SUPPLEMENT

*The Canadian Society for Clinical
Investigation and the Clinician
Investigator Trainee Association of
Canada acknowledges the support of:*

*Canadian Institutes of Health Research
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Bristol-Myers Squibb Canada
Eli Lilly Canada Inc.
University of Ottawa
Burroughs Wellcome Fund*

2011 CSCI/CITAC Young Investigators Forum Abstracts

CSCI/CITAC YOUNG INVESTIGATORS FORUM

for

CLINICAL SCIENTISTS IN TRAINING
CIP and MD/PhD STUDENTS

TRIBUNE DES JEUNES CHERCHEURS

pour

CHERCHEURS CLINICIENS EN FORMATION
ÉTUDIANTS INSCRITS AU PROGRAMMES
PFCC ET MD/PHD

Tuesday, September 13, 2011
Mardi, le 13 septembre 2011

Delta Ottawa City Centre
Ottawa, Ontario

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Clin Invest Med 2011; 34 (6S): 1-40.

1-01 The Role of Slit2 In Vascular Inflammation

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Objective: Vascular inflammation and cellular influx is a hallmark in the pathogenesis of atherosclerosis. Inhibiting monocyte recruitment is beneficial in preventing atherosclerosis and its clinical manifestations. The trafficking signals that recruit cells to areas of inflammation are provided by small secreted proteins called chemokines. These proteins play a major role in the pathogenesis of inflammation, and redundancy among the chemokine signaling pathways means that blocking one pathway will result in another assuming its function. Our research aim is to block a leukocyte's response to a range of chemokine-induced directional migration signals. The effects of Slit2 on monocyte migration, adhesion, phagocytosis, and on the intracellular signaling pathways involved were investigated.

Methods: Transwell migration assays were used to determine the effect of Slit2 on monocyte migration. Monocyte migration in vivo was investigated using a murine model of irritant-induced peritonitis. Functional assays, including adhesion and phagocytosis assays, were utilized to determine the effect of Slit2 on other functions of monocytes. Western blots were used to determine the intracellular signaling events mediating the effects of Slit2. In addition, dynamic adhesion assays under shear conditions were performed to mimic flow conditions in blood vessels.

Results: We have shown that Slit2 treatment can inhibit monocyte migration both in vitro, using transwell assays, and in vivo, using a murine peritonitis model of inflammatory cell influx. This inhibition is shown to be dose- and time- dependent. In addition, Slit2 inhibits monocyte adhesion to TNF- α stimulated endothelial monolayers under static conditions.

Conclusions: These experiments seek to understand the molecular and cellular events that cause blood vessels to become diseased. The ultimate goal is to identify new strategies for preventing and treating heart disease and stroke. This may allow the use of Slit2 as a novel anti-inflammatory therapy to prevent monocytes migration to inflammatory foci.

1-02 The harlequin mutant mouse displays an early biomarker of human aging-associated retinal degeneration relevant to disease etiology and drug testing

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Purpose: Identifying the earliest structural aberrations in the retina can assist in developing next-generation diagnostic and therapeutic approaches to retinal disease. Mouse mimics of human retinal disease provide an experimental framework to characterize functional and structural changes in the retina with disease onset and progression.

Approach: The *harlequin* disease (X^{hqY}) and carrier mouse (X^{hqX}) are two different mimics of human aging-associated retinal degeneration. Our objective was to identify and track the earliest structural change in the *harlequin* disease and carrier retina.

Methods: Nuclear kinesis in the segment layer (SL) and inner plexiform layer (IPL) was scored for the peripheral and central retina of male, age-matched X^{hqY} and wild type (WT) mice at 2, 4, 6, 8 and 10 months of age (moa) and age-matched X^{hqX} and WT mice at 3, 11 and 15 moa. Postmortem, the histology of the right eye of each animal was evaluated. The number of nuclei in the SL and IPL was determined for peripheral and central retina and evidence of apoptosis in displaced nuclei was examined using a TUNEL assay.

Results: Nuclear kinesis to the SL was negligible in WT mice and significant in X^{hqY} mice at 4 moa ($p=0.02$) with increasing severity to 10 moa in peripheral more than central retina. Nuclear kinesis in X^{hqX} mice was modest. Apoptosis was not associated with displaced nuclei. The number of nuclei in the IPL did not differ with mouse genotype.

Conclusions: Retinal nuclear kinesis is an early structural biomarker of aging-associated retinal degeneration prevalent with normal aging in humans. In *harlequin* mice, nuclear kinesis in the SL serves as an early structural marker that precedes retinal thinning. The development of non-invasive, *in vivo* and single-cell resolution imaging for the aging retina is a challenge that clinicians face in early diagnosis of various forms of aging-associated retinal degeneration.

1-03 Reduced Glutathione Inhibits TRPM2 Currents

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Background: TRPM2 (melastatin-like transient receptor potential type 2) channels are non-selective Ca^{2+} permeable ion channels that have been linked to cell death. TRPM2 is expressed and functional in pyramidal neurons of the hippocampus, which are particularly susceptible to neurotoxicity following ischemia and in various neurodegenerative diseases. Therefore, investigating the pathways that regulate TRPM2 function could help elucidate novel mechanisms of neurodegeneration.

Methods: TRPM2 currents were quantified using whole-cell voltage clamp recordings. NMDA receptor of voltage-gated Ca^{2+} channel mediated Ca^{2+} influx was used to elicit TRPM2 currents in cultured hippocampal pyramidal neurons. The patch pipette contained 1mM ADPR, the intracellular ligand for TRPM2, to facilitate current activation and control for any changes in endogenous levels of ADPR with time *in vitro*. TRPM2 mRNA levels were measured by quantitative RT-PCR. Glutathione (GSH) binding was assessed using a TNT reticulocyte expression system and GSH-sepharose pull-down followed by SDS-PAGE.

Results: We demonstrate that TRPM2 current density increases from 2 to 4 weeks *in vitro*. The increase in currents was not associated with a change in TRPM2 mRNA. Chronic GSH depletion following treatment with L-BSO, an inhibitor of GSH synthesis, enhanced TRPM2 currents in neurons at 2 weeks *in vitro*. Upregulation of endogenous GSH with N-acetylcysteine reduced currents in neurons at 4 weeks *in vitro*. These effects are attributed to glutathione levels and not changes in oxidative stress since currents are reduced when GSH is included in the patch pipette, but are not facilitated by treatment with H_2O_2 . Lastly, GSH physically interacts with both the amino and carboxyl intracellular terminal regions of TRPM2.

Conclusions: The increase in TRPM2 currents with time *in vitro* and inhibition by glutathione suggests that the decrease in GSH associated with age may potentiate TRPM2 currents and augment neuronal Ca^{2+} influx. This could increase the susceptibility of neurons to pathological insults in the aging brain.

1-04 A PDK1-independent regulatory pathway for Akt in vivo

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Background/Purpose: The serine/threonine kinase Akt is a key target of phosphatidylinositol 3-kinase (PI3K) signalling. Across phylogeny, PI3K-dependent Akt activity drives cellular growth and survival. In the roundworm *Caenorhabditis elegans*, the pro-survival function of Akt is conserved in the germline, where the worm homologue of Akt, AKT-1, promotes the survival of germ cells exposed to DNA damaging agents. Exactly how germline AKT-1 activity is regulated in the worm remains unknown, but we hypothesized that PI3K signalling might be involved. Normally, the single, conserved worm PI3K pathway - composed of the insulin/IGF-1 receptor DAF-2, the PI3K AGE-1, and the 3-phosphoinositide-dependent protein kinase 1 PDK-1 - plays a central role in the proper development of the worm body. However, worm PI3K signalling has no known role in controlling the fate of germ cells exposed to DNA damage. Thus, using the germline of *C. elegans* as a model for DNA damage-induced cell death, we sought to decipher how, or even if, DNA damage-dependent Akt activity was regulated by PI3K signalling *in vivo*.

Methods: We used genetic epistasis analysis and immunoprecipitation-Western blotting to determine whether AKT-1 promoted the survival of damaged germ cells as a target of worm PI3K signalling.

Results: Surprisingly, the insulin/IGF-1 receptor DAF-2, and PDK-1 functioned independently of their canonical downstream target AKT-1 to promote DNA damage-induced cell death in the worm germline. Furthermore, DAF-2 and PDK-1 functioned independently of each other, revealing a complete fragmentation of PI3K signalling in response to DNA damage. Consistent with these novel findings, DNA damage did not affect phosphorylation of AKT-1 at a conserved PDK-1 phosphorylation site, Thr350 (Thr308 in mammals), and instead increased phosphorylation at Ser517 (Ser473 in mammals).

Conclusion: A novel biochemical pathway regulates DNA damage-dependent Akt activity independently of PI3K signalling in the worm. Conservation of such a pathway may have relevance to the design of therapeutics targeting Akt-dependent tumours.

1-05 Sub-cellular localization of Y-Box Protein 1 regulates proliferation, invasion, and increased mesenchymal phenotype in astrocytomas

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Purpose: Y-Box-Protein-1 (YB1) is a transcriptional and translational regulator implicated in cancer progression. Previously, using gene expression microarray, we established elevated YB1 levels in pediatric GBM, an aggressive high-grade astrocytoma, possibly driving oncogenesis in this cancer. The purpose of this study is to investigate the role of YB1 in astrocytoma genesis and its possible association with poor prognosis.

Methods: We overexpressed or silenced YB1 protein in pediatric GBM (SF188), adult GBM (U87) and normal human astrocytes cell lines (NHA). Proliferation, migration, soft agar colony formation assay were performed in vitro, accompanied by in vivo mice xenograft to assess the tumorigenic and metastatic ability of these cells. Meanwhile, tissue microarrays including 150 pediatric GBM and 70 Grade I Pilocytic Astrocytoma were stained immunohistochemically (IHC) with YB1. YB1 expression and subcellular localization were scored and analyzed in association with patient prognosis and tumor grades.

Results: YB1 silencing using shRNA reduced YB1 level by 90%, and surprisingly increased cell proliferation, in contrary to what's shown in major literatures. As YB1 is predominantly cytoplasmic in physiological conditions in cells, and nuclear YB1 is known to associate with increased cell growth, sub-cellular localization of YB1 was investigated. In the YB1 silenced clones, YB1 was greatly reduced in cytoplasm, while nuclear YB-1 was actually enriched, explaining the increase in cell proliferation. YB1 overexpression was mainly cytoplasmic, and decreased cell proliferation and increased mesenchymal phenotype including migration. Importantly, in both orthotopic and heterotopic mice injections, although YB1 overexpression decreased the size of tumors formed, they increased metastatic ability of tumors into the liver. IHC on tissue mi-

croarrays of patient tumors showed strong YB1 expression in 66% of pediatric GBM samples, but only 8% in Grade I astrocytoma, suggesting a role of YB1 overexpression in a more invasive tumor phenotype. In addition, for the first time, we showed that nuclear YB1 expression indicated worse progression free survival compared to cytoplasmic YB1.

Conclusion: Our results suggest that YB1 modulates cellular proliferation and mesenchymal properties, based on its sub-cellular localization. Nuclear YB1 drives cell proliferation, whereas cytoplasmic YB1 promotes cell migration and metastasis. Showing the association of nuclear YB1 with worse patient prognosis, our data argue for caution in targeting YB1 for therapeutic intervention.

1-06 Distinct responses to prostaglandin E2 in upper and lower segment human myometrial smooth muscle cells

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Background: We have previously demonstrated that prostaglandin E₂ (PGE₂) can repress inflammatory chemokine output from cultured primary human myometrial smooth muscle (HMSM) cells isolated from the lower segment of the pregnant uterus. As it has been suggested that there may be functional differences between regions of the pregnant uterus, we next sought to investigate whether PGE₂ would elicit the same effect in HMSM cells isolated from the upper segment of the uterus.

Methods: Paired upper and lower segment myometrial biopsies were obtained from Caesarean section procedures at term, prior to labour onset (n=3) and were used to isolate HMSM cells. Cells were treated with prostaglandin receptor agonists (10 μ M – 300pM) in the presence and absence of IL-1 β (1ng/ml). IL-8 was output was determined by ELISA.

Results: Treatment of HMSM cells with IL-1 β resulted in a significant increase in IL-8 output compared to non-stimulated controls in both lower and upper segment. IL-8 output was greater in lower segment HMSM cells (180 000 pg/ml) compared to upper segment HMSM cells (115 000 pg/ml). PGE₂ significantly repressed IL-1 β -induced IL-8 output from lower segment HMSM cells (Student's t test, p<0.05 at 30nM); however, this effect was not observed in upper segment HMSM cells.

Conclusions: We demonstrate that within the uterus, smooth muscle cells isolated from two distinct uterine sites are capable

of producing unique responses. We intend to further characterize these cells in order to use them as a model to study different regions of the uterus and improve our understanding of prostaglandin signalling within the myometrium.

1-07 Mitochondrial Inhabitation Alters Calcium Handling in Hippocampal Neurons

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Background: Ca^{2+} deregulation is centrally involved in the pathophysiology of stroke. Physiologically, neuronal Ca^{2+} homeostasis is tightly maintained through chelation by Ca^{2+} binding proteins, extrusion by the PMCA and the Na/Ca exchanger, and uptake into cellular Ca^{2+} stores. Mitochondria have a dual role: they are a source of ATP utilized by the PMCA, and act as a Ca^{2+} store, acting as a transient Ca^{2+} sink during large Ca^{2+} transients. Ischemic stroke directly affects mitochondria, impairing both functions.

The objectives were the following: (1) can the genetically encoded Ca^{2+} indicator, GCaMP4, be used to sensitively measure Ca^{2+} transients in axons of cortical neurons? (2) what is the effect of mitochondrial inhibition on Ca^{2+} transients? (3) how does the arrest of mitochondrial respiration affect basal cytosolic Ca^{2+} concentrations?

Methods: Calcium transients were measured in axons and presynaptic varicosities in a minimally invasive way – using a genetically encoded Ca^{2+} indicator named GCaMP4. Mitochondrial respiration was inhibited with antimycin A.

Results: We found that following the inhibition of mitochondrial respiration, the recovery of action potential-evoked calcium transients was substantially prolonged but their amplitudes remained unchanged. Moreover, we observed apparently spontaneous, transient, localized calcium transients, which may be of functional significance by increasing the frequency of spontaneous neurotransmitter release.

Conclusions: (1) The genetically encoded calcium sensor, GCaMP4, can be used to measure Ca^{2+} transients in axons and presynaptic varicosities in a minimally invasive manner. It leaves the linear range following ~20 action potentials (2) With cessation of mitochondrial respiration, evoked Ca^{2+} transients initially remain unaltered. After 10-15mins, however, the decay of the calcium transient is significantly delayed while the amplitude remains unchanged (3) After 10-15mins of mitochondrial inhibition, basal Ca^{2+} concentrations show transient regional elevations. This may have impact on sponta-

neous neurotransmitter release.

1-08 Sonic hedgehog regulates Bmi1 in human medulloblastoma brain tumor-initiating cells

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Background: Bmi1 is a key stem cell regulatory gene implicated in the pathogenesis of many aggressive cancers, including medulloblastoma. Overexpression of Bmi1 promotes cell proliferation and is required for hedgehog (Hh) pathway-driven tumorigenesis. This study aimed to determine if Sonic hedgehog (Shh) modulates the key stem cell regulatory gene Bmi1 in childhood medulloblastoma brain tumor-initiating cells (BTICs). Although current literature suggests that there is a correlation between Shh pathway genes and Bmi1 expression, it is unclear whether there is indeed a direct regulatory mechanism.

Methods/Results: To address whether Shh induces expression of Bmi1, stem cell-enriched populations from medulloblastoma cell lines and primary samples were treated with Shh ligand and KAAD-cyclopamine (Shh antagonist). Our data indicate that Bmi1 expression positively correlates with increasing Shh ligand concentrations. Chromatin immunoprecipitation reveals that Gli1 preferentially binds to the Bmi1 promoter, and Bmi1 transcript levels are increased and decreased by Gli1 overexpression and downregulation, respectively. Knockdown experiments of Bmi1 in vitro and in vivo demonstrate that Hh signaling not only drives Bmi1 expression, but a feedback mechanism exists wherein downstream effectors of Bmi1 may, in turn, activate Hh pathway genes. These findings implicate Bmi1 and Hh as mutually indispensable pathways in medulloblastoma BTIC maintenance. Recent molecular characterization of medulloblastoma also reveals that Bmi1 is overexpressed across all subgroups of medulloblastoma, particularly in the most aggressive subtypes. Lastly, despite recent identification of BTIC markers, the molecular characterization of these cell populations remains unclear.

Conclusion: In this work, we propose that the BTIC marker CD133 may segregate a cell population with a Hh-receptor phenotype, thus demonstrating a cell-cell interaction between the CD133+ Hh receptor cells and the CD133- Hh-secreting cells.

1-09 High expression of *Hmga2* during fetal and neonatal life is cell-autonomously required for the robust generation of the hematopoietic stem cell compartment

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Background/Purpose: Fetal-stage hematopoietic stem cells (HSCs) differ functionally from their adult counterparts in their ability to undergo a faster rate of expansion post-transplant. To elucidate the mechanistic basis of this difference we looked for a possible HSC self-renewal regulator that was differentially expressed in highly purified murine fetal and adult HSCs independent of the proliferative status of the cells. A set of transcripts were compared between HSC-enriched (EPCR+CD48-CD150+CD45+; E-SLAM) cells isolated from fetal liver and adult bone marrow. High-mobility group A2 (*Hmga2*) was thus identified as a potential candidate due to its stably higher expression in fetal vs adult HSCs. We therefore hypothesized that *Hmga2* is required for the greater in vivo self-renewal of fetal-stage HSCs.

Methods: *Hmga2*^{-/-} adult and fetal mice were compared to their wild-type controls with respect to total hematopoietic organ counts (i.e., bone marrow and fetal liver, respectively). HSC frequencies were determined from FACS-analysis of the E-SLAM population. In vivo HSC reconstitution rates were determined by transplantation of HSCs into sublethally-irradiated *W41/W41* mice followed by secondary limiting-dilution transplantations.

Results: As an initial inquiry into whether *Hmga2* is required for the high self-renewal activity of fetal HSCs, relative and absolute numbers of HSCs were compared between *Hmga2*^{-/-} and *Hmga2*^{+/+} (wt) controls. Although HSC (E-SLAM) frequencies were similar, absolute numbers of HSCs were significantly lower for *Hmga2*^{-/-} mice due to their ~5-fold lower absolute number of bone marrow cells. To determine if the difference in absolute HSC numbers represents an intrinsic deficit in the rate of HSC expansion during the fetal and neonatal period, ~10 *Hmga2*^{+/+} or wt E14.5 fetal liver HSCs were transplanted and the HSC number generated in vivo measured 6 weeks later. These similar numbers of transplanted *Hmga2*^{-/-} vs wt cells produced significantly fewer daughter HSCs in vivo

($p < 0.001$) thereby supporting the hypothesis that *Hmga2* is required for the high self-renewal of fetal HSCs.

Conclusion: High expression of *Hmga2* is a necessary and distinguishing feature of the high self-renewal characteristic of fetal HSCs that is decreased in later life.

(Supported by the Terry Fox Foundation, the Michael Smith Foundation for Health Research and Canadian Institutes for Health Research; M.C. is supported by a BC Cancer Agency-CIHR-UBC MD/PhD Studentship Award and CIHR-Vanier CGS Award.)

2-01 The Role of Heat Shock Protein 90 in *Candida albicans* cell cycle progression

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Background: The trimorphic fungus *Candida albicans* is the leading cause of systemic candidiasis, a disease with poor prognosis affecting immunocompromised patients. The capacity to switch between different growth morphologies is tightly coupled to its ability to cause life-threatening infection. Recently, the molecular chaperone Heat Shock Protein 90 (Hsp90) has been implicated as a major regulator of *C. albicans* morphogenesis via the Ras1-PKA pathway. Filamentous growth induced by Hsp90 inhibition cannot be readily identified as multicellular pseudohyphae or hyphae, but rather bears a striking resemblance to polarized growth induced by cell-cycle arrest. We set out to explore cell cycle perturbation as a potential mechanism of polarized growth induction.

Methods: Hsp90 function was compromised in *C. albicans* both by pharmacological inhibition and by reducing Hsp90 expression. Cells with compromised Hsp90 function were stained to visualize nuclei (propidium iodide) and septa between cells (calcofluor white). Spindle morphology was assessed using indirect immunofluorescence of tubulin. YFP-tagged Cdc3 (a septin) and Mlc1 (a marker of cytokinesis) were used to further assess progression through the cell cycle. To compliment microscopy, FACS analysis using propidium iodide to quantify nuclear DNA content was used to monitor cell cycle progression in a population of synchronous cells. In response to Hsp90 depletion, levels of the yeast cyclin-dependent kinase, Ccd28, and two mitotic cyclins were assessed by Western blot.

Results: Filaments induced by Hsp90 compromise almost exclusively contained two nuclei and no septa. Assessment of spindle morphology suggested these highly-elongated cells were delayed in late anaphase. A second, less common aberrant morphology was identified, a mother cell with a two-lobed daughter cell. This form was multinucleate and had defects in

cytokinesis. Consistent with microscopic observations, FACS analysis demonstrated distinct 4n and higher ploidy populations. Levels of the CDK Cdc28 were reduced in response to Hsp90 compromise, as were levels of the mitotic cyclin Clb4. Together, these results suggest roles for Hsp90 in mitotic exit and cytokinesis in *C. albicans*.

Conclusion: In the major human fungal pathogen *C. albicans*, Hsp90 is known to play important roles in morphogenesis and the evolution and maintenance of drug resistance. In animal models of systemic infection, efficacy of common antifungal drugs against *C. albicans* is increased when Hsp90 function is compromised. Here, we show that Hsp90 plays an important role in cell cycle progression in *C. albicans* such that its depletion causes a significant delay in late mitosis. This work sheds light on Hsp90's many roles in this important pathogen, and supports the therapeutic potential of Hsp90 as a drug target for major *C. albicans* infections.

2-02 Preclinical development of reovirus as a novel therapeutic for renal cell carcinoma

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Background: Reovirus (RV) is a novel oncolytic virus currently being investigated for the treatment of a variety of malignancies. Metastatic renal cell carcinoma (RCC) is an excellent target for RV therapy as this type of cancer responds to immunotherapy and displays upregulated survival signalling, which is a hallmark of RV sensitivity. In this study, we sought to characterize the response of RCC to RV as a monotherapy and in combination with sunitinib (VEGF/PDGF inhibitor), a first line agent currently used to treat metastatic RCC.

Methods: *In vitro*, a panel of RCC cell lines (786-O, A498, ACHN, Caki-1, Caki-2, RENCA) were treated with escalating doses of RV, sunitinib or a combination of these agents. Cytotoxicity, apoptosis and viral progeny release were assessed via the WST-1, TUNEL and plaque titration assays, respectively. *In vivo*, 6-8 week old immunocompetent BALB-c mice bearing subcutaneous RENCA RCC tumours were treated with intravenous RV [1.0×10^8 pfu/injection x 5], daily oral sunitinib [10 mg/kg] or a combination of these agents. Mice were followed for overall survival and tumour burden. Flow cytometric and immunohistochemical analysis on harvested tumour samples and blood was conducted to determine the immunological response to RV/combination therapy.

Results: RV induced cytotoxicity *in vitro* at 40 multiplicity of infection in all cell lines. RV replication and production of oncolytic infection *in vitro* in RCC was confirmed via viral progeny assays. Interestingly, the combination of RV with sunitinib resulted in an antagonistic cytotoxic effect *in vitro* in all cell lines tested [Combination Index > 1]. *In vivo*, intravenous administration of RV to immunocompetent mice resulted in a decreased tumour burden compared to control mice at day 18 [194 ± 25 mm² vs. 136 ± 12 mm²]. Experiments to assess the effects of administering combined reovirus/sunitinib *in vivo* are ongoing.

Conclusion: Our data show that RCC is responsive to RV both *in vitro* and *in vivo*. Further preclinical and clinical experimentation with RV in RCC is warranted. These preclinical results will be used to initiate a phase I/II clinical trial for the clinical use of RV in RCC.

2-03 Avian pathogenic Escherichia coli and human extraintestinal infections

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Background: Human extraintestinal pathogenic *Escherichia coli* (ExPEC) infections have high morbidity and mortality and are increasingly difficult to treat due to the development of antimicrobial resistance. Public health efforts must therefore focus on preventing ExPEC infections by identifying potential sources. Avian pathogenic *E. coli* (APEC), an ExPEC subtype that contaminates retail meat, may promote human extraintestinal infections by transferring virulence genes to ExPEC colonizing the human host thereby increasing its pathogenicity or by directly infecting humans. To evaluate the hypothesis that APEC promotes human extraintestinal infection the virulence gene profiles and sequence homologies of APEC and human ExPEC were investigated.

Methods: 27 APEC isolates collected in Ontario and Quebec were screened for virulence genes by microarray. Their virulence gene profiles were compared with those of 149 human ExPEC isolates collected from clinical urinary tract infections across Canada and 89 commensal *E. coli* isolates collected from avian, bovine, porcine, and human sources collected across Canada that had been previously determined by collaborators. Ten isolates from each source were selected for analysis of the

virulence gene *fimH*, which was amplified by PCR and sequenced. Genetic comparison of *fimH* sequences (n=60) was performed for nucleotide and deduced amino acid sequences.

Results: The virulence gene distribution among the APEC isolates was diverse overall, with conservation of certain virulence genes (*iss*, *gad*, *csgA*) among all isolates. Approximately 65% of virulence genes were present in both APEC and human ExPEC. Sequence analysis of *fimH* indicated no obvious clustering between APEC and human ExPEC at the nucleotide or amino acid level, suggesting little overlap in the *fimH* sequences present in these pathogens. One APEC isolate was observed to share identical *fimH* sequences with four human ExPEC isolates. However, commensal *E. coli* isolates from porcine and avian sources were also present in this genetic cluster indicating that the association was not unique to APEC and human ExPEC.

Conclusion: Transfer of virulence genes from APEC to human ExPEC appears infrequent and likely does not contribute to a major proportion of clinical extraintestinal infection in humans. However, potential genetic similarities were observed between virulence genes of human ExPEC and commensal *E. coli* from pork and chicken, suggesting that additional virulence gene reservoirs promoting human extraintestinal infection may exist in *E. coli* from retail meat.

2-04 Culture-enriched molecular profiling of the cystic fibrosis airway microbiome

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Objectives/Introduction: The microbiome of the respiratory tract, including the nasopharyngeal and oropharyngeal microbiota, is a dynamic community of microorganisms that is highly diverse. The cystic fibrosis (CF) airway microbiome is the polymicrobial communities present in the lower airways of CF patients. It is comprised of chronic opportunistic pathogens (such as *Pseudomonas aeruginosa*) and a variety of organisms derived mostly from the normal microbiota of the upper respiratory tract. The complexity of these communities has been inferred primarily from culture-independent molecular

profiling. We proposed that culture-enriched molecular profiling (CIMP) would reveal the true complexity of the upper airway CF microbiome.

Methods: To evaluate the cultivability of the airway microbiome directly, we examined six sputum samples in depth using culture-enriched molecular profiling, which combines culture-based methods with the molecular profiling methods of terminal restriction fragment length polymorphisms and metagenomic 16S rRNA gene sequencing.

Results: We found that combining culture-dependent and culture-independent approaches enhances the sensitivity of either approach alone. Our techniques were able to cultivate 43 of the 48 families detected by deep sequencing; the five families recovered solely by culture-independent approaches were all present at very low abundance (< 0.002% total sequence reads). 46% of the molecular signatures detected by culture from the six patients were only identified in an anaerobic environment, suggesting that a large proportion of the cultured airway community is composed of obligate anaerobes.

Conclusions: We demonstrate that the majority of bacteria in the CF airway microbiome are amenable to culturing, suggesting that culture-enriched molecular profiling is useful for the recovery of rare members of the human microbiome. Understanding the function of the microbiome in health and diseases will be facilitated by the ability to grow these organisms in either pure or mixed culture.

2-05 Biophysical Investigation of the Potential Antimicrobial Properties of ApoLp-III from Locust Migatoratoria

Chiu, Michael (University of Calgary MD/MSc, Calgary, AB, CAN)

Objectives: Studies with human, bovine and fish plasma have demonstrated bactericidal activity related to ApoA-I and ApoA-II respectively. Apolipoprotein III (apoLp-III) is an apolipoprotein involved in lipid transport in *Locust migatoratoria*. We propose that due to the amphipathic helical nature of ApoLp-III it will preferentially interact with bacterial biomimetic membranes over mammalian models providing insight into its potential antimicrobial activity or as a template for future antimicrobial peptides.

Methods: ApoLp-III was recombinantly expressed in *E. coli*. Large Unilamellar Vesicles (LUVs) mimetic of mammalian membranes were comprised of phosphatidylcholine (PC) and cholesterol mixtures whereas phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) mixtures are used for bacterial

models. ApoLp-III-LUV interactions were monitored using calorimetry, fluorescence and circular dichroism.

Results & Conclusions: Using isothermal titration calorimetry strong interactions were found between the protein and the anionic phosphatidylglycerol vesicles with weaker binding observed for the mammalian systems. Dye leakage assays showed a strong perturbation of the bacterial membranes compared to the mammalian with small quantities of ApoLp-III inducing over 25% leakage. This result coincides with differential scanning calorimetry results, as low corresponding concentrations resulted in complete abolishment of the biomimetic bacterial lipid phase transition. Circular dichroism indicated a helical profile upon binding all vesicle systems. However, an increased helical profile was observed with the bacterial membranes. These studies demonstrate ApoLp-III preferentially interacts with anionic PG lipids (bacterial model membranes) leading to bilayer disruption and leakage of intracellular contents demonstrating its potential for use as an antimicrobial template.

2-06 A microarray-based approach to identifying novel mutations leading to congenital renal dysplasia

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Background: Congenital renal malformations affect three out of every 1,000 babies born. Renal malformations are characterized by failed development of nephrons, the functional units of the kidney. With an insufficient complement of nephrons, a baby may die in utero or may require a kidney transplant during childhood. The genetic basis of congenital renal malformation is unknown in most cases.

Purpose: We aim to identify novel genetic mutations that cause congenital renal malformations in humans by a genome-wide experimental approach.

Methods: Genomic DNA samples from 16 pediatric patients (8 male, 8 female) presenting renal malformation plus two other congenital defects were analyzed by array-based compara-

tive genomic hybridization (CGH). CGH data were compared against public copy-number variant (CNV) databases (e.g., HapMap) to identify mutations not represented in the general population. We considered a CNV to be rare if it is not overlapped more than 50% by a CNV in the control population. We selected genes overlapping with rare CNVs for follow-up gene expression and knockdown analyses in the mouse to test whether they regulate kidney development.

Results: We identified 25 rare CNVs among the 16 subjects. Twelve of these overlap coding regions in the genome. Two of this subset are microdeletions (>1 million base pairs in size) that overlap multiple genes. A 4-million base-pair deletion, seen in a patient with unilateral kidney dysplasia, overlaps 87 genes at 1p36.2. This region has been implicated in Zellweger syndrome (MIM #214100), which includes renal malformations. A second microdeletion, in a patient with unilateral fused kidneys, overlaps 32 genes in 16q24. None of these genes has previously been associated with congenital kidney defects. Each of the 10 other CNVs of interest overlaps only a single gene locus. Seven of the affected genes have a homolog that is expressed in the embryonic kidney (E14.5) of the mouse: *WDR36*, *TSLP*, *WWOX*, *VTAI*, *SIK1*, *LACTB2*, and *PTPRD*. The latter three are specifically expressed in mouse ureteric buds, the source of nephrogenic signals in the kidney, and thus may regulate renal development.

Conclusions: We have used array-based CGH to identify novel genetic mutations that may underlie renal malformations in humans. We are currently investigating the effect of each CNV on kidney development by morpholino-mediated gene knockdown in mouse embryonic kidney explants. Data from these experiments will be discussed.

2-07 The role of MicroRNAs in Cutaneous Squamous Cell Carcinomas

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Background: Non-melanoma skin cancer (NMSC) is the most common form of cancer worldwide. While ultraviolet radiation is the primary risk factor for developing NMSC, much remains unknown about the process of skin carcinogenesis. miRNAs are small, non-coding RNAs that regulate gene expression. Certain miRNAs function as oncogenes or tumour suppressors, yet their role in cancer development remains un-

clear. Herein, we investigate the role of miRNAs in cutaneous squamous cell carcinomas (SCC).

Methods: To compare the miRNA profile of normal human keratinocytes (NHK) and cutaneous SCC cells, total miRNA was extracted from commercially available cell lines. Using μ Paraflo® Microfluidic Biochip technology and probe content based on the Sanger miRBase Version 12.0, a genome-wide miRNA microarray analysis was performed.

Specific miRNAs were exogenously altered in SCC cells using miRNA mimics and inhibitors. The effects of miRNA deregulation on proliferation, differentiation, apoptosis and invasion were examined.

Results: Of 856 miRNAs studied, 27 miRNAs displayed high signal intensities and were significantly up- or down-regulated in the SCC cell lines. While the majority of miRNAs in the genome remain uncharacterized, several of the miRNAs altered in SCCs are implicated in carcinogenesis. Preliminary data suggest that exogenous up-regulation of miR-125b in SCCs induces a loss of cellular differentiation that results in a more invasive phenotype.

Conclusions: The identification of unique miRNA profiles within NHKs and SCCs allows our dataset to serve as a roadmap for future studies of the microRNAome. By characterizing the specific miRNAs associated with SCCs, molecular insight will be gained into the process of skin cancer development.

2-08 The role of *Chrna6* in Chronic Pain

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Background/Purpose: Chronic pain is an exceedingly prevalent and costly health problem. It is widely acknowledged that significant interindividual variability exists in an individual's propensity to experience chronic pain following similar insults. Since much of this variability is genetic, the identification of genes predisposing to severity of symptoms of chronic pain could be of great value in terms of early intervention and drug development.

Methods: To identify potential candidate genes that may account for the great interindividual variability, we conducted an expression genomics study, and observed strong correlations between dorsal root ganglion (DRG) *Chrna6* (cholinergic receptor, nicotinic, alpha 6 subunit) gene expression and neuropathic mechanical allodynia following spared nerve injury (SNI). *Chrna6* null mutant mice and gain-of-function mutant mice were tested for mechanical allodynia following SNI and intraplantar complete Freund's adjuvant (CFA) injection to causally establish a role for *Chrna6* in chronic pain.

Results: Correlation analysis of SNI allodynia in 25 inbred mouse strains with basal DRG expression revealed that the top two correlations, genome-wide, were with two different probes for *Chrna6*. Subsequent RT-PCR experiments revealed that DRG *Chrna6* expression is >10-fold higher than whole brain expression and 2fold greater than eye expression. Behavioural testing for both SNI and CFA revealed that *Chrna6* knockout mice displayed higher overall levels of allodynia as compared to wildtype mice, while *Chrna6* gain-of-function mutant mice displayed significantly less allodynia.

Conclusions: The findings presented here demonstrate the protective role of *Chrna6* in chronic pain. Ultimately, by identifying and manipulating those genes responsible for inducing variable levels of chronic pain, such as *Chrna6*, novel therapeutic approaches and drugs can be developed that target the symptoms of debilitating chronic pain conditions.

2-09 Use of next-generation sequencing to study epithelioid sarcoma

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Background: Advancement of sequencing technologies in the past few years has given rise to the next-generation sequencing era. From the 454, SOLiD, and Illumina platforms to the more recent single molecule real time sequencing, we are now able to sequence whole genomes for lower costs. With access to the Illumina platform, we focused on sarcomas. They serve as excellent paradigms to study cancer biology and sequencing analysis as many seem to have characteristic driving mutations, such as the SSX-SS18 of synovial sarcoma and the EWS-FLI1 of Ewing sarcoma.

A relatively understudied sarcoma is epithelioid sarcoma. This tumor, which is of mesenchymal origin but displays epithelial morphology, metastasizes in 40% of patients (1). The median overall survival is 88 months for those without metastasis and only 8 months with metastasis (1). Current therapy includes surgical resection, non-targeted chemotherapy and radiation (1). The characteristic finding of this disease is loss of INI1 expression (2), a tumor suppressor member of the SWI/SNF chromatin remodeling complex; however, gene sequencing results do not seem to be able to explain this finding at the

DNA level (3) while there have been prior reports of reduced INI-1 transcripts (4).

Methods: Five epithelioid sarcoma samples and a cell-line were submitted for whole transcriptome shotgun sequencing (WTSS). RNA from tumor samples was extracted using miRNeasy minikit (Qiagen) or trizol extraction. DNA from tumor samples was also submitted for SNP 6.0 array analysis. A two step QRT-PCR on the same samples was performed with primers for ex5-6 of INI1. Results were normalized to GAPDH and expressed relative to Hela expression levels. INI-1 IHC was carried out on the available paraffin blocks.

Results: Several fusions were identified in two of the epithelioid sarcomas whose WTSS has been finished including INI1-WASF2 in one case, and ERBB3-CRADD, HNF1A-CMKLR1 in another. These were confirmed by PCR. SNP6.0 results showed reduced copy number of INI1 in the cell line and 2 of 5 tumor samples. IHC analysis carried out in 2 of the 5 samples showed loss of INI1 expression in one case and interestingly the presence of this protein in the case with the INI1-WASF2 fusion (V06). QRT-PCR results revealed reduced expression of INI-1 in 3 of 5 samples and the cell line.

Conclusion: Our results suggest that INI1 has a critical role in the carcinogenesis of epithelioid sarcoma. Loss of copy number and fusion mutations involving this gene were identified. The fusion mutation of V06 could potentially have a dominant negative effect as the expression of INI-1 is maintained based on IHC. Furthermore, expression analysis showed reduced expression in 3 of 5 tumors and 1 cell line. We are currently carrying out transfection and molecular analysis of INI1 in the cell-line to further characterize the role of this gene in epithelioid sarcoma.

3-01 Methods for measuring oppression: A scoping literature review of Aboriginal population health research

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Background: In an evidence-based approach to population health interventions, evidence must emerge from scientifically rigorous and context-relevant studies. The context of Aboriginal women's health includes intersecting sources of oppression from race, gender, social exclusion and the legacy of coloniza-

tion. Oppression can lead to poor health outcomes through pathways including disadvantage along the social determinants of health, barriers to health care access and healthy living, unhealthy coping behaviours, and stress pathophysiology. Oppression is recognized theoretically as crucial for understanding and addressing Aboriginal women's health; however, relatively few quantitative studies assess it. Empirical methods of measurement are required to do so.

Objective: To explore existing methods, we performed a scoping literature review to examine how concepts of oppression have been measured in Aboriginal population health studies. This scoping review is the first step of a project aimed at developing an approach to comprehensively quantify oppression in epidemiological research in Aboriginal women's health.

Methods: Systematic database searches of the peer-reviewed literature were conducted to identify eligible quantitative studies in Canada, USA, Australia and New Zealand. Information from each paper was abstracted and charted. The measurement methods found were summarized and analyzed for strengths and weaknesses in light of relevant theoretical and qualitative literature.

Findings: Perceived racism, racist attitudes, and domestic and sexual violence have been measured with various scales and indicator questions. Individual-level and area-level measures of social and economic disadvantage have been used in studies to indicate social exclusion. The multigenerational impacts of colonization have been assessed with scales on historical loss trauma, and indicator questions on parental residential school attendance. Limited are measurements of gender oppression beyond domestic and sexual violence. Furthermore, little emphasis has been placed on measuring the intersections of sources of oppression.

Significance: Through mapping out existing methods, we have identified specific gaps that need to be addressed to allow for comprehensive and appropriate measurement of oppression in Aboriginal health research. Improved measurement of oppression, and consequent understanding of how it can be addressed, will help in the design of effective interventions.

3-02 Perspectives of intravenous drug users on the harms caused by installing blue lights in public washrooms

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Objective: Blue lights are installed in washrooms to discourage public use of intravenous drugs, yet there are no published

studies of the effectiveness and safety of blue lights in achieving their presumed purpose. To fill some of the gap in published knowledge around this issue we engaged IV drug users from two Canadian cities on what they perceive to be the benefits and harms of installing blue lights in public bathrooms.

Study Design: Between January and March 2011, a cohort of 18 current and former injection drug users was recruited purposively through drug user community advocacy groups in Vancouver and Abbotsford, British Columbia. Participants' perceptions of the impacts of blue lights in washrooms were gathered using semi-structured interviews conducted by 5 medical students. Interviews were transcribed and analysed according to the method of interpretive description, with the goal of generating distinct clusters of related concepts nested within an overarching thematic framework.

Results: The majority of drug users interviewed found injecting under blue lights to be difficult and would, consequently, avoid bathrooms fitted with them. Yet, many described a preference for avoiding injecting in public washrooms, regardless of the presence of a blue light. In situations during which they would violate this preference, namely when in the midst of powerful withdrawal symptoms or when they perceived injecting outside to be their only alternative, many would inject under blue light, even knowing it would be more difficult. Drug users identified four categories of harms from blue lights: 1) they make it difficult for users to access their veins while injecting thereby making injecting more dangerous and more likely to cause physical harm; 2) they make users more likely to miss their veins when injecting, thus they force users to seek their next dose of drug earlier than they would otherwise need to, and, trying to acquire this next dose can cause harms to themselves as well as harms to society; 3) by hindering users' ability to inject in public washrooms, they force users to find other more unsafe and more unhygienic places to inject; finally 4) they make it easier for users to get caught and persecuted while using drugs. Despite these harms, many participants were in favour of blue lights in washrooms. This appeared to be tied to a belief that their injecting in washrooms would be harmful to others, and that their own health and safety were less important than preventing these harms to the public.

Conclusions: Installation of blue lights in public washrooms has harmful effects which are borne disproportionately by those injection drug users who are already most at risk of poor health, namely those who are most strongly addicted, and those without access to private spaces to inject. Yet, due to internalized oppression, drug users may be unlikely to initiate efforts to remove these harmful installations.

3-03 Adhering to Clinical Practice Guidelines in the Emergency Department: A review of Congestive Heart Failure, Acute Exacerbations of Chronic Obstructive Pulmonary Disease, and Community Acquired Pneumonia

Tierney, Sarah (Queen's University, Kingston, ON, CAN)

Introduction: We assessed adherence to clinical practice guidelines for the emergency management of patients with acute exacerbations of chronic obstructive pulmonary disease (AECOPD), acute congestive heart failure (CHF), or community acquired pneumonia (CAP) who returned to the emergency department (ED) within 2 weeks.

Methods: We conducted this prospective cohort study in two Canadian tertiary care EDs. We included patients initially discharged from the ED with AECOPD, acute CHF, or CAP who returned within 14 days, and were subsequently discharged, admitted, or died at 30 days (flagged outcomes). An expert ED panel derived a list of critical actions based on national and international clinical practice guidelines for each illness population. A trained medical student abstracted charts. We systematically reviewed all flagged outcomes for adverse events (adverse outcomes related to health care provided). We calculated the number of critical actions completed on the index visit. We used descriptive statistics for data analysis.

Results: Of 1200 patients enrolled, 68 had flagged outcomes [33 (48.5%) AECOPD, 29 (42.6%) CHF, and 6 (8.8%) CAP]. Mean age was 73.8 (SD 9.5), and 47.1% were female. The expert panel identified 10 CHF, 13 AECOPD and 10 CAP critical actions. Emergency physicians appropriately completed critical actions for CAP patients 93.3% of the time (SD 8.2), CHF patients a mean of 90.3% of the time (SD 7.3) and AECOPD patients 73.4% of the time (SD 13.9). There were 20 adverse events (1.7%, 95%CI: 0.9-2.4) and the proportion of adverse events to flagged outcomes was highest among CAP (3/6, 50.0%) patients followed by CHF (8/29, 27.6%) and AECOPD (9/33, 27.3%). Greater adherence to critical actions was not associated with less adverse events.

Conclusions: We found variation in practice when evaluating treatment of AECOPD, acute CHF and CAP against clinical practice guidelines. Knowledge translation efforts are needed particularly for the AECOPD population.

3-04 Re-evaluation of dyslipidemia treatment for young adults with a low CVD risk score

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Background/Purpose: To explore the issues surrounding short term pharmacological treatment, specifically HMG-CoA reductase inhibitors (statins), for secondary dyslipidemia in young adults who are considered mild to moderately at risk of CVD, a Clinical Vignette is presented along with a review of the current clinical practice guidelines and peer reviewed research. Clinical practice guidelines for young adults remains vague when it comes to pharmacological treatment for secondary dyslipidemia in patients considered mild to moderately at risk of CVD. Lifestyle modifications are considered the primary treatment for prevention of CVD in this population until there CVD risk score is increased, either by age or additional risk factors. The hypothesis is that in young adults (<40 years of age) who have secondary dyslipidemia for whom lifestyle modifications have provided little to no benefit, statin use will decrease their current CVD risk by improving their dyslipidemia, and provide greater long term benefit to their CVD risk than lifestyle modifications alone.

Methods: A review of the current Canadian, US and European clinical practice guidelines, along with a review of the literature (Medline, Embase, Cochrane, and ACP) was conducted.

Results: All sources show a general lack of research and information for ages 20-40, unless associated with a primary dyslipidemia. There is consistent data acknowledging the benefit of statin use to long term CVD risk of children and adults, and data acknowledging the need for CVD prevention in young adults. There is also data supporting that preventative measures (including pharmaceutical interventions) initiated in young adults show a positive cost-benefit result. Potential benefits and risks of statin withdrawal in low risk subjects are not well documented.

Conclusion: Improved risk assessment and management for cardiovascular disease among young adults (<40 years) is needed. Watchful waiting with therapeutic lifestyle changes until further CVD risk factors are established should not be the mainstay of treatment. The sequela of future CVD, including progression of atherosclerotic vessels, can be mitigated through earlier statin use in this cohort. These individuals should be provided the option for short term statin use after counselling on the potential benefits and risks.

3-05 Associates with Eye Care Utilization among Canadian Adolescents

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Purpose: Many but not all Canadian provincial health insurance plans cover eye care services for children younger than 18 years of age. We examined how provincial eye care coverage and selected socio-demographic and health factors affect adolescent's access to eye care providers.

Methods: Data was collected from the Canadian Community Health Survey 2007/2008. Utilization of eye care services was measured using the question "[Not counting when you were overnight patient, in the past 12 months], have you seen, or talked to an eye specialist, such as an ophthalmologist or optometrist?" Respondents aged 12-17 were included in the analysis (n=11,015) since 12 was the youngest in the survey. Associations of interest were assessed by prevalence ratios (PR).

Results: Canada wide, 45.6% of adolescents utilized eye care services over a 12 month period. Utilization varied greatly by provincial insured eye care coverage: significantly higher (46.4%) in provinces with routine eye exams covered by provincial insurance plan (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, and Quebec), lower (35.9%) in provinces with routine eye exams not covered by provincial insurance plan (Newfoundland and Labrador, New Brunswick, Nova Scotia, and Prince Edward Island), and lowest (27.1 %) in the three territories with a dearth of eye care professionals (Yukon, North West, and Nunavut).

Compared to adolescents in provinces with routine eye exams covered, those with routine eye exams not covered were 24% less likely to utilize services (PR=0.76, 95% confidence interval (CI): 0.67-0.85); whereas those in the three territories were nearly 40% less likely to utilize eye care providers (PR=0.63, 95% CI: 0.48-0.83). Significantly lower utilization rates were also found among males (10% less likely than females), those with dwellings not owned by a member of the household (19% less likely than those owned), and those reading less than 3 hours weekly (13% less likely than those reading 3 or more hours) not counting school reading. Adolescents with diabetes were 67% more likely to utilize eye care services (PR=1.67,

95% CI: 1.29-2.15) than those unaffected. Lower level of household highest education, spending less than 1 hour on a computer weekly, and having children aged 5 or younger in the household were all associated with a non-statistically significant reduction in utilizing eye care services.

Conclusions: Inequities in eye care utilization were observed amongst Canadian adolescents. Factors associated with significantly less utilization were: routine eye exams not covered, lack of eye care professionals, male gender, living in dwellings not owned, non-diabetic, and spending less time reading.

3-07 Canadian health professional education lacking in Teaching on Canada's health care system: Health professional student survey

Alston, Jillian (University of Toronto, Toronto, CAN)

Background: Canada's health care system shapes the well-being of patients, health professionals work, and likely their career satisfaction. Thus, it is essential that students in health professional programs be equipped with the necessary knowledge to actively participate in shaping the health care system and to advocate for optimal care and population health outcomes.

Canadian medical students, in particular, are expected to have a basic knowledge of the health care system, which includes an understanding of the principles, federal, and provincial legislative framework and laws governing the health care system.

However, health policy teaching in medical school curricula lags behind the dynamic discussions that the public has pertaining to our health care system and its current issues. The purpose of this study was to assess the opinions of health professional students on the coverage in their formal curriculum of issues pertaining to the Canadian health care system, as well as other avenues through which they sought information.

Methods: Anonymous online surveys were distributed to health professional programs in Ontario. All six medical schools in Ontario were contacted, and surveys distributed electronically over medical student email listservs. The survey assessed the opinions of these students on the coverage in their formal curriculum of issues pertaining to the Canadian health care system, as well as other avenues through which they sought information.

Results: There were 828 students who completed the survey. These students showed an interest in learning more about current issues in health care. The majority of students were unsatisfied with their formal education on Canada's health care system. Those who were more comfortable with Canada's health care system sought out informal sources for information (Figure 1).

ure 1).

Conclusions: This survey suggests that education on this topic is quite lacking. Further formal studies in this area are required to inform curriculum development committees.

3-08 How does the metabolic syndrome defined by two definitions predict Cardiovascular Disease mortality?

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Background: No studies have compared the ability of the National Cholesterol Education Program Adult Treatment Panel (ATP III) and the International Diabetes Federation (IDF) definitions of metabolic syndrome (MetS) to predict CVD mortality.

Objective: The purpose of this study was to examine the prevalence of the component factors of MetS and the relation between MetS and CVD mortality using the ATP III and the IDF definitions.

Methods: The Canadian Heart Health Survey was a cross-sectional study conducted in all 10 Canadian provinces (1986-1992). Statistics Canada linked the CHHS data set to the Canadian Mortality Database. The present study is based on 2553 men and 2644 women from three provinces (Manitoba, Ontario, and Saskatchewan) for whom full anthropometric measurements, mortality data, and data on all components of MetS were available. MetS was defined according to ATP III and IDF definitions. Cox-regression analyses was conducted.

Results: In women with metabolic syndrome according to the IDF definition 81.9%, 76.6%, 71.7%, and 14.4% have low high-density cholesterol, high triglyceride, hypertension, and diabetes, respectively, versus 84.5%, 80.3%, 78.4%, and 19.3% according to ATP III (P-value $\leq$ 0.001). In men, the comparable levels of prevalence are 71.7%, 85.1 %, 71.5%, and 10.5% according to IDF and 78.7%, 89.3%, 85.0%, and 13.1% according to ATP III (P-value $\leq$ 0.001). The hazard ratio (95% CI) of death due to CVD events in women with MetS according to the ATP III and the IDF definitions are 3.96(1.30-12.09) and 2.56 (1.32-4.97), respectively. The comparable numbers for men are 2.21(1.16-4.02) and 2.50(1.50-4.17).

Conclusion: The prevalence of MetS is higher when the IDF definition is applied but the metabolic derangement of individuals identified is less severe. The ATP III definition predicts CVD mortality better in women, while the IDF definition predicts CVD mortality better in men.

3-09 Differentiating between Bipolar Disorder Types I and II: results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC)

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Background: Bipolar Disorder I (BDI) and Bipolar Disorder II (BDII) vary considerably, with differences in symptomatology, management and prognosis. For patients with depression, the distinction between BDI and BDII is not always apparent, and hinges on the differentiation between manic/mixed and hypomanic episodes. The current analysis compares demographics, clinical features, depressive symptoms and co-morbid conditions between these two population groups.

Methods: Data were obtained from the National Epidemiological Survey on Alcohol and Related Conditions, which is a large cross-sectional survey of a representative sample (N=43,093) of the U.S. population. A total of 1,429 subjects were included in our analysis based on DSM-IV criteria, 935 with BDI and 494 with BDII.

Results: Key differences between BDI and BDII were identified in all categories in our comparison of means. In the regression analysis, a number of variables were determined to be predictors of BDI, including unemployment (OR=0.6), taking medications for depression (OR=1.7), a history of a suicide attempt (OR=1.8), depressive symptoms such as weight gain (OR=1.7), psychomotor agitation such as fidgeting (OR=1.5), feelings of worthlessness (OR=1.6) and difficulties with responsibilities (OR=2.2), as well as the presence of specific phobias (OR=1.8) and cluster C personality traits (OR=1.4).

Conclusions: There are significant differences between bipolar disorder types I and II in demographics, clinical features, depressive symptomatology, and co-morbidities that can help distinguish these separate conditions in addition to findings of manic/mixed and hypomanic episodes. Increased recognition of these disorders as distinct clinical entities can lead to substantial improvements in management and prognosis.

3-10 Exploring the optimal design and implementation of computerized clinical decision support.

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Background: Computerized clinical decision support systems (CCDSSs) promise to improve care processes and patient outcomes by delivering timely patient-specific advice to practitioners at the point of care. Unfortunately, results from rigorous evaluations remain mixed and little is known about what makes an effective system. Despite this, clinical decision support functionality has become tied to much public money invested in the adoption of electronic medical records in North America. We set out to determine if CCDSSs are effective at improving care processes or patient outcomes, and to identify features that determine success.

Methods: A systematic review of randomized trials comparing use of CCDSSs to non-CCDSS controls in clinical care settings. We conducted literature searches to January 2010 in bibliographic databases and scanned reference lists for eligible articles. Guided by partnerships with clinicians, senior hospital administrators, and CCDSS researchers, we extracted over 50 trial and system features that may determine success. Our pre-specified logistic model tested the importance of integration with clinical workflow, feedback at the time of care, presenting advice within electronic charting or order entry systems, demanding a reason for ignoring the advice, provision of advice to both patients and practitioners, and the authors of the study also being the developers of the system. A secondary analysis will test the importance of an additional 10 features selected by surveying the primary authors of all included studies.

Results: We included 166 RCTs. 90% (n=150) measured impact on the process of care and 58% (n=87) of these showed improvement. 50% (n=83) measured (typically non-major) clinical outcomes but only 23% (n=19) of them found benefit. Our preliminary statistical model discovered new associations and refuted ones previously thought to be true.

Conclusions: CCDSSs improved the process of care in a small majority of studies but this rarely translated to benefits for patients. Our statistical models test current assumptions and provide much needed empirical guidance on optimal design and implementation of CCDSS. This is the largest review of CCDSS to date with the power to reliably discover more associations than any previous work and the results will be of inter-

est to health system decision makers, system developers, hospital administrators, and clinicians considering local adoption of electronic systems.

3-11 Feasibility Assessment for Implementation of Heart Failure Clinical Caremaps using Electronic Medical Records in Primary Practice

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Background and aims: Heart failure (HF) affects over 350,000 Canadians and is associated with significant mortality and health-care costs. Most community-dwelling HF patients in Canada are followed by family physicians (FP). Current evidence-based treatments for HF management are often not fully implemented in clinical practice. The primary aim of this project is to ascertain the level of use and impact of Electronic Medical Records (EMR) in the family health network in Hamilton, Ontario specifically with respect to management of HF. This project provides necessary pilot work towards successful implementation of HF clinical caremaps in EMRs to provide decision making support for family physicians.

Methods and results: A survey was conducted and sent to 207 family physicians within the Family Medicine Association of Hamilton (FMAH) out of which 42 replies were received. The survey included questions regarding basic demographics of the family practices, specifics about HF patients and their management as well as EMR use, its advantages/ disadvantages and whether the use of EMR has improved management of HF patients. Among the 42 physicians which replied, the significant majority (92.9%) practices in a large urban area such as Hamilton, Ontario and have over 10 confirmed HF patients at their family practice exemplifying the critical need for proper management of HF at the primary care level. In terms of EMR use, the large majority of physicians (71.4%) were using EMR in their clinical practice and the most commonly used EMR for these physicians was practice solutions (60.0%). There was no general consensus on whether EMRs have helped in improving the management of HF patients but there is a definite need for decision making support for physicians in managing HF patients.

Conclusions: There is a large amount of family practices currently employing EMRs and this number is expected to rise in the coming years. As such, there is a definite need for management tools which can be integrated into these EMRs to provide decision making support for physicians in managing HF. Many of the topics and medications regarding HF can be diffi-

cult to manage for family physicians and tools such as caremaps can help provide optimal care as per the Canadian Cardiovascular Society (CCS) guidelines.

3-13 Assessment and Treatment of Thyroid Function in Calgary Heart Failure Clinics

Shafran, Daniel (University of Calgary, Calgary, AB, CAN); Isaac, DL (University of Calgary, Calgary, CAN)

Background: Abnormal thyroid function (TF) is associated with cardiac dysfunction. International Heart Failure (HF) guidelines include routine assessment and treatment of TF. In small single-centre studies only 36% of patients pre-ventricular assist device and 40% of patients in an American HF clinic had TF appropriately assessed. HF clinics provide specialized care according to established guidelines; it is unclear to what extent TF is monitored in Canadian clinics, what the incidence of abnormal TF is in HF patients, and how TF abnormalities impact patient outcomes.

Methods: Retrospective review of electronic records and clinic charts of all patients at 3 hospital-based HF clinics in Calgary from November 2010 to January 2011. Thyroid function testing (TFT), use of thyroid-altering medication, and decompensation of HF (use of IV diuretics, doubling of oral diuretic dose, ER visit or hospitalization for HF in the previous 12 months) was assessed.

Results: Records of 773 patients were reviewed. Of these, 719 (93.0%) had some documentation of TFT; but only 592 (76.6%) had TFT in the previous 12 months. Variability existed between sites; the 3 clinics performed TFT in 69.4%, 84.4%, and 96.6% of patients respectively. Of all 773 patients, 21.3% (165) had documented abnormal TF, with 63% (104) adequately treated (normal TSH on treatment), 18.8% (31) inadequately treated, and 18.2% (30) getting no treatment. Of 658 patients with normal TFT, whether treated or not, 199 (30.2%) decompensated compared with 25 (41.0%) of those with abnormal TFT ($P=0.1109$). Decompensation rates in patients with normal TFT vs patients whose TFT was abnormal or never measured were 199 (30.2%) and 55 (47.8%) respectively ($P=0.0003$).

Conclusion: 1) Calgary HF clinics perform TFT according to guidelines in a greater proportion of patients than reported elsewhere, though variability exists between clinics. Annual TF testing in HF clinic patients should near 100%; this may be achieved via education and standard clinic protocols; 2) Abnormal TF is common in Calgary HF clinic patients; 3) Reducing HF decompensation improves outcomes and reduces health care costs. The rate of HF decompensation is signifi-

cantly lower in patients with normal TFT than in those with unmeasured or abnormal TFT. Additional investigation is required to further evaluate the role of assessment and treatment of TF in reducing HF decompensation.

4-01 CAV3-Kv4 signaling complexes act to control neuronal excitability during fluctuations in extracellular calcium

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Purpose: A-type potassium currents and T-type calcium currents play a critical role in determining neuronal output. Kv4 channels exist as a complex with K⁺ Channel Interacting Proteins (KChIPs). We have recently demonstrated that Cav3 channels are coupled to the Kv4 complex and act as a calcium source to regulate Kv4 inactivation. Here we show that the gain of stellate cell firing is dynamically modulated by physiological fluctuations in extracellular calcium through the action of the Cav3-Kv4 signaling complex.

Methods: A-type and T-type currents were recorded from stellate cells in P15-P20 rat cerebellar slices and from tsA-201 cells co-transfected with Kv4, Cav3, KChIP, and DPP10 cDNAs.

Results: Whole cell recordings of stellate cell *I_A* revealed that Kv4 inactivation followed a sigmoidal relationship with changes in extracellular calcium. Similarly, in tsA-201 cells expressing Cav3-Kv4 complex members, Kv4 inactivation tracked extracellular calcium in a sigmoidal fashion. When KChIP3 was omitted from the transfection, this relationship was abolished. Previously, we demonstrated that internal perfusion of antibody directed at KChIP3 increased the gain of stellate firing by roughly two-fold. Using dynamic clamp, we extend those findings here and show that the gain of stellate firing is dynamically modulated by fluctuations in extracellular gain.

Conclusions: Physiological fluctuations in extracellular calcium dynamically modulate the gain of stellate cell firing through the concerted action of the Cav3-Kv4 signaling complex. Decreases in extracellular calcium negatively influence synaptic transmission, through decreased neurotransmitter release. From our preliminary evidence, we now predict that stellate cells will be able to compensate for reduced neurotransmitter input by increasing their gain of firing through the action of the Cav3-Kv4 signaling complex. Ultimately, this property will allow for stellate cells to maintain a stable output, irrespective of fluctuating synaptic input.

4-02 Development of a lentiviral-based cellular barcoding strategy to examine the spectrum of in vivo growth and differentiation potential of normal human mammary stem cells

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Background: Current evidence supports a hierarchical model of mammary epithelial cell differentiation in which self-renewing bipotent stem cells generate unipotent, lineage-restricted progenitors that ultimately mature into non-dividing myoepithelial and luminal cells incapable of further division. However, in humans, in particular, this model is based on a limited number of in vivo clonal tracking experiments and very little is known about the absolute growth and lineage differentiation potentialities of individual mammary cells.

Methods/Results: To develop a more comprehensive approach to addressing this question than is possible with limiting dilution assays, we have developed a cellular barcoding strategy to track the composition of clonally generated progeny from large numbers of co-transplanted stem cells in a xenotransplant setting. First, a library of non-coding DNA-based cellular barcodes was established in bacterial clones transformed with barcode-containing lentiviral vectors. Sequencing results from a first set of 230 individual clones within a larger barcode lentiviral library established that these were all different without any detectable redundancy or presence of clones lacking a barcode. Preparation of the larger lentiviral library yielded an estimated 1 million unique barcode-containing bacterial clones. Optimization of conditions for lentiviral transduction of primary human mammary epithelial cells minimized the frequency of double barcode integrations, and demonstrated that there was no difference between susceptibility of lentiviral transduction between luminal progenitor and basal mammary

stem-cell enriched fractions. Transduced mammary stem cell-enriched fractions of cells have been transplanted under the kidney capsule of highly immunodeficient NOD-SCID/IL-2R γ C-null mice and the cells regenerated will then be assessed in vitro and in secondary transplants to track the clonal distributions of different lineages of cells produced in primary and secondary transplants.

Conclusions: We have produced a powerful tool and transduction protocol suitable for performing simultaneous clonal tracking studies on a large number of individual normal human mammary stem cells. Application of this methodology to primary and secondary xenotransplants should provide new insights into the proliferative potential of lineage restricted and bipotent cells isolated from the normal human mammary gland.

L.V.N. is supported by a BC Cancer Agency – Vanier Canada Graduate Scholarship – UBC MD/PhD Studentship Award.

4-03 Intravascular Neutrophil Extracellular Traps (NETs) protect against bacterial dissemination in Gram-negative sepsis

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Background: A key feature of the systemic inflammatory response syndrome of severe bacterial sepsis is the profound recruitment of neutrophils into the microcirculation of highly vascular organs such as the liver. The reasons for this profound sequestration of neutrophils within the liver remain unknown. We hypothesized that neutrophils are recruited to the liver as part of a coordinated host defense mechanism to protect against bacterial dissemination during septic infections. Specifically, we hypothesized that neutrophils release neutrophil extracellular traps (NETs, extracellular webs of decondensed chromatin covered in anti-microbial proteins) to ensnare bacteria from the bloodstream and prevent dissemination.

Methods: Spinning-disk confocal intravital microscopy was used to visualize the liver microvasculature of anesthetized experimental mice. Various fluorescently-labeled antibodies and selective dyes were used to visualize and quantify neutrophils, platelet-neutrophil interactions, NETs release, and bacterial trapping *in vivo*. Bacterial dissemination was determined 24 hours post-infection (1×10^7 *E. coli* Xen14, i.p.) by quantifying colony-forming units (CFU) of *E. coli* in various organs.

Results: Within 4 hours after the induction of endotoxemia or intraperitoneal *E. coli* infection, NETs were observed within liver sinusoids. NETs release by neutrophils within the liver was dependent on platelet-neutrophil interactions, as depletion of platelets or disruption of a key adhesion molecule mediating platelet-neutrophil interaction (LFA-1) significantly inhibited the generation of NETs (control serum vs. platelet-depleting serum $p < 0.01$; WT vs. LFA-1 $^{-/-}$ $p < 0.05$). In Gram-negative sepsis (*E. coli*, 1×10^7 CFU i.p.), the disruption of NETs production by platelet-depletion, LFA-1 deficiency, or administration of i.v. DNase (to degrade intravascular NETs) resulted in significantly reduced trapping of bacteria in liver sinusoids, together with significantly increased dissemination of bacteria to distant organs (increased CFU in blood and lungs).

Conclusions: During endotoxemia and Gram-negative sepsis, neutrophils are recruited to the sinusoids of the liver where they cast NETs into the vasculature. Intravascular NETs ensnare bacteria from the circulation of septic mice, and protect against bacterial dissemination during Gram-negative sepsis.

4-04 Utilization of Coronary Revascularization among those with and without Schizophrenia

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Objectives: Those with schizophrenia have been shown to be at high risk for coronary artery disease (CAD). Despite a high risk status, this cohort is offered less revascularization than the general population. What is not known is whether the schizophrenia population is under-treated, or whether people with schizophrenia have higher rates of non-occlusive coronary disease. This project sought to determine: 1) The rates of coronary revascularization among those with CAD, with and without schizophrenia; 2) a description of their clinical profiles and coronary anatomy.

Methods: Patients admitted to hospital with a primary diagnosis of schizophrenia between the years of 1995-2009 were identified using the Alberta Health Services Discharge Abstract Database. This cohort of patients was linked to the APPROACH database. People in the schizophrenia cohort were matched with controls (1 case per 4 controls, N=1144) on age, gender, indication and year of catheterization. Extent of coronary disease was assessed using: 1) left ventricular ejection fraction (LVEF); 2) the Duke Jeopardy Score; and 3) the Duke index, an assessment of coronary anatomy risk.

Results: People with schizophrenia carry a higher burden of co-morbid disease than the general population, especially congestive heart failure (21.4% vs. 13.4% $p < 0.003$). Patients with schizophrenia are more likely to have a LVEF in the 20-35% range (9.0 vs. 4.6% $p < 0.004$). Those with schizophrenia are more likely to have “normal” coronaries at catheterization (33.6% vs. 28.5%, $p < 0.27$) and are less likely to have a “high risk” coronary profile (19.7% vs. 26.2%, $p < 0.27$). A smaller proportion of people with schizophrenia received revascularization within one year of catheterization, (45.4% vs. 52.9%, $p < 0.04$). After controlling for co-morbid illness and CAD the hazard ratio is 2.34 (95% CI 1.67-3.28 $p < 0.001$).

Conclusions: People with schizophrenia carry a large burden of co-morbid disease and have a larger rate of systolic dysfunction. This dysfunction is in the context of a less atherosclerotic coronary anatomy profile than the general population. A smaller proportion of people with schizophrenia are treated surgically, and more are treated medically, despite an increased burden of disease. This discrepancy can be partially explained by their healthier coronary anatomy. Overall, there is an increased mortality among those with schizophrenia after controlling for differences in co-morbid illness and CAD.

4-06 Association of Interleukin-6 and Interleukin-8 with Poor Prognosis in Elderly Chronic Lymphocytic Leukemia Patients

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Purpose: Chronic lymphocytic leukemia (CLL) is a disease of the elderly with the median age at diagnosis being 72 years. Prognosis is highly variable with the relative survival progressively worsening after age 70 years. In this study we have evaluated whether the age-related cytokines, IL-6 and IL-8, play a role in the poor survival of elderly patients with CLL.

Methods: The plasma levels and biological effects of the age-related cytokines, IL-6, IL-8 and TNF- α , were evaluated in 193 CLL patients of varying ages. These cytokines were correlated with standard clinical parameters and survival. Causes of death were ascertained to determine whether the elderly died from

CLL-related causes or other comorbidities associated with advanced age. Patient samples were chosen in a sequential order to obtain a cohort that reflects the age and sex-distribution observed in Manitoban population.

Results: As predicted from population studies, the survival of patients progressively worsened with age, with the primary causes of death being CLL or one of its complications, i.e. infection or second tumours. Both IL-6 and IL-8 correlated positively with patient age, and high levels were associated with reduced overall survival. IL-6 was a particularly strong prognostic factor for overall survival in patients aged ≥ 70 years, being a stronger factor than IgVH mutational status. Both IL-6 and IL-8 levels were higher in CLL patients with positive life-time history of cardiovascular diseases, which could be a source of IL-6 and IL-8. The biological effect of higher IL-6 or IL-8 levels in CLL patients leading to reduced survival may be related to the tumor microenvironment. We found that IL-6 and IL-8 could increase adhesion of CLL cells to stromal cells in vitro indicating more aggressive disease.

Conclusions: Taken together, this study indicates that IL-6 and IL-8 are important prognostic markers in elderly patients and might contribute to more aggressive disease. This might be an attractive target for improving the outcomes in elderly patients with CLL.

4-07 Does Perioperative Non-Steroidal Anti-Inflammatory Drug (NSAID) Use Increase the Risk of Anastomotic Leak in Elective Colorectal Surgery?

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Background: To determine whether perioperative non-steroidal anti-inflammatory drug (NSAID) use increased the risk for anastomotic leaks in elective colorectal surgery.

Methods: A case-control study evaluated colorectal surgery patients at a tertiary hospital from July 2006-November 2010. Cases were defined as patients over 18 years with an anastomotic leak within 30-days postoperatively, excluding diverting stomas, pelvic exenterations, and emergency resections. Controls were defined as patients without an anastomotic leak and were matched 3:1 to cases. NSAID exposure was defined as any

NSAID use in the perioperative period. Analysis was done with odds ratios (OR) and 95% confidence intervals (CI).

Results: 60 cases and 180 controls were identified. Cases were 65% male and had a mean age of 65 (SD 17) years. 58% had open surgery, 33% had rectal surgery, and 82% were for malignancy. Cases had a median length of stay (LOS) of 21 days (IQR: 14, 29.5) with 8% in-hospital mortality. Controls had a median LOS of 6 days (IQR: 4, 8) and had 0% in-hospital mortality. There was no significant increased risk of anastomotic leak with NSAID use in the perioperative period, OR 0.67 (95% CI: 0.35, 1.28). There may be an association at high NSAID dose, OR 1.11 (0.41, 2.95), if given adequate power. In a subgroup analysis, celecoxib use had an adjusted OR of 1.00 (95% CI: 0.51, 1.96) and ketorolac use had an adjusted OR of 1.28 (95% CI: 0.69, 2.35).

Conclusion: This study failed to identify a significant relationship between perioperative NSAID exposure and anastomotic leaks in elective colorectal surgery. There may be an association at high NSAID dose given adequate power and the associations may be NSAID specific. Further, larger studies are required to clarify this relationship.

4-08 Do Longer Wait Times Lead to Poor Outcomes in Patients with Ureteral Stones Waiting for Ureteroscopy?

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Introduction and Objectives: Surgical wait times have become a contentious quality indicator in universal health care systems. The potential for cannibalization towards higher priority surgical cases has led to concerns for the prolonged wait times for non-priority urologic procedures. This may be particularly relevant given the expanding use of medical expulsive therapy for ureteric stones. We describe and evaluate the effect of surgical wait times on the early outcomes and peri-operative complications of elective ureteroscopic laser lithotripsy for non-urgent ureteral calculi.

Methods: All patients undergoing ureteroscopic laser lithotripsy for non-urgent ureteral stones at a single centre between 2008 and 2010 were included. Wait times were determined utilizing a prospectively collected administrative database reporting a summary OR wait times. Further determination of wait times as well as clinical data was supplemented by extensive chart review. Associated outcomes investigated included

additional hospitalization or urgent care visits while on the wait list and success of lithotripsy.

Results: One hundred and sixty four patients were identified. The mean stone size was 8.7 mm and only 29% of patients were started on medical expulsive therapy at the time of presentation. The reported success at lithotripsy was 96%, with 6 (4%) cases of unsuccessful access/fragmentation. The mean number of extra hospital visits after initial presentation was 1.0 (range 0-6) and 22 (13%) patients had more than 2 urgent care visits. We are meeting our government-defined wait time targets (figure), however the median wait time from OR booking to case completion was 50 days. There was a significant trend to increasing urgent care visits with prolonged wait times (0.69 visits/person compared to 1.57 visits/person at a cut-off at the 25th percentile (90 days), $p < 0.05$). There was a non-significant trend to decreased success of lithotripsy with wait times.

Conclusions: The overall wait time for elective ureteroscopy was prolonged, likely influenced by multiple factors including specialist use of medical expulsive therapy. We have met our government-defined wait time targets however; our median wait times to be discouraging for patients waiting for stone management. Success of ureteroscopy was high despite prolonged wait times.

4-09 Indwelling pleural catheters and chemical pleurodesis for malignant pleural effusions: a cohort study

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Background: Malignant and paramalignant pleural effusions are important complications of many malignancies. Management options include insertion of an indwelling pleural catheter (IPC) to achieve chronic effusion drainage and hospitalization with tube thoracostomy and chemical pleurodesis (CP) with talc or doxycycline to prevent fluid reaccumulation. We aimed to compare management of malignant pleural effusions with IPC insertion or CP with regards to survival, success of pleural effusion management.

Methods: We designed a retrospective cohort study comparing patients with malignant and paramalignant pleural effusions and ECOG performance status ≤ 4 managed either with IPC insertion or CP during non-contemporary 3-year periods at The Ottawa Hospital. The CP group was identified through

the prescription of talc or doxycycline and the IPC group from the IPC Program database.

Results: The IPC and CP groups comprised 193 and 168 consecutive patients respectively. Median survival from the date of catheter insertion was longer in the IPC group (148 days vs 133 days in the CP group, log-rank $p < 0.05$). The pleural effusion control rate at 6 months was higher in the IPC group (52.7% vs 34.0% in the CP group, $p < 0.01$), but rates of freedom from catheter at 90 days and pleural effusion at 180 days were not significantly different (IPC: 25.8%, CP: 34.0%, $p = 0.14$).

Conclusions: We found an intriguing possible survival benefit favouring management of malignant or paramalignant effusions with IPC insertion. Given an uncertain explanatory mechanism and possible biases due to study design, this needs to be confirmed in a randomized controlled trial.

4-10 Morphological Effects of Chemotherapy on Ovarian Serous Adenocarcinoma

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Background: Ovarian carcinomas are treated either with debulking surgery and post-operative chemotherapy, or neo-adjuvant chemotherapy followed by interval debulking surgery. Chemotherapy effects on ovarian carcinomas have not been well studied. We sought to 1) assess chemotherapy changes in only ovarian serous adenocarcinomas by comparing pre- and post-chemotherapy samples; and 2) examine grading post-chemotherapy.

Materials and methods: Archival cases of serous adenocarcinomas were reviewed: one group included pre-treatment biopsies ($n=7$) with comparison to subsequent post-chemotherapy resections; other group included resections prior to chemotherapy ($n=14$). Cases evaluated for treatment effects (necrosis, bizarre nuclei, epithelial-stromal ratio, old hemorrhage, giant cell reaction, fat necrosis, foamy changes, ballooning and cholesterol clefts); treatment response (none, minimal or marked) and grading (Silverberg).

Results: The epithelial to stromal ratio was increased in all treated cases and correlated with the degree of treatment response. Four cases showed lobular carcinoma-like features. Bizarre nuclei, cholesterol clefts, giant cell reaction, foamy macrophages and intra-tumoural lymphocytes were nearly exclusively seen in treated cases. Post- compared to pre-chemotherapy grade remained the same or increased in 6/7 cases. Bizarre nuclei were a pitfall for nuclear atypia post-

treatment.

Conclusion: Our study highlights specific morphological changes in serous adenocarcinomas treated with chemotherapy, which may be linked to treatment response. Grading post-treatment was similar to pre-treatment (remains high grade 2 or 3).

4-11 Liver Transplantation in Human Immune-deficiency Virus Infected Recipients: the British Columbian Experience

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Background: Human Immunodeficiency virus (HIV) no longer precludes patients from consideration for solid organ transplantation. Despite evidence of good clinical outcomes internationally, HIV-infected Canadians with end-stage liver disease are still prohibited from undergoing transplantation. A notable exception is the British Columbia Transplant Program.

Purpose: There is a need to evaluate the experience in BC to determine the issues surrounding liver transplantation in HIV-infected patients, and examine clinical strategies to provide them with the optimal care. The purpose of this project is to assess for quality assurance in terms of management of this complex patient population, and provide vital information for this growing subset of Canadians.

Methods: We reviewed charts of HIV positive patients ($N=28$) referred to BC Transplant for liver allograft assessment. Data was collected on a) HIV and Liver Disease Status at time of assessment b) Initial Transplant Assessment and c) Clinical Outcomes. University of British Columbia Behavioural Research Ethics Board (H11-00316).

Results: Of 28 patients referred, 23 were seen in the clinic by our specialist team. Of patients whose files were activated, three were successfully transplanted while one died from acute

renal failure while waiting for transplant. The remained of patients died at the pre-assessment stage (n=10) or were deemed unsuitable (n=10). The commonest reason for rejection was stable liver disease not requiring transplantation (n=4). While majority of patients were BC Residents, some were from other provinces and one from the US.

Majority of referred patients had a primary liver disease diagnosis of Hepatitis C (n=16) or Hepatitis B (n=5). Most had undetectable HIV viral loads and all but one had taken HAART at some point in their disease process. The most common medical comorbidities were anxiety and mood disorders (n=4) and haemophilia (n=4).

Conclusion: This is the first Canadian report of liver transplantation amongst HIV-patients in Canada. The optimistic outcomes of this study may persuade other Canadian centres to consider HIV positive patients for liver, and other solid organ, transplantation.

4-12 Incidence of Retinopathy of Prematurity among premature babies at a NICU from 2006-2010

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Introduction: To study the incidence of retinopathy of prematurity (ROP) in a neonatal intensive care unit (NICU) and obtain information on risk factors associated with ROP.

Methods: Data was retrospectively extracted from The Canadian Neonatal Network that maintains clinical information about neonates. Infants screened for ROP had either a birth weight of <1500g and/or gestational age < 32 weeks. On special request from the neonatologist, babies who did not meet these criteria were also screened. Risk factors were compared among screened children who had ROP and those that did not have ROP by univariate and multivariate analysis.

Results: 423 infants were screened for ROP in the NICU. The incidence of ROP was 40.4%, 39 infants (9.2 %) had severe ROP, 27 infants (6.4%) needed laser treatment. Mean gestational age (26+/-0.13 vs 28.55 +/- 0.12 weeks; p<0.0001) and birth weight (840.5+/-17.49 vs 1190.24+/- 20.20 grams; p<0.0001) were significantly lower among children with ROP versus those without ROP. Multivariate logistic regression showed that low birth weight (p<0.001), gestational age (p<0.001), ventilation therapy (p=0.039), necrotizing enterocolitis (p=0.019) were independent risk factors for ROP.

Conclusions: The incidence of severe ROP and treatment were similar to other studies conducted in highly developed countries. Gestational age and birth weight were the most signifi-

cant independent risk factors for developing ROP. Our study population had an elevated percentage of extremely low birth weight infants but there was not a corresponding increase in severe ROP incidence and treatment which could mean that having a better understanding of ROP pathogenesis and developing site specific strategies for managing extreme premature infants may decrease severe ROP incidence and treatment.

4-13 Changes in the Streptococcus Antibiotic Resistome of Adult Cystic Fibrosis Patients Results from Mutation Rather than Horizontal Gene Transfer

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Background: Cystic fibrosis (CF) is the most common lethal genetic disease among Caucasians, afflicting greater than 4000 Canadians. 90% of CF patients succumb to pulmonary failure due to chronic respiratory infections. Traditionally, research has focused on a narrow spectrum of microorganisms thought to be principal pathogens in the CF lung such as *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, and *Staphylococcus aureus*. However, emerging species of bacteria, such as those from the *Streptococcus* genus, have been shown to be overlooked pathogens. Within the streptococci, there is the potential for an antibiotic resistome due to constant pressure from several prescribed antibiotics as well as the naturally competent nature of the bacteria. Earlier studies done on the microbiome within CF has shown the presence of multiple isolates from several novel species of streptococci, of which may play a role in CF exacerbation. This study aimed to determine rates of resistance among commonly used antibiotics in CF as well as to elucidate molecular mechanisms by which this occurs.

Methods: In this study, 297 streptococcal isolates, both novel and viridans grouping previously isolated from adult CF sputum, underwent susceptibility testing for nine antibiotics. Molecular mechanisms of resistance for the macrolides were determined by a PCR-based screen or by DNA sequencing.

Results: Resistance rates were the greatest for macrolide antibiotics at 60% for erythromycin and 63% azithromycin. Among the novel streptococci, the resistance rates were even higher at 81% and 77%, respectively. Due to these results, macrolide resistance was looked at in closer detail. The two most common mechanisms of macrolide resistance within the streptococci, the *mef* (efflux pump) and *erm* (target site methylation) accounted for only 45% of resistant isolates. Interestingly, the 23S ribosomal point mutations accounted for 52% of macrolide resistance. The remaining 3% of resistant isolates may be due to another mechanism that requires further study.

Conclusions: The prevalence, species distribution and influence of therapy on resistance profiles suggest complex ecological interactions among the streptococci in the airways of CF patients with mutation, rather than horizontal gene transfer, being the primary mechanism of acquired antibiotic resistance in some species within this community. This study also illuminates the potential roles for the *Streptococcus* genus in CF disease progression.

5-01 Late outcomes following grafting of the severely burned face: A quality improvement initiative

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Introduction: Many approaches to surgical management of the severely burned face are described but there are few objective outcome studies. Our purpose was to perform a detailed evaluation of the late outcomes in adult patients who have undergone grafting using a standardized surgical and rehabilitation approach for full thickness (FT) facial burns in order to identify areas for improvement in our treatment strategy.

Methods: This was a prospective observational study in which patients who had undergone grafting for FT facial burns by the senior investigator at a regional burn centre between 1999 and 2010 were examined by a single evaluator. The surgical approach included tangential excision based on the facial aesthetic units, temporary cover with frozen allograft then autografting with scalp skin preferentially, split grafts for the upper eyelid and FT grafts for the lower eyelid. Rehabilitation included compression (uvex and or soft cloth), scar massage, and silicone gel sheeting.

Results: Of 35 patients with facial grafts 14 subjects (age 43 ± 16 yr with 22 ± 21 % TBSA burns) returned for late follow up

at 40 ± 33 mo (range 5-91 mo). A mean of 4 facial aesthetic units per patient were grafted (range 1-9 units), with 6 full facial grafts performed. Scalp was used as donor in 10/14 cases. Scalp donor sites were well tolerated with minor alopecia visible in only 1 case although the donor site visibly extended slightly past the hairline in 2 cases. Color match with native skin was rated at 8.8 ± 0.8 out of 10 when scalp skin was used compared to 7.5 ± 1.6 with other donor sites ($p=0.06$). On the lip and chin, hypertrophic scars were significantly worse compared to the rest of the facial grafts (VSS 8 ± 2 Vs 3 ± 1 , $p<0.01$). Sensory recovery was poor with overall moving 2 point discrimination at 11 ± 3 mm (range 4-15), and monofilament light touch was 3.8 ± 0.6 . Graft borders were significantly more elevated than graft seams. On the forehead the most notable problem was a gap between the graft and hairlines of the frontal scalp and eyebrows (range 0-40mm). Grafted eyelids required one or more subsequent ectropion releases in the majority of cases. The most common problem for the nose was asymmetry of the nostril apertures.

Conclusion: The most problematic late outcomes we identified following facial grafting for FT facial burns included relatively poor sensory return, elevation of graft edges, eyelid ectropion, gaps between grafts and hairline, and marked hypertrophic scarring around the mouth and chin. Our results indicate that possible areas for quality improvement include greater attention to the limits of scalp harvest, more attention to pressure application to graft borders and the lip and chin during rehabilitation, greater accuracy in excision and graft placement on the forehead to avoid gaps with the hairlines, and counselling of the patient regarding the high probability of diminished facial sensation.

5-02 PPIs: Are we prescribing them appropriately?

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Introduction: Proton pump inhibitors (PPI) are a widely prescribed class of drugs costing the US economy an estimated 14 billion dollars annually. It is a class of drugs with few immediate adverse affects; however, longer term use has been linked with a increased incidence of *C. Difficile* diarrhea, pneumonia and fracture risk. Objective: We therefore asked whether PPIs were being prescribed according to approved indications on the clinical teaching unit (CTU).

Methods: We conducted a retrospective chart review of patients discharged from the CTU between March 2010 and January 2011 who were admitted on, or were started on a PPI (n=110). We excluded those who were started on a PPI for treatment of upper GI bleeding (n= 12). We then looked for the following FDA and Health Canada approved indications: gastroesophageal reflux disease, peptic ulcer diagnosed within the previous 8 weeks, upper GI bleeding within the previous 8 wks, primary prophylaxis against GI bleed in high risk patients with previous episode of GI Bleed plus a need to be on NSAID/anticoagulation/glucocorticoids, H. Pylori treatment, and Zollinger-Ellison syndrome. We set the “gold standard” to be that 75% of CTU patients should have an acceptable indication for a PPI. With 110 patients, if our mean were at or higher than this proportion, our lower 95% would be 60%. We could be 95% certain that at least 60% of our patients had an acceptable indication. We compared the proportion of patients admitted to CTU taking a PPI, with our “gold standard” or ideal proportion (75%), using Fishers Exact Test.

Results: We found that only 28% of CTU patients had an approved indication for a PPI (Figure 1). This is much lower than our gold standard of 75% ($p<0.0001$) OR 7.65 (4.14-14.13). Furthermore, 96% of patients with no indication had their PPI prescription continued at the time of discharge. We recommend that CTU physicians review all discharge prescriptions for PPI's, and discontinue them in patients with no approved indication. and Zollinger-Ellison syndrome. We set the “gold standard” to be that 75% of CTU patients should have an acceptable indication for a PPI. With 110 patients, if our mean were at or higher than this proportion, our lower 95% would be 60%. We could be 95% certain that at least 60% of our patients had an acceptable indication. We compared the proportion of patients admitted to CTU taking a PPI, with our “gold standard” or ideal proportion (75%), using Fishers Exact Test.

5-03 On the illusory perceptual compression of visual space during eye-head gaze saccades

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Background: In primates, inspection of a visual scene is typically interrupted by frequent gaze shifts, occurring at an average rate of three to five times per second. Perceptually these

gaze shifts are accompanied by a compression of visual space towards the saccade target, which may be attributed to an oculomotor signal that transiently influences visual processing. While previous studies of compression have focussed exclusively on saccadic eye movements made with the head artificially immobilized, many brain structures involved in saccade generation also encode combined eye-head gaze shifts, where gaze = eye-in-head + head-in-space. In order to understand which brain areas are responsible for perisaccadic visual perception, is thus important to determine whether compression is towards the end-point of gaze saccades made head-unrestrained (HU).

Methods: Using a standard compression paradigm (Lappe M et al., Nature, 2000), we studied mislocalization in HU human subjects who made horizontal saccadic eye-head gaze shifts of 40 to 60 degrees. Subjects were instructed to report the perceived position a briefly (12ms) flashed vertical bar presented over a range of horizontal positions in a time-window ± 200 ms around gaze-shift onset.

Results: We found a powerful compression of visual space that depended on the time at which the vertical bar was presented relative to the onset and time-course of the gaze shift. Importantly, compression was toward the intended gaze target, rather than to the spatial location of the initial eye movement. We also found that the duration of compression was nearly constant across gaze-shift amplitudes.

Conclusions: Our previous model of compression (Richard A et al., J Neurosci, 2009) could be extended to the HU condition and time domain by invoking time-filtered interactions, on a logarithmic map of visual space, between two populations of neurons that encode respectively the gaze-shift vector and visual stimulus position relative to the fovea. The present findings expand on what is known about the extra-retinal mechanisms that maintain perceptual constancy across gaze saccades, and have the benefit of making specific predictions for further electrophysiological experiments in the awake-behaving primate.

5-05 HIV exposed uninfected (HEU) South African infants express a hyperinflammatory innate immune profile

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Background: 300,000 HEU infants born in South Africa every year may be at a higher risk of morbidity and mortality, and aberrant immune responses likely contribute to increased susceptibility to infection. We present data from a prospective cohort study on the immune function of South African HEU infants in response to pathogen-associated stimuli.

Methods: Biological samples were collected at 2 and 6 weeks, 6, 12 and 18 months of age. HEU or unexposed (UE) blood was treated with multiple pathogen-associated stimuli and immune function was assessed. Flow cytometric data on markers of inflammation is presented for conventional dendritic cells (cDC), plasmacytoid dendritic cells (pDC) and monocytes (Mo).

Results: HEU (n=31) and UE (n=29) infants who participated in the study were from the same communities. Statistical differences were found over the first year of life for various combinations of mono and polyfunctional cytokine responses. The most substantial differences between HEU and UE groups were with HEU cDCs expressing higher levels of TNF α or TNF α & IL12p40 at 2 weeks of age and with pDCs producing more IFN α or IFN α & TNF α by 6wks. Of note, all statistical differences that were detected demonstrated a more pro-inflammatory profile in HEU infants.

Conclusion: South African HEU infant innate immune responses are hyperinflammatory. This striking finding provides the first evidence of aberrant innate immune responses in HIV exposed but uninfected infants. Delineating etiological factors underlying these immune abnormalities will be essential to curb high levels of morbidity and mortality seen in HEU infants.

5-06 Basic to applied: Assessing auditory recognition memory using event related potentials

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Background: Patient assessment following acquired brain injury is critical for rehabilitation, however the use of traditional assessment tools breaks down when therapists are confronted with non-communicative patients (e.g. global aphasia, and vegetative state). Thus there is a need for reliable alternatives to current neuropsychological tests which rely on behavioural

responses. One viable option is the use of event-related potential (ERP) technology to identify specific reliable markers of cognition. One such marker, particularly with respect to memory, is the old/new ERP effect which describes the measurement of more positive-going brain potentials to repeated stimuli relative to novel stimuli. While much basic research has been conducted on this effect, relatively little work has been done to explore its application as a diagnostic tool. Thus the purpose of this study was to adapt a subtest of the Wechsler Memory Scale IV (a widely-used neuropsychological test) into a computerized format and evaluate participant (n=27) memory performance through both electrophysiological and behavioural means.

Methods: Subjects listened to two standardized stories and answered 60 true/false statements after a 20-30 minute interval. Adaptation of the test to the computerized format was done while retaining the psychometric properties of the original test. We hypothesized that certain ERP components would be sensitive to repeated, and thus remembered, stimuli relative to novel stimuli; an effect consistent with the old/new ERP effect seen in the literature. Specifically, we hypothesized the presence of a late positivity (500-800 milliseconds post-stimulus onset) associated with explicit recollection of repeated/true statements, as well as the presence of an earlier positivity (300-500ms) associated with a subject's "sense" of familiarity with the information contained in a repeated/true stimulus item.

Results: Based on the behavioural score, two groups emerged: a poor-performing group (below 60% accuracy) and a well-performing group (above 60% accuracy). Only the latter group showed evidence of the late positivity, suggesting that the test is capable of distinguishing memory task performance using event-related potentials. This effect is seen both at the group and individual levels.

Conclusion: Ultimately, these results are very promising and given further investigation, may be applied toward the development of new diagnostic tools for special clinical populations.

5-07 Delineating the functional neuroanatomy of anosognosia or lack of illness awareness in schizophrenia using Fmri

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Background: Anosognosia or lack of illness awareness occurs in both schizophrenia and right hemisphere lesions due to stroke, dementia, and traumatic brain injury. In the latter conditions, anosognosia is associated with unilateral hemispheric dysfunction or interhemispheric disequilibrium, which provides an anatomical starting point for exploring anosognosia in other neuropsychiatric disorders, such as schizophrenia. Anosognosia in schizophrenia is associated with increased rates of relapse, hospitalization, violence, greater symptomatology, and social and occupational dysfunction. The functional neural correlates of anosognosia in schizophrenia have yet to be described. As such, the aim of this study was to identify the functional neuroanatomy of illness unawareness in schizophrenia using functional MRI.

Methods: Clinically stable patients with schizophrenia or schizoaffective disorder had their level of illness awareness assessed using the Schedule for the Assessment of Insight, Expanded Version (SAI-E). Subjects underwent an experiment designed to challenge illness awareness during functional magnetic resonance BOLD imaging acquisition. During scanning, subjects answered either “yes, agree” or “no, disagree” to questions or statements specific to the various domains of illness awareness (i.e. global illness awareness, symptom awareness, awareness of need of treatment or awareness of negative consequences of the illness) or a neutral condition. Analyses were performed using SPM8.

Results: According with the hypothesis that anosognosia arises from right hemispheric dysfunction or interhemispheric disequilibrium, poor illness awareness in schizophrenia was associated with cerebral activations in the left medial and dorso-lateral prefrontal cortex, left anterior cingulate cortex ($t = 4.54$, $p < 0.001$), and left parietooccipital sulcus and precuneus ($t = 6.44$, $p < 0.001$). By comparison, there was notably less activation in these regions in the right hemisphere.

Conclusion: This is the first functional imaging study of illness awareness and schizophrenia. Identification of the neural correlates of anosognosia in schizophrenia would provide putative regions for treatment intervention with focal techniques, such as transcranial magnetic stimulation. Modulation of illness awareness may ultimately lead to improved treatment outcomes (i.e. better medication adherence, lower rates of rehospitaliza-

tion and relapse).

5-08 Mood Instability is a better predictor for Depression and Anxiety than Neuroticism

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Background: The concept of neuroticism is widely used in psychiatry and psychology to indicate a personality construct that predicts the later development of anxiety and depression. All personality questionnaires include a neuroticism factor or an equivalent concept. We have proposed that Mood Instability is a more empirically and clinically useful concept than neuroticism. We previously measured neuroticism with the Neuroticism Scale from the Eysenck Personality Inventory.

Question: The hypothesis was that Mood Instability would be a better predictor of anxiety and depression than the Neuroticism-Extraversion-Openness to experience Personality Inventory (NEO PI-R)

Method: 28 forensic psychiatric patients and 30 graduate students completed the NEO PI-R, the TEMPS temperament scale, and Visual Analogue Scales on mood twice a day for a week. Calculation of the Mean Square Successive Difference (MSSD) statistic from the Visual Analogue Scale gives a measure of Mood Instability. The patients gave signed consent and the students volunteered and gave assent.

Results: An 8 item measure of mood instability derived from the TEMPS was validated as a measure of unstable moods by correlation with the MSSD. The measure of mood instability was a better predictor of anxiety and depression than the NEO PI-R. In mediation analysis, mood instability mediates the relationship between neuroticism and negative affect, but neuroticism does not mediate the relationship between mood instability and negative affect.

Conclusion: Mood instability is a better predictor of negative affect than neuroticism. This has now been shown for neuroticism scales from the Eysenck Personality Inventory and the NEO. Item and factor analysis of the neuroticism scale from the NEO PI-R indicates that it consists of mild symptoms of negative affect. This suggests that mild symptoms predict severe symptoms. The conclusion is that mood instability is a more clinically and empirically useful concept than neuroticism.

5-09 Can a Patent Ductus Arteriosus be Determined to be Hemodynamically Significant, Using Serum Brain-type Natriuretic Peptide and a New PDA Score?

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Abbreviations: BNP – Brain-type natriuretic peptide; ECHO – Echocardiogram; HSPDA – Hemodynamically significant Patent ductus arteriosus

Background: The determination of whether or not a PDA is hemodynamically significant, requiring treatment, poses a dilemma to the clinician.

Purpose: To determine whether BNP, together with a high PDA score measured between 48-72 hours of life, predict a hemodynamically significant PDA (HSPDA) requiring closure in infants <31 weeks GA.

Study Design and Methods: Infants <31 weeks GA, admitted August 2010 to March 2011, to NICU in Winnipeg, Canada, had, following parental consent and blinded from the treating team, bedside serum BNP assay, echocardiogram and a novel PDA score determined between 48-72 hours postnatally. BNP was correlated with echocardiographic parameters and PDA score. Reference standard for HSPDA was a PDA with diameter >1.5mm by ECHO and with left to right non-restrictive shunt

Results: Forty three out of hundred and fourteen eligible neonates were studied. HSPDA was present in 18. Mean GA(weeks) for the HSPDA group was 26.6 ± 1.3 and for non HSPDA group was 28.7 ± 1.2 ; mean birth weight (grams) for the HSPDA group was 888 ± 161 and for the non HSPDA group was 1225 ± 279 ; median(IQ range) PDA score for the HSPDA group was 11.5 (9,14.5), and for non HSPDA group was 3(2,5); the mean of BNP for HSPDA group was 360 ± 305 pg/ml and for non HSPDA group was 18.8 ± 16.6 pg/ml; all were significantly different.

For HSPDA, sensitivity, specificity, positive and negative predictive values for both PDA score and BNP were 88%, 100%, 100%, 92%, respectively.

Conclusions: Both BNP and PDA score predict HSPDA requiring treatment. Cut off values were 90pg/ml, score >7 respectively.

5-10 Visual function and vision-related quality of life after macular hole surgery with short-duration 3 day face-down positioning

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Purpose: In a limited-resource setting, such as the Canadian healthcare system, it is necessary to evaluate the effectiveness of interventions from a surgical and patient perspective in order to justify surgery and expenditure. This study looked to investigate the relationship of vision-related quality of life (VRQOL) and visual function in patients undergoing macular hole (MH) repair with and without combined cataract surgery with short-duration 3 day prone posturing. While previous communications have assessed VRQOL in European and Japanese populations, this is the first study investigating VRQOL after MH surgery with short duration face-down positioning in a North American population.

Methods: 20 consecutive eyes in 19 patients with stage 2 and 3 idiopathic macular holes were enrolled in this prospective interventional case series. 15 eyes received combined cataract and MH surgery and 5 eyes received MH repair alone. Patients completed the self-administered National Eye Institute (NEI) 25-item Visual Function Questionnaire (VFQ-25) before and after surgery. All patients received full ocular examinations pre- and post-surgery. Along with questionnaire scores, macular hole closure rates, complications, visual acuity (VA), and intraocular pressure were examined.

Results: Macular hole closure was achieved in 20 of 20 eyes (100%). Mean postoperative logMAR decreased (i.e., improved) by -0.303 (95% confidence intervals (CI) -0.501 - -0.105, $p=0.0047$). The VFQ-25 composite score rose from 82.019 ± 12.612 standard deviation (SD) to 88.499 ± 7.963 SD ($p=0.012$). Subscale scores including general vision, near activities, mental health, role difficulties, and dependency were all significantly improved ($p<0.05$). Only 7 subjects had decreased composite scores after surgery, and all but one of the 7 had increased VA postoperatively (this subject's VA remained unchanged). Out of the 20 eyes, 3 eyes had worse VA but still demonstrated an increase in the VFQ-25 composite score. No complications or IOP increases were observed.

Conclusions: Macular hole surgery followed by short-duration 3 day face-down positioning significantly improved VRQOL and VA in a North American group of patients. The use of

VRQOL tools alongside anatomical outcomes provides a more comprehensive overview of patients' experience and satisfaction after surgery.

5-11 Management of HER2+ breast cancer: Is treatment with Trastuzumab (Herceptin) beneficial for small tumours?

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Background: Breast cancers are classified into different subtypes based on their expression of three receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). The overexpression or amplification of HER2 receptor (HER2+) is seen in about 15% of breast cancers and has been associated with poor prognosis in terms of recurrence and overall survival. In 2005, HER2-targeted therapy in the form of Trastuzumab (Herceptin) became available for HER2+ tumours >1 cm in size, transforming the management of patients with this breast cancer subtype. Recent data suggest patients with smaller tumours may also benefit from this treatment.

Purpose: To compare recurrence-free survival (RFS) and overall survival (OS) rates between patients with early stage HER2+ tumours who did and did not receive Herceptin.

Methods: Electronic patient records were reviewed for all new breast cancer patients at Sunnybrook Health Sciences Centre, Toronto, Canada, who were seen by a medical oncologist between January 1, 2005, and December 31, 2006. Eight hundred and seventy charts met these initial criteria, and 533 were included in the analyses. Tumour pathology, medical and surgical treatments, and recurrence and mortality dates were collected. RFS and OS rates were calculated and then analyzed with respect to tumour subtype and treatment regimen.

Results: Ninety-five patients had HER2+ tumours, of which 79% (n=75) received Herceptin treatment. The most common reason for not receiving Herceptin was tumour size <1 cm (50%; n=10). Patients with HER2+ tumours who did not receive Herceptin had lower risk pathology in terms of stage and grade compared to those who did receive Herceptin (Average

Stage 1.4 vs 2.13, Average Grade 2.45 vs 2.55). Despite having lower pathological risk, the 5-year recurrence-free survival was lower for patients who did not receive Herceptin (Received Herceptin: RFS: 0.799, 95% CI 0.674-0.880), vs. (No Herceptin: RFS 0.690, 95% CI 0.428-0.850). There was no difference in OS.

Conclusions: The advent of HER2 targeted therapies has significantly impacted the clinical course for breast cancer patients with HER2+ disease. Our data suggest that even patients with < 1cm HER2 positive tumours may derive a benefit from Herceptin.

6-01 Interleukin-1 blockade improves human islet amyloid polypeptide-induced pancreatic islet graft dysfunction

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Introduction: Human islet amyloid polypeptide (hIAPP) is a 37-amino acid peptide co-secreted with insulin by pancreatic beta-cells. hIAPP aggregation contributes to beta cell dysfunction type 2 diabetic and transplanted islets, both characterized by inflammation with macrophage infiltration. hIAPP aggregates share a common cross beta-sheet structure with other amyloids known to induce a potent pro-inflammatory response mediated by macrophage release of interleukin-1 (IL-1). To determine whether hIAPP induces a similar innate immune response in transplanted islets, we evaluated the effect of the IL-1 receptor antagonist (IL-1Ra) anakinra on hIAPP-induced islet graft dysfunction.

Methods: Islets from wild-type or hIAPP-expressing transgenic mice were transplanted into diabetic recipient mice implanted with a mini-osmotic pump containing IL-1Ra (50 mg/kg/d) or saline. Graft function was evaluated by intraperitoneal glucose tolerance testing after eight weeks. Amyloid deposition was assessed by Thioflavin-S staining of grafts and intra-graft macrophages were quantified by staining for the glycoprotein F4/80.

Results: Islet grafts expressing hIAPP contained amyloid deposits in close association with F4/80-expressing macrophages. Transgenic grafts contained 50% more macrophages than wild-type grafts, an effect that was significantly inhibited by IL-1Ra. Furthermore, a five-fold reduction in amyloid area was observed in transgenic grafts from IL-1Ra-treated recipients, suggesting that hIAPP aggregation may not only contribute to IL-1 release but may also be a consequence of islet inflammation. Recipients of transgenic islet grafts displayed impaired glucose tolerance eight weeks following transplantation compared to recipients of wild-type grafts (area under the curve (AUC)=1940±80 vs. 1260±60, $p<0.001$). Administration of IL-1Ra significantly improved graft function in recipients of transgenic grafts (AUC=1470±160 vs. 1940±80, $p<0.005$) but not wild-type grafts, suggesting an important role for IL-1 in mediating hIAPP-induced islet inflammation and dysfunction.

Conclusions: hIAPP aggregation promotes macrophage recruitment and islet dysfunction in an IL-1-dependent manner. Thus, anti-IL-1 therapy may improve graft function in human islet transplant recipients by inhibiting hIAPP-induced inflammation or attenuating amyloid formation. Importantly, these data point to a common mechanism of innate immune activation associated with diverse forms of amyloid disease.

6-02 A Pilot Study Evaluating the Efficacy of Traction Therapy for Peyronie's Disease on a Novel Rat Model

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Background: The efficacy of traction therapy for the treatment of Peyronie's disease is controversial without basic histological support but appears to be gaining in popularity and widespread use. This pilot study evaluates the morphological, histological and functional changes associated with traction therapy on a novel rat model for Peyronie's disease.

Methods: Adult male Sprague-Dawley rats aged 20-24 weeks received intratunical injections of TGF- β -1 and Tetradecyl Sulphate at Day 0 and Day 7 for induction of a durable penile plaque. At 4 weeks, the rats were divided into a traction and control arm. The traction arm underwent microscopic surgery where 2 plicating horizontal mattress sutures were placed on

each side of the stable plaque to exert longitudinal stress. At week 6, an additional plicating suture was placed on each side to assure adequate tension. The control arm received no interventions. Penile pressures were measured in triplicates on all rats using cavernous nerve electrostimulation. They were sacrificed for gross, histological and immunohistological analysis at week 8.

Results: Gross examination showed penile curvature with a palpable plaque on both arms. There was no significant difference between the traction and control arms. On histological examination, the plaque is shown as an area of increased non-polarized collagen deposit within the tunica albuginea (Figure 1). This area is smaller with less non-polarized collagen in the traction arm compared to the control arm. Immunohistology showed recovery of smooth muscle α -actin and decrease of TGF- β -1 in the traction arm. Lastly, the traction arm achieved higher peak penile pressures than the control arm with cavernous nerve electrostimulation.

Conclusion: Traction therapy is a novel approach for the treatment of Peyronie's disease. The results of this pilot study shows evidence of histological and functional improvement with traction on this novel rat model for Peyronie's. The mechanism may involve remodeling of the penile plaque. However, further study is warranted.

6-03 Peripheral Tregs regulate sickness behaviour development in inflammatory liver disease

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Background: Peripheral organ-centred inflammatory diseases are commonly accompanied by debilitating non-specific symptoms. These symptoms include fatigue, malaise and a loss of social interest, and have been collectively termed sickness behaviours. Regulatory T cells (T_{reg} s) have been broadly implicated in regulating inflammation; however, a role for peripheral T_{reg} s in modulating sickness behaviour development during peripheral organ-centred inflammatory diseases, such as hepatic inflammation, has not been evaluated.

Methods: For these experiments a mouse model of inflammatory liver disease due to bile duct ligation (BDL) was used as the model of peripheral organ-centred inflammation.

Flow cytometry of CD4⁺Foxp3⁺ T_{reg} cells was conducted in the peripheral blood and liver of BDL and sham mice. The effects of peripheral T_{reg} depletion (using an anti-CD25 antibody) and T_{reg} adoptive transfer upon sickness behaviour, circulating cytokine levels and hepatic mRNA levels were determined. Sickness behaviour was quantified in a social exploration study. Serum cytokine levels were measured using Luminescence beads. Hepatic mRNA levels were determined via real-time PCR.

Results: BDL mice demonstrated a significant reduction in peripheral blood, and an increase in hepatic, T_{regs} compared to sham mice. BDL resulted in the development of sickness behaviours characterized by decreased social investigative behaviour and increased immobility of BDL mice. Elimination of functional peripheral T_{regs} in BDL mice resulted in worsening of BDL-associated sickness behaviours, an effect that was reversed with T_{reg} cell adoptive transfer. Hepatic mRNA and plasma levels of IL-6, a cytokine implicated in inflammation-related sickness behaviours, were elevated in T_{reg}-depleted BDL mice. In contrast, adoptive transfer of T_{regs} decreased circulating IL-6 protein and liver IL-6 mRNA levels. Sickness behaviour development in IL-6 knockout BDL mice was markedly reduced compared to wildtype BDL mice.

Conclusion: Peripheral T_{regs} modulate sickness behaviour development in the setting of peripheral inflammation, an effect driven through T_{reg} inhibition of hepatic IL-6 production and, subsequently, reduced liver-to-brain signalling via IL-6 circulating in the peripheral blood.

6-04 Novel Methods in Retrograde Labeling of Peripheral Nerves

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Background: Nerve injuries are devastating. While the treatment of nerve injuries has advanced over the last several decades, outcomes often remain disappointing. Most meaningful innovations have been pioneered in animal models. Interventions

intended to improve nerve regeneration following injury must be able to be measured and compared with controls. As these interventions become increasingly complex, so must our imaging modalities.

Purpose: The goal of this study was to develop a novel technique to image multiple nerve branches at one time. The ability to multiplex imaging may be of critical importance in evaluating nerves with multiple branches (sensory and motor) such as the facial and sciatic nerve. By labeling different branches, one can assess the extent, pattern and accuracy of nerve regeneration. Here we demonstrate the successful application of multiplex labeling of peripheral nerves.

Methods: A unilateral transection of the tibial and common peroneal nerve was performed on 20 rats. We then applied retrograde tracers, including True Blue (TB), Fluorogold (FG), Fluororuby (FR) and Fluoroemerald (FE) to the transected sites in peripheral nerves. The retrograde tracers are taken up by the nerves over the course of 7 days. Spinal cords then undergo fluorescent microscopy to determine the amount and location of the labeled motor neurons.

Results: We simultaneously imaged regions of the spinal cord that innervate multiple separate nerves. We were able to distinctly resolve the neuron cell bodies. On average 811 tibial and 398 common peroneal motor neurons were labeled using various retrograde markers. The motor neurons were successfully single, double and triple labeled.

Conclusions: The neuronal tracers FG, FR, FE and TB are effective in labeling tibial and common peroneal motor neurons. Our results demonstrate the possibility of multi-labeling of motor neurons after one week. With careful experimental design, multi-labeling of neurons may provide further insight and allow for characterization.

6-05 Glucosamine-supplementation promotes accelerated atherogenesis in apoE-deficient mice

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Background: Despite being a major independent risk factor for cardiovascular disease and stroke, the mechanisms by which diabetes mellitus causes the accelerated progression of athero-

sclerosis remain to be elucidated. In a hyperglycemic mouse model, it has previously been shown that elevated concentrations of glucosamine can promote endoplasmic reticulum (ER) dysfunction in vascular cells. Glucosamine is a naturally-occurring aminosugar that plays a variety of roles in metabolic pathways. It is also available as a nutritional supplement; however, its effects on metabolic outcomes are not well-studied.

Purpose: To test the atherogenic potential of glucosamine supplementation in an apolipoprotein E (apoE)-deficient mouse model, in order to gain a better understanding of the molecular and cellular pathways linking diabetes to the development and progression of atherosclerosis.

Methods: Five week-old ApoE-deficient female mice were separated into three groups of eight: control, glucosamine and mannitol. The control group drank tap water; the glucosamine group drank tap water with 5% glucosamine added to it; and the mannitol group drank tap water with 5% mannitol added to it. At 15 weeks of age, paraffin-embedded serial aortic root sections (4µm thick) were collected per mouse on glass slides for measurement of lesion size. The sections were stained with standard Harris' hematoxylin and Eosin. Lesion area was quantified using computer-assisted morphometry. Whole-blood glucose levels were measured using a DEX glucometer. Plasma glucose and lipid levels were determined using colorimetric diagnostic kits for total cholesterol, triglycerides and glucose.

Results: ApoE-deficient mice in the 5% glucosamine group had larger atherosclerotic lesions, by area, in their aortic root cells than the control and 5% mannitol groups. There were no significant differences in glucose levels between the three groups. There was a significant difference in measured cholesterol levels between the 5% glucosamine and 5% mannitol groups ($p < 0.05$, $n = 12$ for each group).

Conclusion: Glucosamine supplementation in apoE-deficient mice promotes accelerated atherogenesis, producing more atherogenic lesions in arterial walls.

6-06 Skin Derived Precursor Cells (SKPs) improve remyelination within a model of focal adriamycin-induced tibial nerve demyelination

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Introduction: Skin derived precursor cells (SKPs) can mimic the phenotypic appearance of Schwann cells when predifferentiated in vitro (SKP-SCs), and are a reliable autologous source of Schwann cells that survive in considerable numbers when

micro-injected into nerve grafts and denervated nerve. Our hypothesis is that SKP-SCs can produce morphologically and electrophysiologically functional myelin as they ensheath axons. We are testing this hypothesis in a model of focal demyelination of the rat tibial nerve.

Methods: We unilaterally injected 500,000 GFP labeled SKP-SCs into the tibial nerves of 10 adult Lewis rats, while the contralateral tibial nerve received media injection. This was done one week after a demyelinating bilateral tibial nerve lesion was created using a 30ul injection of 12.5ug/ml Adriamycin (1). This model provides a reproducible timeline of demyelination, followed by remyelination (fig 1a, Day 0 Adriamycin, $n = 5$). All animals are followed for compound motor action potentials (CMAPs) every three days until 50 days post-initial injection. Animals from identical cohorts of each group are being periodically sacrificed for teased fibre, morphological, and immunohistological analysis using anti-voltage gated sodium channel, anti-voltage gated potassium channel, as well as anti-CASPAR antibodies. Also, these animals are being periodically sacrificed for immunohistological analysis of axial tibial nerve sections looking for myelination of tibial nerve axons by labeled SKP-SCs, using a combination of frozen sections, semi-thin Epon sections, as well as ultrathin EM cryosections.

Results and conclusions: In our preliminary work, we have demonstrated that SKP-SCs promoted a significantly lower G-ratio when analyzed against either media or Schwann cell injection in this model (Day 33 Epon morphometry, $N = 5$ /group, Figure 1b,c). We have also demonstrated preliminary evidence of myelination by DiI labeled SKP-SCs (Day 33 teased nerve fibre, Figure 1d; Green = DiI, Red = Nile Red [compact myelin stain], Blue = NaV 1.6, Grey = Neurofilament). Results concerning the electrophysiological data are pending. We expect to see a more rapid return to baseline function in the SKP-SC injected group. We also predict that SKP-SCs will participate in functional nodes of Ranvier formation, as demonstrated by confocal microscopy. Finally, we predict that prelabeled SKP-SCs will be seen to conclusively myelinate tibial nerve axons on immunohistological analysis.

1. England, JD., Rhee, EK., Said, G., Sumner, AJ: Schwann cell degeneration induced by doxorubicin (adriamycin). Brain 111: 901-913, 1988

6-08 The Role of Three-Dimensional Visualization in Robotics-Assisted Cardiac Surgery

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Background/Purpose: The objective of this study is to determine the value of three-dimensional visualization in robotics-assisted cardiac surgery using a teleoperated robotic system with seven degrees-of-freedom and three-dimensional visualization.

Methods: A cardiac surgery test bed was constructed to measure forces applied by the da Vinci surgical system (Intuitive Surgical, Sunnyvale, CA) during mitral valve annuloplasty. The tissue test bed consisted of a porcine mitral valve mounted on a six-axis ATI Industrial Automation force/torque sensor to measure applied forces. Sutures were passed through the porcine mitral valve annulus in a transverse mattress fashion at predetermined points by participants with different levels of experience in robotics-assisted surgery. The sutures were subsequently passed through an annuloplasty band and tied in place robotically. These trials were repeated alternating between two-dimensional and three-dimensional visualization.

Results: At this time, a novel apparatus for measurement of forces applied by the da Vinci Surgical System during robotics assisted mitral valve annuloplasty has been developed. Using this model, we have shown three-dimensional visualization significantly reduced the time required to complete both suturing and knot tying to tie each suture in all levels of surgical expertise by as much as 50.1s per knot ($P = 0.033$). In addition, there was more marked decrease in time in participants with increased experience in robotics-assisted cardiac surgery. Further experiments are in progress.

Conclusion: We expect that cardiac surgery simulations with three-dimensional visualization will lead to faster and more efficient learning curves and operative performances with faster operative times.

6-09 Three-dimensional representation of the fiber bundle architectural pattern of extensor carpi radialis longus and brevis as parametric b-spline curves

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Introduction: Muscle architecture, the arrangement of fiber bundles relative to the force generation axis within the muscles have been attributed as the “best predictors of force generation” (Lieber and Friden 2000). To date, biomechanical models of skeletal muscles have used single or multiple linear segments or generic fiber bundle pattern to represent the internal muscle architecture. Ravichandiran et al. (2009) published architectural parameters of extensor carpi radialis longus (ECRL) and brevis (ECRB) as whole muscles and in parts. However, due to the complexity of the fiber bundle architectural arrangement and the number of fiber bundles involved, it was only feasible to publish mean parameters for the muscles and their regions despite the parameters being determined at the fiber bundle level.

Purpose: To develop computational techniques for mathematically defining the 3D fiber bundle distribution pattern of ECRL and ECRB that can be used to determine the architectural parameters from the mathematical model alone.

Methods: Previously digitized coordinate fiber bundle data was used to create b-spline representation of each fiber bundle. The b-splines were optimally simplified using a recursive computer script until the simplest representation was obtained without affecting the accuracy. Fiber bundles with statistically similar architectural parameters were then grouped into regions and each region was represented as a parameterized b-spline model. Sets of regional parameterized b-spline models were used to represent each muscle as a whole.

Results/Conclusions: ECRL with a relatively less complex architectural arrangement of fiber bundle requires less parameters to represent the muscle architecture than a complex muscle such as ECRB. Further work is still required to develop the parametric b-spline muscle architectural model that is feasible for publication as a series of mathematical equations.

6-10 Muscle architecture and innervation pattern of the superior head of the lateral pterygoid: A three dimensional analysis.

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Background: The lateral pterygoid is divided into superior (SLP) and inferior heads. The SLP has attachments to the disc-capsule complex of the temporomandibular joint (TMJ) and has been implicated in TMJ disorders. Despite its clinical significance, few studies have examined the intramuscular innervation and architecture relative to the muscle attachment sites.

Purpose: To investigate the intramuscular innervation and muscle architecture of SLP using 3D modelling.

Methods: Following exposure of SLP superiorly and laterally in 19 formalin embalmed cadaveric specimens, fibre bundles were serially dissected and digitized. In an additional 10 specimens, the intramuscular innervation was digitized throughout the muscle volume. The digitized data from both parts of the study were used to reconstruct 3D models using Maya® with laboratory developed plugins. From these models, muscle architecture and innervation pattern were analyzed and quantified.

Results: The SLP consisted of a superior part that inserted into the articular disc ($25.4 \pm 6.8\%$ of SLP) and TMJ capsule ($33.9 \pm 5.7\%$ of SLP), and an inferior part that inserted into the pterygoid fovea ($40.7 \pm 6.9\%$ of SLP). The superior part was subdivided into four quadrants by innervation: anterolateral, middle deep temporal (MDT) nerve ($n=6$) with contributions from buccal ($n=3$) and posterior deep temporal (PDT) nerves ($n=1$); anteromedial, buccal ($n=6$) or MDT ($n=4$) nerves; posteromedial, masseteric nerve ($n=6$) with contributions from buccal ($n=2$), MDT ($n=1$) and PDT ($n=1$) nerves; posterolateral, no predominant pattern (buccal, MDT or PDT nerves). The buccal nerve supplied the inferior part of SLP in all specimens. Additional innervation to this part was provided by masseteric and MDT nerves.

Conclusion: During the masticatory cycle the inferior part of SLP, inserting into the pterygoid fovea, moves the mandible while the superior part may position the disc capsule complex. Division of the superior part into quadrants based on innervation

pattern may indicate that movements of the disc capsule complex can be finely controlled to minimize forces occurring at the TMJ. Improper activation of the quadrants may result in excessive anteromedial and anterolateral movement of the disc capsule complex which may play a role in the progression of TMJ disorders. This is supported by Liu et al. (2010) who found excessive anteromedial and anterolateral displacement of the articular disc in patients suffering from TMJ disorders.

6-11 Establishing a platform for MRI-assisted laser robotic surgery

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Background: Recent advances in laser technology include fiber-guided lasers for improved surgical ergonomics, novel diode systems that are safe for intracranial tissue, and development of contact technology to provide tactile feedback to the surgeon. NeuroArm is uniquely equipped with 3 DOF haptic feedback to transmit forces of surgical dissection to the surgeon at the human-machine interface. Our goal was to develop a surgical robotic system with integrated laser technology.

Methods: A pre-clinical tool was manufactured to allow neuroArm to manipulate a 980nm fiber-guided contact diode laser, and applied in a rodent model ($N=50$). Following pre-clinical studies, neuroArm ($N=20$) and the laser ($N=4$) were independently integrated into surgical procedure, in a step-wise fashion. The pre-clinical tool served as a prototype for clinical grade manufacture.

Results: Overall, preliminary evaluation in rodents shows that laser technology allows faster pathological dissection when compared to a bipolar/microscissors combination in low and high vascular pathology. Good results have been obtained with case experience for both robotic ($N=20$) and laser ($N=4$) cases, with acceptance by the surgical team.

Conclusions: Our data demonstrate the first successful integration of contact laser technology with a haptic-enabled surgical robotic system. The clinical tool is presently being manufactured for simultaneous use of the technologies in clinical practice.

6-12 The association between quadriceps muscle parameters and patellar cartilage volume in knee osteoarthritis

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Background: Quadriceps weakness and reduced distal vastii function (vastus medialis, VM; vastus lateralis, VL) have been postulated to contribute to the degenerative changes associated with patellofemoral knee osteoarthritis (PFOA). The purpose of this study was to determine whether quadriceps strength, whole muscle volume or distal vastii volumes are associated with magnetic resonance imaging (MRI)-derived measures of patellar cartilage volume (VC) in patients with knee OA.

Methods: Twenty-three (13 female) community dwelling participants with knee OA completed isometric maximal voluntary knee extensor contractions for determination of quadriceps strength. MRI using 3D multi-echo spoiled gradient echo imaging sequences and a multi-point fat-water separation method were used to acquire images of the quadriceps and the PF joint. Semi-automated segmentation methods were used to derive measures of VC and muscle volume. The determinants of VC were obtained from a stepwise multiple linear regression model.

Results: The study parameters significantly ($p < 0.001$) associated with patellar VC were distal VM volume ($r^2 = 0.62$), distal VL volume ($r^2 = 0.23$), quadriceps volume ($r^2 = 0.63$), isometric strength ($r^2 = 0.64$) and sex ($r^2 = 0.48$). A stepwise model including distal VM volume ($\beta = 0.77$), strength ($\beta = 0.50$) and distal VL volume ($\beta = -0.46$) predicted the greatest proportion of variance in VC (adjusted $R^2 = 0.74$, $p < 0.001$). VM volume alone explained 61% of the variance in patellar VC in this model. Demographic factors such as age, height, body mass and pain (measured with the subscale score of the Western Ontario and McMaster Osteoarthritis Index) were not significantly associated with patellar VC.

Conclusions: Weakness of the distal VM may allow for mal-tracking of the patella during knee flexion. Although preliminary, these results provide support for this mechanism as a

large proportion of the variance in patellar VC was explained by distal VM volume.

6-13 Pulse Oximetry on the Ear is Likely Inaccurate

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Introduction: Pulse oximetry allows clinicians to detect hypoxemia non-invasively and is used as the standard of care on the hospital ward. Arterial blood oxygen saturations (O₂sats) are generally recorded using a finger clip, which has been validated against arterial blood gas. However, other locations, which are not well validated, are often used. We encountered a medical ward case in which intensive care intervention was delayed due to an inaccurate reading using the Nellcor tape on the forehead. This case prompted us to investigate the accuracy of pulse oximetry at different sites.

Methods: We measured O₂sats using a finger clip on all patients and concurrently compared this reading to O₂sat readings on the ear, toe, forehead, and another finger. In addition to the clip on these locations, we used the Nellcor tape on each location and a specialized Nellcor forehead sensor for the forehead. This gave us eight O₂sat comparisons to the clip on the finger. We reasoned that in order to be considered well correlated, other sites should measure O₂sats within four percent of the finger clip. We recruited patients from the internal medicine clinical teaching unit, at Royal University Hospital in Saskatoon, who required supplemental oxygen.

Results: We recruited twenty-three patients to our study. The majority of patients required oxygen to maintain their O₂sats greater than ninety-two percent. Most patients in the study group were over sixty years of age and had been admitted for COPD, CHF or pneumonia. Figure 1 shows that, with the exception of other finger readings, there was more than a 4% difference between finger clip readings and the readings from other locations more than 20 % of the time. Although the ear tape and specialized forehead Nellcor varied, on average, by more than 4%, the confidence intervals overlapped our 4% threshold for clinical significance. However, the non-specialized Nellcor forehead tape and ear clip were found to be inaccurate by more than 4% with a 90% confidence.

Conclusion: Previous studies have shown that finger clip readings correlate within $\pm 2\%$ of arterial blood gas measurements when the O₂sat is 82-96%. Our results show that the Nellcor

tape on the forehead and the ear clip both consistently overestimate finger clip readings by more than 4%. These results were statistically significant; therefore, the use of these sites cannot be recommended. Furthermore, the Nellcor taped ear and specialized forehead Nellcor, on average, varied from the finger clip by more than 4%, but these results were statistically insignificant. However, these methods should be used with caution, especially given the large standard deviation seen with the specialized forehead Nellcor. As expected, the finger clip correlated well with the Nellcor tape on the end or side of the finger. Our results suggest that the toe may be the most reliable alternative to the finger clip.

6-14 Osteoarthritic synovial fluid deficient in proteoglycan 4 demonstrates decreased boundary lubricating ability

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Background: Proteoglycan 4 (PRG4) proteins in synovial fluid (SF) and at the surface of articular cartilage function as boundary lubricants; they reduce friction, preventing wear and degradation, when cartilage surfaces are in contact with each other. SF deficient in PRG4 lacks normal boundary lubricating ability. We hypothesised that PRG4 levels can be diminished during osteoarthritis (OA).

Purpose: (1) Quantify PRG4 and hyaluronan (HA) content in normal (NL) and chronic OA human synovial fluid (hSF). (2) Assess the normal human cartilage boundary lubricating ability of OA hSF with deficient and elevated PRG4 concentration compared to normal hSF, with and without supplementation of PRG4±HA.

Methods: OA hSF was aspirated from patients receiving therapeutic injection. Sandwich ELISA was used to measure PRG4 (custom assay) and HA (commercially available assay) concentration. Human cartilage boundary lubricating ability of OA hSF, supplemented hSF, and NL hSF was assessed using a previously characterized cartilage-on-cartilage friction test. Data presented as mean±SEM.

Results: OA hSF deficient in PRG4 ($N = 4$, $293.6 \pm 70.8 \mu\text{g/mL}$) compared to NL ($N = 12$, $508.5 \pm 41.3 \mu\text{g/mL}$, $p < 0.05$) failed to lubricate; lubricating ability was restored by supplementation with PRG4. OA hSF with elevated PRG4 ($N = 4$, $774.6 \pm 69.2 \mu\text{g/mL}$, $p < 0.05$ vs NL) lubricated as well as NL hSF and lubricating ability was not significantly altered by supplementation.

Conclusion: Normal PRG4 levels may not be present in all chronic OA hSF. Some post-injury and chronic OA patients may benefit from PRG4 supplementation as a biotherapeutic treatment.

6-15 A 3D model of the musculotendinous architecture of piriformis: An implication in piriformis syndrome?

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Background: Many causes of Piriformis Syndrome (PS) are possible due to the complicated and variable nature of the anatomical relationship between piriformis, sciatic nerve (SN), and surrounding tissue. The literature has identified two potential principal etiologies (Hopayian, 2010). It is thought that with normal anatomy, the SN becomes entrapped as it passes inferior to the hypertrophied piriformis muscle. Conversely, an anomalous relationship between the piriformis muscle and SN may also contribute to the formation of PS. However this mechanism is still elusive due to conflicting evidence. **PURPOSE** To characterize and quantify the musculotendinous architecture of piriformis, and its relationship to the SN and greater sciatic foramen to enhance the anatomical foundation for these proposed etiologies.

Methods: Five formalin embalmed cadaveric hips were dissected to reveal piriformis, obturator internus, gemelli, and the osseofibrous greater sciatic foramen. Piriformis was then serially dissected and digitized using a Microscribe™ G2XDigitizer. Digitized data was imported into Autodesk Maya™ and 3D models were created with customized plugins developed in our lab. The muscle bundle architecture and extent of internal aponeurosis was analyzed within the compiled model. The musculotendinous architecture was then related to the position of the SN and greater sciatic foramen.

Results: Piriformis is a fan shaped muscle with a large intramuscular aponeurosis that is continuous with its tendon. The fibre bundles throughout the muscle volume insert into the aponeurosis which is thickest at its inferior border. The SN,

where it exits the pelvis, was found to be bound by the bone of the greater sciatic notch and the thick inferior part of the aponeurosis, positioning the nerve between dense connective tissue and bone. The SN was further restricted as it exited the pelvic cavity in the one anatomic anomaly found within our group where the common fibular division pierced both the muscle belly and aponeurosis.

Conclusions: There is a growing body of evidence supporting the mechanism of impingement of the SN as it exits the greater sciatic foramen. Our findings are in alignment with Kanakis et al. (2010) who found histological evidence of SN changes at its point of exit from the pelvis. The musculotendinous architecture of piriformis provides support for the anatomic impingement of the SN as it passes between piriformis and the sciatic foramen contributing to PS.

6-16 Diffuse non-ischemic microvascular disease in asymptomatic patients with type 2 diabetes

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Background: Type 2 diabetes is an independent risk factor for adverse cardiac events. The subclinical development of diabetic heart disease portends diffuse microvascular disease, prior to onset of clinically evident ischemic heart disease. Non-invasive screening methods, such as Cardiovascular Magnetic Resonance (CMR) imaging, are important for evaluation and risk stratification of these high-risk, yet asymptomatic patients.

Methods: The cross sectional pilot study includes data from 2 groups of subjects: Patients diagnosed with type 2 diabetes (HbA_{1c} 7.5-9.9%; mean age 56.1 ± 7.58 ; $n=10$), and healthy, non-diabetic age-matched controls (mean age 50.0 ± 9.8 ; $n=15$). Subjects were excluded if they had evidence of uncontrolled hypertension ($>140/90$ mmHg) or ischemic heart disease, determined by medical history and ECG. Qualified subjects underwent a CMR-Adenosine stress perfusion scan. Standard CMR protocols were used to assess cardiac function, morphology, and presence of fibrosis.

Results: Subendocardial perfusion delays were observed in 6 of 10 diabetic patients, and only 1 of 15 healthy controls ($p < 0.05$). Importantly, patients exhibited primarily diffuse, circumferential perfusion defects (Figure 1). Controls were normotensive (mean $124.8/79.9$ mmHg), and patients had normal or controlled blood pressure (mean $130.6/81.2$ mmHg), primarily through ACE inhibitors. Patients and controls had normal, comparable cardiac functional parameters (Left Ventricular Ejection Fraction $57.7\% \pm 2.4$ and $57.9\% \pm 3.6$, respec-

tively).

Conclusion: The observed perfusion abnormalities support previous nuclear imaging findings and pathophysiological research of diabetic heart disease, and are indicative of diffuse microvascular disease. Further studies are required to assess the pathophysiologic context and prognostic impact of these findings.

6-17 Accounting for variable lung density in whole-body PET/MRI attenuation correction

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Present attenuation correction (AC) algorithms in whole-body PET/MRI do not generally account for variations in lung density, either within or between patients. As lung density varies widely in a healthy population and even more so in the presence of pathology, failing to account for said variability may adversely impact accurate quantitation. In this work, a technique to incorporate patient specific lung density information into MRI-based attenuation maps is developed and compared to approaches that assume uniform lung density.

Methods: Five beagles were imaged with ^{18}F -FDG PET/CT and MRI. The MRI was comprised of two TurboFLASH sequences, one to visualize the whole body and the other to visualize lung parenchyma. The MRI of the lungs was registered to the CT to infer a mapping of MRI signal to CT signal. Subsequently, this mapping was used to convert the lung MRI into a pseudo lung CT. The whole-body MRI was segmented into three tissue types (air, lung, and soft tissue). The air and lungs were assigned a constant CT number, while the lungs were replaced with the pseudo lung CT. PET images reconstructed using MRI-based attenuation maps both with and without patient specific lung information were compared to PET images reconstructed with a CT-based AC method.

Results: Incorporating patient specific lung information into MRI-based attenuation maps was found to be favourable with respect to the quantitative accuracy of the PET in the lungs and for tissues in close proximity to them, including the myocardium. However, there exists a tradeoff between how much spatial information is incorporated into the estimates of lung density and how much noise is introduced into the MRI-based attenuation map.

Conclusions: A means of using MRI to infer the spatial distribution of CT number in the lungs was developed and applied to augment whole-body MRI-based attenuation maps. This technique been shown to have a desirable impact on the quantitative accuracy of PET within the lungs and clinically important tissues.

ORAL-1 New mechanisms of action of atypical antipsychotic quetiapine: implications to the treatment of psychiatric neurodegenerative disorders

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Background: Studies have shown myelin defects and oligodendrocyte (OL) dysfunction present in the patients with schizophrenia. However, the effects of antipsychotics on these disturbances remains unknown. In this project, we studied the effects of quetiapine (QTP), an atypical antipsychotic drug, on the OL development in vitro, and its effects on demyelination and remyelination in vivo.

Methods: Demyelination: C57BL/6 mice were fed a 0.2% cuprizone (CPZ)-containing or control diet for 5 weeks. QTP (10mg/kg/day, P.O) or vehicle (water) was co-administrated with CPZ for 5 weeks. Remyelination: CPZ 0.2% (w/w) was fed to C57BL/6 mice for 12 weeks to induce chronic demyelination and OL degeneration. At week 13 CPZ was withdrawn to allow remyelination and QTP (10mg/kg/day, P.O) or vehicle (water) was administrated for up to 4 weeks. Mice fed with normal chow for 12 weeks served as control. Locomotor activity was assessed by photobeam in a mouse chamber and spatial working memory was evaluated using the Y maze. Immunohistochemistry staining was used to detect morphological and biological changes in the brain. The in vitro study used rat embryonic neural stem cells cultured from E17 fetus brain and

treated the cells with QTP (0.1-1 μ m) for up to 6 days. Electron microscopy (EM) was used to detect myelination in cell culture.

Results: Exposure of male C57BL/6J mice to CPZ in the diet produced significant demyelination and cellular activation. CPZ administration resulted in hyperactivity and impaired spatial working memory compared to control mice. QTP co-administration significantly reduced the severity of behavioural impairments, demyelination and cellular response in the demyelinated regions. Remyelination occurred spontaneously when CPZ was withdrawn from the diet. QTP treatment resulted in improved working memory and myelin restoration compared to mice that received vehicle. QTP decreased OL accumulation but accelerated the repopulation of mature OLs in the demyelinated areas compared to vehicle. QTP facilitates the maturation of OL and myelin forming in neural stem cell culture, which is mediated by activation of extracellular signal-regulated kinase 2 (ERK-2) pathways. Both the in vitro and in vivo studies found the transcription factor olig2 was highly expressed in the OL progenitor cells but poorly expressed in the mature OLs.

Conclusion: These studies found that CPZ produced demyelination and schizophrenia-like behaviour. QTP treatment during the remyelination process resulted in improved working memory and reduced hyperactivity compared to vehicle treated mice. QTP facilitated the repopulation of mature OLs and appeared to facilitate the down regulation of olig2 in the process of cell maturation. The results suggest the role of OL dysfunction in schizophrenia pathogenesis and revealed a new feature of QTP in schizophrenia therapy.

ORAL-2 Disruptive Behaviour Disorders and Road Trauma in Adolescent Males

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Background: Adolescent male drivers contribute to a large number of serious road crashes despite low rates of driving and excellent physical health. We examined the amount of road trauma involving adolescent males that might be explained by prior disruptive behaviour disorders (Attention-Deficit Hyperactivity Disorder, Conduct Disorder, Oppositional Defiant Disorder).

Methods: We conducted a population-based case-control study of consecutive male youth between age 16 and 19 years hospitalized for road trauma (cases) or appendicitis (controls) in Ontario, Canada over 7 years (April 1, 2002 through March

31, 2009). Using universal health care databases, we identified prior psychiatric diagnoses for each individual during the decade before admission.

Results: Overall, a total of 3,421 patients were admitted for road trauma (cases) and 3,812 for appendicitis (controls). A history of disruptive behaviour disorders was significantly more frequent among trauma patients than controls (767 of 3,421 versus 664 of 3,812), equal to a one-third increase in the relative risk of road trauma (odds ratio = 1.37, 95% confidence interval 1.22–1.54, $p < 0.001$). The risk was evident over a range of settings and after adjustment for measured confounders (odds ratio 1.38, 95% confidence interval 1.21–1.56, $p < 0.001$). The risk explained about one-in-20 crashes, was apparent years before the event, extended to those who died, and persisted among those involved as pedestrians.

Conclusion: Disruptive behaviour disorders explain a significant amount of road trauma in adolescent males. Programs addressing such disorders should be considered to prevent injuries.

ORAL-3 Surgical stress promotes the development of cancer metastases by a coagulation-dependent mechanism involving Natural-Killer cells in a murine model.

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Background: Surgery precipitates a hypercoagulable state and has been shown to increase the development of cancer metastases in animal models, however mechanism(s) responsible for this are largely unknown. We hypothesize that the prometastatic effect of surgery may be secondary to the postopera-

tive hypercoagulable state.

Objective: To determine if surgical stress promotes the development of cancer metastases by increased formation of tumor cell emboli (TCE) associated microthrombi.

Methods: Surgical stress was induced in Balb/c or C57Bl/6 mice by laparotomy and partial hepatectomy (liver surgery) or laparotomy and left nephrectomy (left kidney resection), preceded by tail vein injection of CT26-LacZ colon cancer or B16F10-LacZ melanoma cells to establish pulmonary metastases with or without perioperative anticoagulation with tinzaparin, dalteparin (low-molecular-weight-heparins), warfarin (Vitamin-K dependent factor II, VII, IX and X inhibitor), hirudin (thrombin inhibitor) or antibody that depletes mouse platelets in vivo. Mice were euthanized at day 3 post tumor cell injection and their lung tumor burden was quantified. Fibrinogen and platelets were fluorescently labeled prior to surgical stress to evaluate TCE associated fibrin and platelet clots. Role of Natural-Killer (NK) cells in post-operative tumor metastases was evaluated using anti-asialo antibody and confirmed using transgenic mice deficient of NK cells.

Results: Surgery resulted in a two-fold increase in metastases while anticoagulation with all five agents attenuated this effect at 3-days post surgery. Fibrin and platelet clots were associated with TCE significantly more frequently in mice that underwent surgery, as compared to mice with no surgery or pretreatment with anticoagulation. In mice with intact NK cells, surgery significantly increased lung tumor burden, however, this effect was lost when mice were depleted of NK cells either pharmacologically or genetically.

Conclusions: Surgery promotes the formation of fibrin and platelet clots around TCE and this appears to be the mechanism for the increase in metastases seen following surgery. Anticoagulation abrogates this prometastatic effect. NK cells seem to play an important role in the development of post-operative tumor metastases. Therapeutic interventions aimed at reducing peritumoral clot formation and enhancing NK cell function in the perioperative period will have important clinical implications in attenuating metastatic disease.

ORAL-4 The role of SHIP-2 in CEACAM1-dependent host cellular responses to *Neisseria gonorrhoeae*

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Background: The Carcinoembryonic Antigen-related Cellular Adhesion Molecule-1 (CEACAM1), expressed on the surface of epithelial cells, acts as a receptor for *Neisseria gonorrhoeae*. CEACAM1 functions as a co-inhibitory receptor via immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic domain that recruit tyrosine phosphatases which dephosphorylate components involved in responses to pathogens. Inhibiting these responses is one mechanism by which *N. gonorrhoeae* evades the immune system. Several cellular receptors have been shown to recruit both tyrosine and lipid phosphatases and thereby participate in multiple pathways. A bioinformatic screen suggested that CEACAM1 may act in such a fashion; however, this function has not yet been investigated. SHIP-2 is a 5'-inositol phosphatase considered to be a negative regulator of activating phosphoinositide pathways. We hypothesize that SHIP-2 associates with CEACAM1 in response to infection by *N. gonorrhoeae* and negatively regulates phosphoinositide signaling pathways.

Methods: In an epithelial cell model, the association of SHIP-2 with CEACAM1 and the levels of membrane-localized phosphoinositides were assessed by microscopy-based techniques. Pro-inflammatory mediator secretion was assessed by ELISA.

Results: SHIP-2 colocalizes with *N. gonorrhoeae*-bound CEACAM1 but not with bacteria adhering to other receptors. SHIP-2 colocalizes with extracellular but not intracellular bacteria. Similarly, PIP₃ colocalizes with extracellular bacteria whereas 3,4-PIP₂ colocalizes with intracellular bacteria, presumably generated by the degradation of PIP₃ by SHIP-2. SHIP-2 expression has both enhancing and inhibitory effects on pro-inflammatory mediator secretion (IL-6 and IL-8, respectively) by epithelial cells in response to *N. gonorrhoeae*.

Conclusion: SHIP-2 associates with neisserial-bound CEACAM1 in an epithelial cell model. Our observations suggest that this association alters the levels of phosphoinositides during bacterial uptake which in turn has variable effects on pro-inflammatory mediator secretion. The CEACAM1-dependent recruitment of SHIP-2 upon *N. gonorrhoeae* binding suggests that CEACAM1 may regulate phosphoinositide pathways and thereby contribute to neisserial inhibition of immune cells.

ORAL-5 The NLRP3 inflammasome induces cardiac dysfunction and arrhythmogenesis in calcineurin-overexpressing mice through Protein Kinase C

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Background: Chronic diseases of diverse etiology share a systemic, low-grade sterile inflammation that contributes to disease progression. For example, persistent cardiac inflammation post myocardial infarction can result in heart failure and is associated with adverse clinical outcomes including arrhythmic deaths. IL-1b is a pro-inflammatory cytokine released in response to danger signals. On recognition of danger associated molecular patterns (DAMPs) such as ROS, ATP and urate, a multiprotein complex termed the inflammasome is activated. This leads to activation of the cysteine protease caspase-1, which cleaves pro-IL-1b into its active form. Cardiac specific overexpression of the calcium dependent protein phosphatase calcineurin (CN/Tg) activates a range of hypertrophic signals. CN/Tg mice develop massive cardiac hypertrophy followed by inflammation, ventricular dilatation, heart block and episodes of ventricular tachycardia. Given the role of inflammation in other chronic disease states, we hypothesized that the inflammasome is activated in the CN/Tg heart failure model and that IL-1b contributes to myocardial dysfunction and arrhythmias.

Methods and Results: We detected multiple NLRP transcripts in WT and CN/Tg hearts. Cardiac tissue from CN/Tg mice had elevated NLRP3 mRNA and increased conversion of pro-IL-1b, IL-18 and pro-caspase-1 to their active forms. In vitro IL-1b prolonged action potential duration and induced a slowly inactivating component of INa in WT myocytes, which was abolished on PKC inhibition with BIS. Blockade of PKC in CN/Tg ventricular myocytes resulted in restoration of INa. In vivo, CN/Tg mice treated with recombinant IL-1Ra had significantly improved systolic performance (p<0.001) and reduced episodes of heart block (p<0.00001). Lastly, inhibi-

tion of isozyme specific PKC- β 2 in CN/Tg mice resulted in markedly reduced caspase-1 activation and myocardial infiltrate.

Conclusions: Taken together, our results indicate that the inflammasome is active and contributes to myocardial dysfunction and arrhythmogenesis in CN/Tg mice through a PKC dependent pathway. Blockade of IL-1 β reversed these phenotypes. These results identify the inflammasome as a potential novel target for subsequent therapeutic exploitation in the treatment of heart failure.

ORAL-6 Integrin-Linked Kinase (ILK) is required for TGF- Receptor type II signalling in dermal myofibroblasts

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Background: Cutaneous wound closure requires the transition of dermal fibroblasts to myofibroblasts, cells that display smooth muscle-like properties. This process requires stimulation of transforming growth factor (TGF)- β receptors. TGF- β 1 is involved in multiple aspect of cell function, and abnormal regulation of TGF- β 1 signalling leads to severe pathological conditions, including lethal tissue inflammation, cancer, and fibrosis. In the dermis, we have established that TGF- β 1 induction of myofibroblast differentiation is dependent on integrin-linked kinase (ILK). Thus, the purpose of this study was to determine the mechanisms involved in modulation of TGF- β 1

signalling by ILK.

Methods: Dermal fibroblasts were isolated from 3 day-old Ilk^{fl}/f mice and cultured. Ilk gene inactivation was induced by Cre-lox recombination using adenovirus mediated expression of Cre recombinase. Fibroblast populated collagen lattice cultures were utilized to assess cell contractility, and levels of phosphorylated Smad2 was used to measure TGF- β 1 signalling. Co-immunoprecipitation, immunoblotting, and quantitative polymerase chain reaction were used to assess protein interactions, changes in protein levels, and mRNA levels, respectively.

Results: ILK deficiency resulted in impaired Smad2 phosphorylation in response to TGF- β 1, which was associated with decreased interaction between TGF- β receptor type I and type II. ILK-deficient cells displayed reduced levels of TGF- β receptor type II accompanied by increased receptor ubiquitination and turnover. Inhibition of the proteasome restored TGF- β receptor type II protein levels, although this led to only a partial rescue of Smad2 phosphorylation in response to TGF- β 1 stimulation. Collectively, this study demonstrates a novel role of ILK in regulating TGF- β 1 signalling by affecting TGF- β receptor type II stability. We further identified an interaction between ILK and TGF- β receptor type II and determined that this interaction does not require association with β 1 integrins. In this capacity, ILK appears to promote TGF- β 1 signalling in dermal fibroblasts by protecting against TGF- β receptor down-regulation, potentially via direct interactions.

Conclusion: ILK is necessary to transduce signals implicated in the transition of dermal fibroblasts to myofibroblasts, through regulation of TGF- β receptor type II turnover and signaling.