using a multiple linear regression.

Results: In the first phase of the study, with over 56 months of recruitment using traditional sources, 35 women were enrolled in the study, resulting in a mean rate of ±0.62 recruits per month. In the 6 months implementing recruitment through social media, 45 women were recruited, for a 12-fold higher rate of ±7.5 recruits per month (p<0.0001). Attrition rates remained constant, suggesting that social media mainly had a positive impact on recruitment.

Conclusions: Clinicians and scientists recruiting for clinical studies should learn how to use online social media platforms to improve recruitment rates, thus increasing recruitment efficiency and cost-effectiveness.
Cluster Randomized Trial of the Effect of a Prenatal Education Module on Pain Management during Routine Infant Immunizations

Sarah Smart BSc¹, Chaitya Parikh BSc¹, Vibhuti Shah MD², Matthuschka Sheedy RN ICCE², Woojin Yoon MSc² and Anna Taddio PhD¹
¹University of Toronto, Toronto, Canada; ²Mount Sinai Hospital, Toronto, Canada

Background: Unmitigated pain in infants has the potential to impact long-term health outcomes. There is ample literature outlining effective pain management strategies for immunization including psychological, physical and pharmacological interventions; however their use in clinical practice has been limited. Directing knowledge translation activities to parents in order to improve current analgesic practices is a novel approach that is ideally suited to address the current gap between research evidence and clinical practice. Teaching parents about these strategies in an enriching setting such as prenatal education classes has the potential to increase utilization of these strategies, and empower parents to take on a more active role in the management of their infant’s pain during routine immunization.

Objective: To assess the effect of teaching parents in the prenatal education environment and the impact it has on the number and types of pain-management strategies utilized during the 2-month immunization appointment.

Methods: This single-blinded cluster-randomized controlled trial enrolled expectant mothers from prenatal classes at Mount Sinai Hospital. Classes were randomized to receive a 30-minute interactive presentation on either immunization pain management (intervention group) or general immunization information (control group) and outcomes will be assessed during follow-up telephone surveys after each infant’s two-month immunization appointment.

Results and Timeline: Two-hundred and four mothers from 28 prenatal classes participated in this study. Follow-up data collection is completed for 110 mother/infant dyads and it is expected that data collection will be complete by July 2013. Subsequent data analysis will identify differences between control and intervention groups and their use of pain-management strategies during their infant’s two-month immunization appointment as well as any reported barriers parents experienced to implementing these strategies. No interim analyses are planned.
The Safety of Inhaled Corticosteroid Use in Pregnancy and Childhood

Laura Smy, Dr. Bruce Carleton, Dr. Gideon Koren

Background: Asthma is a common chronic condition affecting approximately 235-million people worldwide. Inhaled corticosteroids (ICS) are preferred for long-term control of asthma, but previous research has found their use is associated with adrenal suppression resulting in decreased cortisol levels in the body. The effect of long-term cortisol deficit in children is a concern because symptoms of decreased cortisol include chronic fatigue, muscle weakness, and hypoglycemia. Additionally, during pregnancy, cortisol naturally increases in third trimester and the increase is required for proper fetal lung development. The effect of decreased maternal cortisol is currently unknown. Therefore, providing further information regarding the effect of ICS use on adrenal function in childhood and pregnancy is pivotal.

Objective: To identify type- and dose-specific effects of inhaled corticosteroids on adrenal function in pregnancy and childhood by measuring hair cortisol as a novel biomarker of systemic exposure to these medications.

Hypothesis: Hair cortisol will effectively detect adrenal suppression caused by ICS use during pregnancy and childhood, and identify whether certain ICS are more toxic than others to the HPA axis.

Methods: Patients are being recruited through the Motherisk Program counselling line, SickKids asthma clinic, and the nation-wide Drug Safety and Effectiveness Network. A total of 750 children and 300 pregnant women, inclusive of patients and controls, will be enrolled from all participating sites. After obtaining consent, a questionnaire and hair collection form is used to collect pertinent medical, asthma, and hair care information. Hair samples will be segmented for particular time points of interest and analyzed for cortisol using an ELISA assay. Statistical analysis will be performed as appropriate.

Status: Currently recruiting.
Volumetric Characterization of Tri-n-acetylglucosamine binding to lysozyme

Ikbae Son, Yuen Lai Shek, David N. Dubins, and Tigran V. Chalikian
Leslie Dan Faculty of Pharmacy, Department of Pharmaceutical Sciences, Toronto, Ontario, M5S 3M2, Canada

Volumetric characteristics of protein recognition events determine the direction of pressure-induced shifts in the recognition reaction, while also providing insights into the structural, dynamic, and hydration changes. We report changes in volume, \( \Delta V \), and adiabatic compressibility, \( \Delta K_S \), accompanying the binding of tri-N-acetylglucosamine \([\text{GlcNAc}]_3\) to lysozyme. We interpret our measured changes in volume and compressibility in terms of changes in hydration and dynamic properties of the protein. Based on our \( \Delta V \) data, we find that 79±44 water molecules are released to the bulk from the hydration shells of the protein and the ligand. Our \( \Delta K_S \) data suggest a 4±2 % decrease in the mean-square fluctuations of the intrinsic volume of the protein, \( \langle \delta V_M^2 \rangle \) (or 2 % decrease in \( \delta V_M \)). Thus, the trisaccharide-bound state of the enzyme is less hydrated, more rigid, and less dynamic compared to the unbound state. In general, we discuss the importance of volumetric insights into the molecular origins of protein recognition events.
Cost-Effectiveness Analysis of Screening for Lung Cancer Using Low Dose Computed Tomography: The Toronto Experience

Mallory Thao, Jeffrey S. Hoch, Peter C. Coyte

Background: Lung cancer is the number one killer among cancers. Recently, the U.S. National Lung Screening Trial found that at risk participants who received annual low-dose computed tomography (“LDCT”) scans for lung cancer had a 20 percent lower risk of dying from the disease compared to standard chest X-rays. By using the clinical data of the Toronto-based one-arm trial of the Lung Cancer Screening Study undertaken by the University Health Network team, this paper is the first Canadian cost-effectiveness study to analyze the use of LDCT for detecting early stage lung cancers.

Method: The study was designed from the perspective of the Ontario Ministry of Health and Long-Term Care (“MoHLTC”) to evaluate whether stage shifting from early detection of asymptomatic lung cancer through LDCT screening versus late stage detection of lung cancer in symptomatic individuals in the usual care setting could be cost effective for at risk individuals. A model using a decision tree structure to capture the screening probabilities and a transitory Markov model to determine the health stages was created to estimate the difference between the expected benefits gained and the expected costs incurred. To test for variability of key parameters, one-way sensitivity analyses were performed. To test for uncertainty within the model, a probabilistic sensitivity analysis was undertaken.

Results: LDCT lung cancer screening was shown to be cost effective in the Canadian environment. Our deterministic results show an incremental cost of Cdn. $25,437 per life year and Cdn. $31,317 per QALY making the programme cost effective assuming a willingness-to-pay threshold of Cdn. $50,000.

Conclusion: Despite the high costs of screening tests and the large population to be tested, a screening programme for lung cancer among at risk individuals can be cost effective from the perspective of the MoHLTC.
**Informed Consent for Chiropractic Care: The Patient’s Experience**

Winterbottom, M., Boon, H., Facey, M., Mior, S. & Caulfield, T.

**Background:** Chiropractors are required to obtain informed consent from patients prior to treatment. Uncertainty surrounding adverse events and contested risk estimates may present a challenge for both practitioners and patients during risk disclosure.

**Objectives:** The objective of this study was to explore how chiropractic patients experience the process of informed consent.

**Methods:** A descriptive qualitative study design was used. Eligible participants were new to the Canadian Memorial Chiropractic College teaching clinics, over 18, and not employed as health care professionals. Open-ended telephone interviews were conducted and transcripts were analyzed using constant comparative analysis.

**Results:** Data collection is approximately 50% completed thus only preliminary findings are available. This consent process involves setting the stage, risk disclosure, signing a consent form, and knowledge exchange throughout treatment. These stages along with outside influences (e.g., previous experiences, media influence etc.), shape patients’ perspectives of the risks associated with chiropractic care. The discussion that takes place during risk disclosure is more important to patients than the consent form; patients value openness during risk discussion and perceive the consent form as a tool to protect the practitioner. Micro-conversations about the amount of pain patients experience during treatment often occur; these conversations may influence the level of risk associated with treatment. The risks are perceived as rare and minimal, and many patients cannot recall specific risks that were disclosed.

**Conclusion:** Discussions about the amount of pain and pressure applied during treatment are not perceived as part of the informed consent process in the academic literature. This study contributes to the literature by reconceptualising the process of informed consent to include these conversations. Moving forward, interviews with patients from private clinics will be conducted to compare to these results.
Modelling Autism Spectrum Disorder Through Maternal Immune Activation in Mice

Ingrid Cong Yang Xuan¹, Amy J. Ramsey², David R. Hampson¹, ²
¹Department of Pharmaceutical Science, University of Toronto, Toronto, Ontario, Canada
²Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction and communication as well as ritualistic repetitive behaviours. Apart from genetic predisposition, epidemiological studies suggest that maternal immune activation (MIA) during pregnancy may also be a risk factor for ASD. This theory is supported by the presence of activated astrocytes and microglia in human post-mortem brain samples and changes in cytokine levels in the sera of ASD patients. To study MIA in a laboratory setting, we injected mouse dams (C57BL/6) with lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly I:C) during mid-gestation to mimic a bacterial or viral infection, respectively. The offspring produced (i.e. LPS 1X or Poly IC 1X) were compared with offspring from dams that were injected during two consecutive pregnancies (i.e. LPS 2X or Poly IC 2X). Once the offspring reached adulthood, male and female mice were analyzed separately and compared with saline controls. We investigated ASD-associated behaviours including motor activity, social interaction and repetitive behaviour using the automated activity box, modified three-chamber paradigm, and marble burying test, respectively. The results indicate the presence of several ASD-like behaviours and pathologies in the MIA offspring, some of which are sex and treatment-dependent.
Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, is a fatal neurodegenerative disorder for which there are currently no effective therapies. It was recently reported that an expansion of a (GGGGCC)•(GGCCCC) hexanucleotide repeat within a non-coding region of C9orf72 causes ALS and FTD. Unaffected individuals have 2-19 repeats and individuals with as few as 20-25 repeats may show symptoms of disease, while those affected can have 250-1600 repeats. We have demonstrated that the (GGGGCC)n RNA forms extremely stable G-quadruplex structures. We also report the binding of TMPyP4 to the ALS-FTD r(GGGGCC)8 repeat using gel mobility-shift assays, circular dichroism (CD) spectroscopy and UV spectroscopy. Additionally, we report that TMPyP4 competes with and can displace ASF/SF2 and hnRNPA1 purified proteins from the ALS-FTD-associated r(GGGGCC)8 repeat.