The Westmead Association

2018 HOSPITAL WEEK

RESEARCH, NURSING & MIDWIFERY, ALLIED HEALTH RESEARCH ABSTRACTS

AUGUST 29th 30th & 31st

40 Years of Excellence 1978-2018
# RESEARCH ABSTRACTS

<table>
<thead>
<tr>
<th>Topic</th>
<th>ABSTRACT No’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent Medicine</td>
<td>1 - 5</td>
</tr>
<tr>
<td>Brain Dynamics &amp; Mental Health</td>
<td>6 - 10</td>
</tr>
<tr>
<td>Cancer &amp; Haematology</td>
<td>11 - 29</td>
</tr>
<tr>
<td>Cardiology &amp; Cardiovascular Disease</td>
<td>30 - 49</td>
</tr>
<tr>
<td>Dermatology</td>
<td>50 - 53</td>
</tr>
<tr>
<td>Diabetes &amp; Endocrinology</td>
<td>54 - 55</td>
</tr>
<tr>
<td>Embryology</td>
<td>56 - 57</td>
</tr>
<tr>
<td>Genetics &amp; Gene Therapy</td>
<td>58 - 70</td>
</tr>
<tr>
<td>Hepatology &amp; Liver Disease</td>
<td>71 - 74</td>
</tr>
<tr>
<td>Immunology</td>
<td>75 - 82</td>
</tr>
<tr>
<td>Infectious Diseases</td>
<td>83 - 111</td>
</tr>
<tr>
<td>Neurology &amp; Neuroscience</td>
<td>112 - 115</td>
</tr>
<tr>
<td>Oral Pathology</td>
<td>116</td>
</tr>
<tr>
<td>Paediatrics &amp; Neonatology</td>
<td>117 - 123</td>
</tr>
<tr>
<td>Public Health &amp; Health Services Research</td>
<td>124 - 131</td>
</tr>
<tr>
<td>Renal</td>
<td>132 - 148</td>
</tr>
<tr>
<td>Surgery &amp; Transplantation</td>
<td>149 - 154</td>
</tr>
<tr>
<td>Women's Health</td>
<td>155 - 156</td>
</tr>
</tbody>
</table>

# NURSING & MIDWIFERY ABSTRACTS

# ALLIED HEALTH ABSTRACTS

Acknowledgement of Assessors
2018 RESEARCH ABSTRACTS
<table>
<thead>
<tr>
<th>Time</th>
<th>Presenter</th>
<th>Abstract No.</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.30 – 10.40</td>
<td>Noa Lamm-Shalem</td>
<td>16</td>
<td>Actin polymerization alters nuclear architecture in response to DNA replication stress to maintain genome stability</td>
</tr>
<tr>
<td>10.40 – 10.50</td>
<td>Rebecca Poulos</td>
<td>20</td>
<td>Characterisation of prostate cancer prognosis by machine learning of 1,566 prostate proteomes generated by PCT-SWATH mass spectrometry</td>
</tr>
<tr>
<td>10.50 – 11.00</td>
<td>Koon Hiang Lee</td>
<td>22</td>
<td>Ex vivo expanded PRAMEspecificT lymphocytes for tumour immunotherapy exhibit an extended cytokine profile</td>
</tr>
<tr>
<td>11.00 – 11.10</td>
<td>Benjamin Nash</td>
<td>58</td>
<td>Genomics, molecular diagnoses and genotype-phenotype insights in the inherited retinal dystrophies</td>
</tr>
<tr>
<td>11.10 – 11.20</td>
<td>Anais Amaya</td>
<td>61</td>
<td>Successful in vivo editing of patient-derived primary human hepatocytes</td>
</tr>
<tr>
<td>11.20 – 11.30</td>
<td>Christopher Denes</td>
<td>92</td>
<td>Mapping the egress pathway of HSV-1 by determining the interactome of viral envelope glycoprotein gE</td>
</tr>
<tr>
<td>11.30 – 11.40</td>
<td>Aleksandra Fabijan</td>
<td>98</td>
<td>Bacteriophages as adjuvant therapy to knock down the bacteraemic burden in severe Staphylococcal sepsis</td>
</tr>
<tr>
<td>11.40 – 11.50</td>
<td>Ratna Wijaya</td>
<td>100</td>
<td>Hepatitis B vaccination or infection induces novel antigen-specific human memory natural killer cells</td>
</tr>
<tr>
<td>Time</td>
<td>Presenter</td>
<td>Abstract No.</td>
<td>Title</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>12.00 – 12.05</td>
<td>Isabella Breukelaar</td>
<td>9</td>
<td>Differences in cognitive control brain activation between euthymic bipolar and remitted unipolar depressed individuals</td>
</tr>
<tr>
<td>12.05 – 12.10</td>
<td>Nipu Jayatilleke</td>
<td>24</td>
<td>A novel predictive tool for heavy axillary nodal involvement in sentinel node positive breast cancer</td>
</tr>
<tr>
<td>12.10 – 12.15</td>
<td>Amin Sabri</td>
<td>60</td>
<td>Functional genomic and mouse model studies in characterisation of a novel retinal ciliopathy</td>
</tr>
<tr>
<td>12.15 – 12.20</td>
<td>Ruebena Dawes</td>
<td>69</td>
<td>Mendelian disease gene analysis: Animal phenotypes surpass scores of genetic constraint in guiding novel gene discovery</td>
</tr>
<tr>
<td>12.20 – 12.25</td>
<td>Di Yuan</td>
<td>88</td>
<td>HIV and the colorectal mucosa-investigating the early interactions of HIV with mucosal target cells in Situ</td>
</tr>
<tr>
<td>12.25 – 12.30</td>
<td>Darcii Terre</td>
<td>99</td>
<td>Mycobiome of Bondi Beach sand – a clinical connection?</td>
</tr>
<tr>
<td>12.30 – 12.35</td>
<td>Hanwen Chen</td>
<td>101</td>
<td>Synergetic effects of antibiotics against multidrug resistant gram-negatives</td>
</tr>
<tr>
<td>12.35 – 12.40</td>
<td>Vanessa Marcelino</td>
<td>111</td>
<td>Antibiotic resistance genes in the microbiome of wild birds</td>
</tr>
<tr>
<td>12.40 – 12.45</td>
<td>Natasha Rogers</td>
<td>13</td>
<td>CD47 blockade modulates fibrosis in chronic kidney injury</td>
</tr>
<tr>
<td>12.45 – 12.50</td>
<td>Titi Chen</td>
<td>145</td>
<td>Targeting CD103+ dendritic cells using FLT3 inhibitors for treatment of kidney disease; relevance to human kidney disease</td>
</tr>
</tbody>
</table>
Efficacy of Very Low-Energy Diets for Weight Loss: A systematic review of intervention studies in Children and Adolescents with Obesity

Authors: Sarah Andela,1 Tracy L Burrows,2 Louise A Baur,1,3 Daisy Coyle,4 Clare E Collins,2 Megan L Gow1,3

Affiliations: 1The University of Sydney, 2The University of Newcastle, 3The Children’s Hospital at Westmead, 4Sax Institute

Aim: Very low-energy diets (VLEDs) are designed to achieve rapid weight loss. This systematic review aimed to determine the efficacy and safety of VLEDs for weight loss in children and adolescents with obesity.

Methods: A systematic search of six health and medical databases up until November 2017 identified 24 eligible English-language VLED intervention studies in children and adolescents (£18-years) with obesity. Quality appraisal was conducted using The Academy of Nutrition and Dietetics quality criteria checklist and study data extracted to customised tables. Meta-analysis was performed using Comprehensive Meta-Analysis software.

Results: Sixteen pre-post, four non-randomised comparison, two randomised controlled trials and two chart reviews studied a 3-24 week VLED intervention providing £800kcal/day (3350kJ) or <50% daily estimated energy requirements. Weight was the most frequently reported weight-related outcome (20 studies). Meta-analysis of these 20 studies indicated 8.1kg mean weight loss (95% confidence interval [CI]: 7.1 to 9.0kg, p<0.001) following the intervention. Adolescent only studies had greater weight loss compared with studies including child and adolescent participants (17.7kg [CI: 9.9 to 25.6kg, p<0.001, n=4] compared with 7.9kg [CI: 7.0 to 8.9kg, p<0.001, n=16] weight loss). Meta-analysis of seven studies reporting weight outcomes beyond the intervention period (20-weeks to 14.5-months from baseline) indicated 5.2kg mean weight loss (CI: 2.7 to 7.7kg, p<0.001) at latest follow-up compared with baseline. Only 12 studies reported side effects: five reported no adverse side effects and seven reported mild adverse side effects (e.g. fatigue, hunger, nausea).

Discussion: The evidence to date suggests VLEDs are safe and effective for treating children and adolescents with obesity. Weight loss may be more pronounced in adolescents. A VLED may lead to greater weight loss compared with more traditional dietary interventions. Future studies should determine strategies for maintaining weight loss following a VLED intervention and comprehensively assess adverse effects associated with VLED adherence.
Weight Loss Outcomes for Adolescents with Obesity in a Tertiary Clinic
Sarah Young1, Alicia Grunseit2, Kerryn Chisholm2, Natalie B Lister3,4
1University of Sydney, Nutrition and Dietetics Group, School of Life and Environmental Sciences, The Charles Perkins Centre, University of Sydney 2006
2Nutrition and Dietetics Department and Department of Weight Management Services, The Children’s Hospital at Westmead, Westmead, New South Wales, Australia
3Institute of Endocrinology and Diabetes, The Children’s Hospital at Westmead, Westmead, New South Wales, Australia
4University of Sydney, Discipline of Child and Adolescent Health, Westmead NSW 2145, Australia

Background: Evidence for the management of obesity in adolescence is growing, and treatment principles focus on long-term behaviour change, management of obesity associated complications and a developmentally appropriate approach. However, data demonstrating the effectiveness of weight management in adolescents in “real-world” clinics is lacking.

Aim: The aim of this study is to explore the weight related outcomes of a multidisciplinary weight management clinic for adolescents with obesity in a tertiary hospital.

Method: A retrospective medical chart review of the Weight Management Service at The Children’s Hospital at Westmead between October 2014 to December 2017 was conducted. A total of 57 participants aged 13-17y were identified and anthropometric data was extracted and used for index calculations. Statistical analysis was intention to treat, and sub-group analysis was completed to compare genders, dietary interventions and attendance.

Results: Overall, weight related outcomes significantly improved from baseline until last contact with the clinic: BMI z-score (-0.05±0.45, p=<0.001), weight (-2.32±6.49kg, p=0.021), waist circumference (WC; -2.3±6.49cm, p=0.03), BMI (-1.06±2.08kg/m2, p=0.001), centile (-0.69±1.75, p=0.029), BMI expressed as a percentage of the 95th percentile (BMI95; -7.89±12.19%, p=<0.001) and Waist-to-Height ratio (WtHR; -0.02±0.16, p=0.01). Attendance decreased over time but increased contact with the clinic significantly improved all weight related outcomes. Adolescents who completed the 6-month program (n=10) had significant reductions in weight related outcomes, including BMI z-score (-0.15±0.13, p=0.021); centile (-0.45±0.45, p=0.035) and BMI95 (-7.63±7.46%, p=0.043). Girls had greater reductions in BMI z-score and centile (-0.11±0.06, p=0.02 and -0.35±0.17, p=0.017) compared to boys. Changes in weight related outcomes were greatest when adolescents were following a very low energy diet, particularly for BMI z-score (-0.12±0.11) and BMI95 (-5.44±6.19%).

Discussion: These data demonstrate the effectiveness of the real world clinical practice for management of adolescent obesity and emphasise the importance of clinic attendance for improvement of weight related outcomes.
**Intermittent energy restriction in adolescents with obesity: a pilot study**

H Jebeile1,2, ML Gow1,2, NB Lister1,2, K Chisholm3,4, A Grunseit3,4, S Alexander4, CT Cowell2,5, LA Baur1,4, SP Garnett1,2,5  
1The University of Sydney, Discipline of Child and Adolescent Health, Sydney, Australia  
2Institute of Endocrinology and Diabetes, The Children’s Hospital at Westmead, Sydney, Australia  
3Department of Nutrition and Dietetics, The Children’s Hospital at Westmead, Sydney, Australia  
4Weight Management Services, The Children’s Hospital at Westmead, Sydney, Australia  
5Kids Research, The Children’s Hospital at Westmead, Sydney, Australia

**Introduction:** Obesity rates in adolescents remain high and novel treatment approaches are required. In adults, intermittent energy restriction (IER), popularised as the 5:2 diet, is as effective for weight loss as continuous energy restriction. We investigated the use of IER in adolescents with obesity.

**Methods:** During weeks 1-12, participants followed an IER dietary plan including a Very Low Energy Diet (VLED) 3 days/week (500-600kcal/day) and a healthy diet 4 days/week. For weeks 13-26, participants were given a choice to continue with 1-3 days of VLED/week or follow a healthy diet. Outcomes measured at 0, 12 and 26 weeks were body composition, cardiovascular risk, and diet acceptability.

**Results:** 30 participants, aged 12-17 years (mean [SD] 14.5yrs [1.4], female n=25) with a median BMI 34.9kg/m² (range: 27.7-52.4), were recruited. Compared with baseline, body weight and BMI expressed as a percentage of the 95th percentile (BMI%95th) were significantly reduced at 12 weeks (n=23, -3.3kg [3.7] p<0.0001, -5.4(2.2), p<0.0001), with reduced BMI%95th maintained at 26 weeks (n=21, -5.0 [9.3], p=0.02). Improved body composition, with reduced fat mass (n= 17, -4.31kg [6.4], p=0.025) and maintenance of fat free mass (p=0.937), was seen at 26 weeks. Triglycerides were reduced at 26 weeks compared with baseline (n=21, -0.22mmol/L[0.31], p=0.008). At 12 weeks, all participants chose to continue IER with 2 days/week (n=12) or 3 days/week (n=10) of VLED. Adolescents found IER acceptable, rating it as easy (mean [SD] n=19, +2.1 [1.2]) and pleasant (n=19, +1.9 [1.2]) on a Likert scale from -4 to +4.

**Conclusion:** Intermittent energy restriction is an effective and acceptable dietary intervention in adolescents with obesity. A randomised controlled trial is required to compare IER with continuous energy restriction.

This study was funded by the Financial Markets Foundation for Children and the Heart Foundation of Australia Vanguard Grant.
How do marginalised young people navigate the Australian healthcare system?

Fiona Robards1, Melissa Kang1,2, Lena Sanci3, Catherine Hawke4, Marlene Kong5, Stephen Jan6, Kate Steinbeck7 Tim Usherwood1,6

Department of General Practice, The University of Sydney, Westmead
University of Technology Sydney, Ultimo
Department of General Practice, University of Melbourne, Carlton
School of Rural Health, University of Sydney, Orange
The Kirby Institute, University of New South Wales, Randwick
The George Institute for Global Health, Sydney
Discipline of Paediatrics and Adolescent Health, University of Sydney

Aim: Disparities in health and wellbeing exist among those who are socially and economically marginalised. Access, engagement and navigation around health systems are key components of universal health coverage. Research about healthcare access and navigation of healthcare systems by marginalised young people, including their use of technology, is limited.

Method: Funded by NSW Health to inform policy, the Access 3 project focused on marginalised young people aged 12-24 in NSW. Part of Access 3, this qualitative longitudinal study involved 3-4 interviews over 12 months. Semi-structured interviews were guided by questions about health literacy, health service access and navigation through the health system over time. Analysis used Nvivo software and Grounded Theory (Corbin and Strauss, 2015).

Results: We interviewed 41 young people who were gender and/or sexuality diverse (n=20), rural (n=20), refugee (n=9), homeless (n=9) and/or Indigenous (n=5), with 22 belonging to one marginalised group; 16 belonged to two; and three belonged to three groups, allowing an exploration of intersectionality. A retention rate of over 85% was achieved.

Marginalised young people have complex lives and lack preparation in their role as health system navigators. Their healthcare journeys are challenged by system demands and complexity and experiences of discrimination. Five themes emerged: Young people’s health literacy embraces our connected, digitally disrupted world. Costs are often prohibitive and hidden. The ability to pay and knowledge of free healthcare services determines the path chosen by young people. Engagement is all about the people and relationships. Welcoming, respectful service environments and structures offer marginalised young people a safe haven from an often hostile and confusing health system. Health system navigation must be assertively supported.

Discussion: In addition to welcoming and respectful services, young people require active navigation support and technology solutions. Professionals can engage young people as agents for change.
Investigating the Influence of Digital Health Information on Adolescents’ Interactions with Health Professionals

Tiffany B. Allen, Karen M. Scott, BEd, MA, PhD, and Patrina H. Y. Caldwell, BMed, FRACP, PhD

Society’s increased access to and use of digital health information has created an informed patient who desires to be in control of their health. A shift in the patient’s role within a health consultation has been shown to alter the dynamic of the patient – health professional relationship. The adult experience in the face of this change has been extensively discussed, however an understanding of the adolescent experience still appears to be lacking.

Understanding the unique adolescent – health professional relationship is important as the nature of this interaction determines the development of health behaviours that endure into adulthood. This study aims to further understand this relationship from the perspective of the adolescent as influenced by the increased use of digital sources of information to answer questions related to health.

The study will employ a qualitative research methodology and thematic analysis. Semi-structured one-on-one interviews will be conducted either at the Children’s Hospital at Westmead or via Skype according to participant type and preference. Participants will be 12-18 years and make up two cohorts; ‘Patient’ and ‘External’, those from the Children’s Hospital at Westmead and those not currently being treated within the hospital respectively.

Recruitment techniques will differ between cohorts as the role of the researcher in recruitment will alter from approaching and inviting participants, too allowing participants to independently express their interest in being involved via contacting the researcher. Interviews will be audio recorded and transcribed verbatim.

Techniques developed from the grounded theory approach will guide thematic analysis of the data. Key words and phrases will be further classified into broader concepts that will form the overall themes and sub-themes of the study. The data will be extensively reviewed throughout by researchers to decrease and resolve all discrepancies.

The results of this study will be presented.
Does White Matter Microstructural Integrity differ in the combined and inattentive subtypes of ADHD? A Diffusion Tensor Imaging Study.

Saad, J.F.1,2, Griffiths, K.R.1, Kohn, M.R.1,3, Clarke, S.1,3, Williams, L.M.4,5, Korgaonkar, M.S.1,2

Brain Dynamics Centre, The Westmead Institute for Medical Research, and Sydney Medical School, Sydney, NSW, Australia
The Discipline of Psychiatry, Sydney Medical School, The University of Sydney, Westmead Hospital, Sydney, NSW, Australia
Centre for Research into Adolescents’ Health, Department of Adolescent and Young Adult Medicine, Westmead Hospital, Sydney, NSW, Australia
Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
MIRECC, Palo Alto VA, Palo Alto, CA, USA.

Aims: Converging evidence indicates that dysfunctional brain network connections are concordant with aberrant neuroanatomical and functional features; these findings extend support for differential neural mechanisms underlying the ADHD subtypes. However, diffusion tensor imaging (DTI) studies investigating microstructural white matter (WM) properties between the ADHD subtypes are limited and have shown equivocal results.

Methods: We used DTI data from 35 ADHD participants defined using DSM-IV criteria as combined (n=19) or as predominantly inattentive type (n=16), aged 8-17 years, and 28 matched neurotypical controls. We performed tract-based spatial statistical (TBSS) analyses on DTI derived measures of fractional anisotropy (FA), mean (MD), radial (RD), and axial (AD) diffusivity to assess differences in WM microstructural integrity between the two ADHD subtypes and controls.

Results: None of the DTI measures (FA, MD, RD, and AD) were significantly different between the ADHD subtypes, or for each subtype relative to controls.

Discussion: Adding to the paucity of DTI studies examining ADHD subtypes, this study did not observe WM differences distinguishing the ADHD subtypes. These findings contrast with our previous results from the same cohort which demonstrated distinguished organizational profiles between subtypes using structural covariance network measures, which are characteristic of functional connectivity.

Together, this may suggest that functional network patterns may better account for the clinical symptoms that characterize ADHD subtypes. Further, whole brain connectome and graph theory analyses to explore topological differences in WM network organization between ADHD subtypes will be undertaken.
**ABSTRACT 7**

**Amygdala activation and connectivity to emotional processing distinguishes patients with euthymic bipolar disorders and remitted unipolar depression.**

May Erlinger1, Isabella A. Breukelaar1, Philip Boyce2, Philip Hazell2, Cassandra Antees1, Sheryl Foster3,4, Stuart M. Grieve5,6, Lavier Gomes3, Leanne M. Williams1,7,8, Anthony W.F. Harris1,2, Gin S. Malhi2,9, Mayuresh S. Korgaonkar1,2

**Background:** Mechanistically based neural markers such as amygdala reactivity offer one approach to addressing the challenges of differentiating bipolar and unipolar depressive disorders, independently from mood state and acute symptoms. Although emotion-elicited amygdala reactivity has been found to distinguish bipolar from unipolar patients in depressed states, it remains unknown whether this distinction is trait-like and present in the absence of an acutely depressed mood. We addressed this knowledge gap by investigating euthymic bipolar (BP) and unipolar major depressive disorder (MDD) populations assessed in remission (rather than in acutely unwell states).

**Methods:** 81 participants (31 BP and 25 MDD patients matched for age-gender, number of depressive episodes and severity; 25 age-gender matched healthy individuals) completed fMRI tasks measuring supra- and subliminal emotion processing of threat, sad, happy and neutral faces. We compared groups for neural activation and connectivity for the amygdala.

**Results:** BP participants had lower left amygdala activation relative to MDD during supraliminal and subliminal threat, sad and neutral processing and for subliminal happy faces. BP participants also exhibited lower connectivity between the amygdala and insula for threat and between the amygdala and medial orbito-frontal for happy supraliminal and subliminal processing. BP participants also demonstrated greater amygdala-insula connectivity for sad supraliminal and subliminal face processing.

**Conclusion:** Independent of valence or level of emotional awareness amygdala activation and its connectivity during facial emotion processing can distinguish BP and MDD patients. These findings provide evidence that this neural substrate could be a potential trait-marker to differentiate these two disorders largely independent of illness state.
Prevalence of sensory and emotional features in children with tic disorders.

Nicolette Soler1,3, Chris Harwick1, Dr Iain Perkes 4, Dr Shekeeb S Mohammad2,3, Dr Paula Bray3, Prof Russell Dale2,3
1 The Children's Hospital at Westmead, Department of Psychological Medicine.
2 The Children's Hospital at Westmead, Department of Paediatric Neurology.
3 The Children's Hospital at Westmead Clinical School, University of Sydney.
4 Faculty of Science, The University of New South Wales

Background: Tic disorders negatively interrupt a child's daily participation. Broader somatic hypersensitivity has been described in this population. Therefore, tics may involve sensorimotor phenomena, rather than a 'pure' movement disorder.

Aims: This prevalence study aimed to explore the relationship between sensory and emotional control features in children with tic disorders.

Methods: A 163 children, (with tic disorders (n=103) and healthy controls (n=60)) were recruited through the Tic Clinic at the Children's Hospital at Westmead. Parents completed 5 questionnaires that assessed their child's sensory preferences, executive functioning and quality of life. A staff specialist completed the Yale Global Tic Severity Scale and the Premonitory Urge to Tic Scale.

Results: Compared with healthy controls, participants with tic disorders reported experiencing: Significantly more sensory sensitivities as measured on the Sensory Processing Measure and Short Sensory Profile 2 (SSP2) (T=9.256; Mean=15.360; p<0.001; T=9.398; Mean=15.360; p<0.001).

Significantly greater emotional control difficulties as measured using the Behaviour Rating Inventory of Executive Function 2 (BRIEF2) (Emotional Regulation Index) (T=11.786; Mean=13.204; p<0.001).

There was a strong relationship between sensory sensitivities (SSP2) and emotional control difficulties (ERI) in children with tic disorders (r=0.718).

Discussion: This study provides useful information for clinicians relating to the sensory profile and specific sensory sensitivities of children with tic disorders. Further research into the inclusion of sensorimotor and emotional control strategies to complement existing first-line treatment for tic disorders is required.
Differences in cognitive control brain activation between euthymic bipolar and remitted unipolar depressed individuals

Isabell Breukelaar1, May Erlinger1, Anthony Harris12, Philip Boyce2, Gin S. Malhi23, Philip Hazell2, Stuart Grieve14, Cassandra Antees1, Sheryl Foster 67, Lavier Gomes6, Leanne M. Williams89, Mayuresh S. Korgaonkar12

1Brain Dynamics Centre, The Westmead Institute for Medical Research, The University of Sydney, Westmead, Sydney, Australia
2Discipline of Psychiatry, Sydney Medical School, University of Sydney, NSW, Australia
3CADE Clinic, Department of Psychiatry, Royal North Shore Hospital, Sydney, NSW, Australia
4Sydney Translational Imaging Laboratory, Heart Research Institute, Charles Perkins Centre and Sydney Medical School, University of Sydney, NSW, Australia
5Department of Radiology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia
6Department of Radiology, Westmead Hospital, Westmead, NSW, Australia
7The Discipline of Medical Radiation Sciences, Faculty of Health Science, The University of Sydney, NSW, Australia
8Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
9MIRECC, Palo Alto VA, Palo Alto, CA, USA

Introduction: Impairment of cognitive function is known to occur in bipolar mood states and persists in euthymia. The same has been shown in both symptomatic and remitted major depression. This study investigated potential differences in the neural substrates of persistent cognitive dysfunction in euthymic bipolar and remitted unipolar depressed populations by comparing brain function during cognitive control related tasks.

Methods: 23 euthymic bipolar (BP), 23 remitted major depressive disorder (MDD) and 23 healthy control (HC) participants, matched on age and gender, completed a functional magnetic resonance imaging (fMRI) task measuring response inhibition, working memory and auditory attention. Brain activations and connectivity in ROIs of the cognitive control brain network (bilateral dorsolateral prefrontal (DLPFC), posterior parietal cortices (PPC) and the dorsal anterior cingulate (dACC)) for each task were analyzed in SPM8 and compared between groups, at a cluster-level FDR of p < 0.05, k > 71 voxels.

Results: The BP group had significantly greater activation in the PPC compared to MDD during both auditory attention (left only, p = 0.009) and response inhibition tasks (p = 0.001) but had significantly lower activation in the left PPC during working memory (p = 0.002). For connectivity during working memory, BP had significantly greater left PPC to the right DLPFC connectivity relative to MDD (p = 0.002). For attention, BP had significantly greater left to right DPC connectivity (p = 0.003) and left DPC connectivity to the left DLPFC (p <0.001) and the dACC (p<0.01) than MDD.

Conclusion: The left parietal cortex appears to have a central role in differentiating responses of BP and MDD participants during cognitive tasks; however these differences appear to be task specific. However, further research is needed to better elucidate what might be driving the brain differences in these illnesses and why these differences are dependent on the task.
Cognitive and Emotional Biomarkers of Anxious Major Depressive Disorder: An iSPOT-D Report

Taylor A Braund1,2, Anna Campain,3 Donna M Palmer1,2, Leanne M Williams,4,5 Anthony W F Harris1,2,6

Brain Dynamics Centre, The Westmead Institute for Medical Research, Sydney, NSW, Australia
Discipline of Psychiatry, Sydney Medical School, University of Sydney, Sydney, NSW, Australia
The Brain Resource Company, Sydney, NSW, Australia
Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
Sierra-Pacific Mental Illness Research, Education, and Clinical Center (MIRECC) Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA
Department of Psychiatry, Westmead Hospital, Western Sydney Local Health District, NSW, Australia

Background: Major depressive disorder (MDD) commonly co-occurs with one or more anxiety disorders or with clinically significant levels of anxiety symptoms. While evidence suggests these anxious forms of MDD present with more severe clinical profiles and are prognostic of poorer antidepressant treatment outcomes, little is known of their cognitive and emotional functioning. We compared cognitive and emotional functioning in various forms of anxious MDD and assessed whether functioning could predict antidepressant treatment outcome.

Method: 1,008 adults with MDD and 336 healthy controls completed IntegNeuro: a computerized cognitive and emotional functioning assessment battery. Participants were then randomised to one of three antidepressants and reassessed at 8 weeks regarding 17-item Hamilton Depression Rating Scale (HRSD17) and 16-Item Quick Inventory of Depressive Symptomatology-Self-Rated (QIDS-SR16) remission and response. Anxious MDD was defined using common criteria (i.e., MDD with one or more anxiety disorders, or a HRSD17 Anxiety subscale score > 7) and theoretically driven novel criteria (i.e., MDD with severe/extremely severe Depression Anxiety Stress Scales (DASS) Anxiety and Stress subscale scores).

Results: People with syndromally defined anxious MDD had poorer working memory and people with DASS defined anxious MDD had poorer working memory, cognitive flexibility and greater emotional bias compared to their non-anxious counterparts. Cognitive and emotional functioning in people with HRSD anxious MDD predicted QIDS-SR16 response with 74% cross-validated accuracy.

Conclusion: Cognitive and emotional functioning impairments in anxious MDD vary depending on its definition, but generally compound existing MDD deficits with anxiety related deficits (i.e., executive functions). Subtyping by anxious MDD can also help develop cognitive and emotion based treatment prediction biomarkers.
NUCLEAR IGFBP-3 IS A POTENTIAL BIOMARKER FOR RESPONSE TO EGFR-SPHINGOSINE KINASE TARGETED THERAPY IN BASAL-LIKE TRIPLE-NEGATIVE BREAST CANCER (TNBC)

Sohel M Julovi1*, Janet L Martin1, Robert C Baxter1

1, Kolling Institute for Medical Research, University of Sydney
*Westmead Institute for Medical Research, current address

Background: TNBC has no approved targeted therapy and is commonly treated with adjuvant chemotherapy including doxorubicin. Oncogenic signaling involving sphingosine kinase-1 (SphK1) and EGFR is initiated in TNBC cell lines by IGFBP-3, which is highly expressed in basal-like TNBC tumors and prognostic for poor recurrence-free survival. Inhibitors of SphK (fingolimod) and EGFR (gefitinib) together (F+G) induce a strongly synergistic cytostatic effect in TNBC cell lines, prevented by IGFBP-3 downregulation. Therefore, we evaluated IGFBP-3 as a response biomarker in TNBC xenograft tumors after combination treatment ± doxorubicin.

Methods: Tumors established in mice from human basal-like TNBC cell lines HCC1806 and MDA-MB-468, were treated with F+G ± doxorubicin (2 mg/kg/week, the MTD). Tumors were analyzed by IHC, and cell proliferation in vitro by live-cell imaging.

Result: In vitro, F+G acted synergistically with doxorubicin to markedly inhibit proliferation. In vivo, F+G significantly inhibited tumor growth and enhanced mouse survival, but doxorubicin had minimal effect alone, and no significant incremental effect with F+G. Tumor IGFBP-3 staining was predominantly nuclear, positively correlated with Ki67, and significantly downregulated by F+G, with no added doxorubicin effect. High nuclear IGFBP-3 IHC scores were strongly associated with worse mouse survival, while high apoptosis (cleaved caspase-3) scores were associated with better survival.

Conclusions: Blocking IGFBP-3-dependent oncogenic signaling with F+G is highly growth-inhibitory to basal-like TNBC tumors but synergism with doxorubicin was not seen in vivo, possibly because of dose-limiting doxorubicin toxicity. Nuclear IGFBP-3 staining may have utility as a biomarker of treatment response in TNBC. Supported by Cancer Council NSW.
The impact of social deprivation on stage of presentation of colorectal cancer in a western Sydney population

MacDermid E1, Pasch J1, Fok K2, Pasch L3, Premaratne C1, Kotecha K2, Barto W1, El Khoury T2.
Nepean Hospital NSW.
Westmead Hospital NSW.
University of Western Sydney.

Aims: The adverse effect of socioeconomic deprivation on outcomes after surgery for colorectal cancer has been well described for populations in the US and Europe, with no clear cause being found in terms of relative stage at presentation. The divide between socioeconomic groups in Australia continues to grow, and needs to be considered when devising national health strategies.

Methods: Patients from a database of colorectal cancers resected from 5 hospitals in Western Sydney from 2010 to 2016 (n = 1596) were stratified into socioeconomic centiles using their post-code at time of surgery, and a Socioeconomic Deprivation Index available from the Australian Bureau of Statistics. Patients from the lowest and highest centile were compared. Chi-squared and Mann-Whitney U testing were used to compare factors including demographics, tumour site, size and differentiation, the presence of neurovascular invasion, and tumour and nodal-status stage between the least and most socioeconomically deprived groups.

Results: 322 (20.2%) patients from the most socioeconomically deprived centile, and 275 (17.2%) patients from the least deprived were identified. Patients from the most deprived centile were more likely to present with stage T4 (p = 0.002), node positive (p = 0.02) and circumferential (p=0.001) cancers and at a younger age (p = 0.032) than those from the least. There was no significant difference with regards to tumour size, differentiation or neurovascular invasion at the time of surgery.

Conclusion: Patients from the most socioeconomically deprived post-codes in western Sydney are more likely to present with higher T-stage and node-positive colorectal cancers, and at a younger age than those from the most affluent. These findings have implications for resourcing colorectal cancer screening and diagnostic services in western Sydney.
Role of long noncoding RNA TUG1 in liver cancer

Xiaoqi Huo1 Jacob George1 and Liang Qiao2
1Westmead Institute for Medical Research, University of Sydney and Westmead Hospital
2Storr Liver Centre, Westmead Institute for Medical Research (WIMR), the University of Sydney and Westmead Hospital, Westmead, NSW 2145, Australia

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer related death worldwide. Drug resistance and tumour recurrence significantly contribute to the poor prognosis of this malignancy. Long noncoding RNAs (lncRNAs) are a group of functional RNAs (>200nt in length) without protein coding ability. A large number of dysregulated lncRNAs have been identified and show a close relationship with liver cancer initiation, progression, and therapeutic outcomes. Our preliminary studies have shown that the lncRNA TUG1 is highly relevant to liver cancer formation, and thus could be a potential therapeutic target.

Aim: We aimed to study the biological role of TUG1 in HCC.

Methods: The expression pattern of TUG1 was tested in HCC tissues and adjacent non-tumourous liver (n=42), as well as in liver cancer cell lines by quantitative real time PCR (qPCR). The expression of TUG1 in two liver cancer cells (SNU182 and SNU423) was knocked down by specific siRNA against TUG1, and the impact of TUG1 downregulation on the proliferation, colony formation, migration and invasion ability of these cells was studied. Propidium iodide staining was used for cell cycle analysis.

Results: TUG1 was overexpressed in HCC tissue compared with adjacent non-tumourous liver (p<0.001). In two liver cancer cell lines (SNU182, SNU423), TUG1 was significantly up-regulated compared with a normal liver cell line (IHH). Silencing of TUG1 by siRNA markedly decreased the proliferation and colony forming ability of liver cancer cells (p<0.05). Knockdown of TUG1 reduced the ability of cancer cells to migrate (p<0.05) and invade (p<0.05) and led to cell cycle arrest at G0/G1 (p<0.05).

Discussion: TUG1 is overexpressed in liver cancer. TUG1 may promote the progression of HCC by increasing cancer cell proliferation, migration and invasion. TUG1 may be a promising target for the treatment of liver cancer.
The Biological Function of Constitutive Androstane Receptor and Its Possible Role In Liver Tumorigenesis

Sarah Bae1, Kyung-Jin Kim1, Liang Qiao1

1 Storr Liver Centre, Westmead Institute for Medical Research, the University of Sydney

Background: Liver cancer is the fifth most common cancer worldwide. It is a very aggressive type of cancer known for its recurrent and drug resistant characteristics that attributes to the very low survival rate. Constitutive androstane receptor (CAR) is a cytoplasmic protein that is involved in drug metabolism and most importantly, liver tumorigenesis in mice. However, the role of CAR in human liver tumorigenesis is far from being clearly defined. Hence, there is a need to further study the biological function of CAR in human liver tumorigenesis as it is a possible drug target for novel therapeutics.

Aim: We aim to compare the biological features of CARhigh and CARlow expressing liver cancer cells as well as their regulatory role on liver tumorigenesis.

Methods: Six hepatocellular carcinoma cell lines (SNU-182, SNU-423, Huh7, PLC, HepG2, Hep3B) and one normal hepatocyte cell line (IHH) were cultured and flow cytometry used to quantify the basal level of CAR. The biological characteristics of CARhigh and CARlow expressing cells will be studied with functional assays along with CAR specific agonist (CITCO) and CAR siRNAs. Gene expression patterns of CARhigh and CARlow cells will be analysed by RNAseq.

Results: The majority of the cell populations express CAR and the difference in CAR expression levels were shown by the comparison of geometric mean of fluorescence intensity (gMFI) measurements. Results of the functional assays will be presented.

Discussion: The possible role of CAR in human liver tumorigenesis will be revealed through the comparison of experimental results from functional assays and analysis of gene expression patterns by RNAseq.
Replication stress induces mitotic cell death through cohesion fatigue and mitotic telomere deprotection

V. Pragathi Masamsetti¹, Ka Sin Mak¹, Ronnie Ren Jie Low¹, Chris D. Riffkin², Noa Lamm¹, Jan Karlseder³, David C.S. Huang², Makoto T. Hayashi⁴ & Anthony J. Cesare¹

¹Children’s Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia. ²Walter and Eliza Hall Institute of Medical Research, Cancer and Haematology Division, Parkville, Victoria 3052, Australia. ³The Salk Institute for Biological Studies, Molecular and Cell Biology Department, La Jolla, California 92037, USA. ⁴Department of Gene Mechanisms, Graduate School of Biostudies/The Hakubi Center for Advanced Research, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, 606-8501, Japan

Inducing DNA replication stress or targeting pathways that respond to replication stress is a prominent approach for chemotherapeutic cancer treatment. Lethal replication stress has previously been associated with “mitotic catastrophe”, a broad descriptor encompassing the complex and poorly understood mechanisms that connect genomic insult to mitotic disruption and cell death.

While low dosages of replication stress drive genome instability through the passage of damaged DNA and chromosome segregation errors during mitosis, direct mechanisms connecting lethal replication stress to cell death remain unclear. Here we show that lethal replication stress induces mitotic cell death, in the same cell cycle, through the two parallel pathways of cohesion fatigue and telomere deprotection.

We found that p53 compromised cells treated with pharmacological replication stress-inducing compounds, undergo a prolonged S/G2 phase followed by spindle assembly checkpoint (SAC)-dependent mitotic arrest. The SAC is engaged because WAPL promotes impaired centromeric cohesion during the prolonged S/G2, which is then passed into mitosis. Continued mitotic arrest then drives WAPL-dependent cohesion fatigue and cell death. As an independent and parallel mechanism to induce cell death, the SAC also engages Aurora B- and ATM-dependent mitotic telomere deprotection regulated by TRF2.

These results identify cell death due to replication stress primarily as a mitotic event and thereby reveal the mechanism of replication stress associated mitotic catastrophe. We identify novel roles for WAPL in regulating cell death and pinpoint a non-canonical telomere function, where chromosome ends regulate cell death independent of telomere length, in response to genomic DNA replication stress.
Actin polymerization alters nuclear architecture in response to DNA replication stress to maintain genome stability

Lamm-Shalem N1, Biro M2 and Cesare AJ1

1Genome Integrity Unit, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales 2145, Australia.

2EMBL Australia Node in Single Molecule Science, School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia.

Impediments that slow the rate of DNA replication are collectively referred to as "replication stress". Replication stress is the main driver of genome instability in oncogenesis and is recognized as a hallmark of cancer. To cope with replication stress and maintain genomic health, eukaryotic cells have evolved a sophisticated replication stress response pathway that mediates the repair and restart of stalled replication forks. Understanding the molecular basis of replication stress, and the pathways that respond when replication is impaired, is crucial for the understanding of tumorigenesis.

Actin is a cytoskeletal protein that forms filaments to provide cells with mechanical support and driving force for movement. While actin is traditionally considered a cytoplasmic protein, recent evidence indicates actin polymerization can also occur inside the nucleus. However, the role for nuclear actin fibres, and the mechanism(s) triggering their polymerization, remain unclear.

Using live-cell and super-resolution imaging, chromatin fibre analysis, biochemistry, cell and molecular biology, we discovered that actin polymerization plays a prominent role in the DNA replication stress response. We have found that following replication stress, the mTOR signalling pathway is activated to drive actin polymerization in the nucleus. Strikingly, actin polymerization drives nuclear volume changes, and directed-movement of DNA replication forks along actin fibres to the nuclear periphery where certain repair and restart factors are compartmentalized. Thus, by inducing architectural changes actin polymerization allows replication fork repair.

Altogether, we have discovered a novel mTOR-dependent mechanism that mediates repair of stalled replication, to maintain a healthy genome. Cancer cells rely heavily on such repair pathways that allow them to tolerate replication stress. Therefore, our data suggest that combining replication-stress inducing agents with pharmacologically mTOR inhibitors (both classes of agents are currently used in the clinic) to induce synthetic lethality might be a very promising approach to treat cancer.
FANCM is required for survival of ALT cells by suppression of a “hyper-ALT” phenotype

Robert Lu1, Alexander P. Sobinoff1, Joshua A. M. Allen1, Christopher Nelson1, Hilda A. Pickett1

1Telomere Length Regulation Group, Children's Medical Research Institute, University of Sydney, Westmead NSW Australia

Cancer cells are fundamentally limited by the shortening of telomeres at the ends of chromosomes. They overcome this by activating a telomere maintenance mechanism of which 15% of cancers utilise a telomerase-independent mechanism known as alternative lengthening of telomeres (ALT). The importance of understanding telomere maintenance in ALT cells arises from treating ALT-positive tumours when treating cancers with telomerase inhibitors.

AIMS: We sought to identify the role and mechanism of FANCM in maintaining telomere integrity in ALT and telomerase cells. This is because telomeres are repetitive regions which are prone to replication stress and that FANCM is a key replication fork protein, which canonically functions in DNA crosslink repair in the FA pathway.

METHODS: Cells utilising ALT exhibit heterogeneous telomere lengths, telomere dysfunction, enrichment of ALT-associated PML bodies, and small free-floating telomeric DNA species. These criteria will be assessed in telomerase-positive and ALT cell lines following FANCM depletion, and expression of FANCM mutants with disrupted functional domains.

RESULTS and DISCUSSION: We have found that FANCM depletion unleashes a “hyper-ALT” effect or phenotype coinciding with a dramatic increase in C-Circles and small, partially single-stranded C-rich telomere fragments. However, FANCM depletion does not promote ALT activity per se, as defined by telomere lengthening, and is detrimental for cell growth in both ALT and telomerase cells.

The “hyper-ALT” phenotypes are dependent on BLM and POLD3 and is primarily suppressed by the MM2 domain of FANCM. Our findings indicate that FANCM plays a major role in protecting replication forks at telomeres and regulating the production of small telomeric species in ALT cells. Future directions would investigate the mechanisms of DNA processing which become engaged in the absence of FANCM. In light of recent literature, we speculate that FANCM depletion may be a novel ALT therapy by potentiating an immune response to extracellular telomeric DNA.
Mechanosensitive Regulation of the Epidermal Growth Factor Receptor and its role in High Grade Gliomas

Vogelzang P1,2, Prior V2,3, Sarker FA2, Turner K2 and O’Neill GM1,2,3.
1Applied Medical Science, School of Medical Science, Faculty of Medicine and Health, 2Children’s Cancer Research Unit, The Children’s Hospital at Westmead, 3Discipline of Child and Adolescent Health, University of Sydney.

High Grade Gliomas (HGG) are the most abundant, aggressive and diffuse primary brain tumours. Despite medical intervention, patient prognosis remains poor with survival ranging from 12 to 18 months. New therapeutic options are therefore urgently required.

Due an almost ubiquitous expression, upregulation and unique constitutively-active mutation (EGFRvIII), the Epidermal Growth Factor Receptor (EGFR) shows great promise as the next target for HGG. Though the implementation of EGFR-targeting agents have been proven successful for colorectal and non-small cell lung cancers, clinical trials reviewing the use of these drugs in HGGs have largely failed.

Reasons cited for these failures often involve poor pharmacokinetic features, but it has become increasingly realised that the biomechanic features of tissue also influence pharmacological responsiveness. Mechanopharmacology is a term that describes the influence of tissue forces such stress, strain and ECM stiffness on the ability of a drug to promote a biological response. Therefore, to truly examine the effectiveness of a drug we must first understand its efficacy in a clinically-relevant tissue context.

This is of particular concern for brain cancer therapies, given that the brain tissue is a uniquely soft environment that is orders of magnitude softer than plastic. Our prior preliminary analyses of the stiffness-dependent activation of kinase activity has suggested that EGFR activity is mechano-sensitive.

Therefore, the aim of this project is to determine whether EGFR activity is suppressed on a soft brain-like environment, thus potentially facilitating resistance to anti-EGFR therapies. To investigate this question, the project will use primary patient-derived tumour cell lines and substrates that resemble brain-like rigidity. The results of these experiments will be presented.

The significance of this study is to emphasize the critical need to consider tissue context when identifying potential new therapies.
The Myoepithelium as Risk Sentinel in Ductal Carcinoma In Situ of the Breast

Gemma M. Wilson1, Barbara J. Guild1, Nirmala Pathmanathan2, Christine L. Clarke1, J. Dinny Graham1,2

1 – Centre for Cancer Research, The Westmead Institute for Medical Research, The University of Sydney, Westmead, NSW 2145
2 – Westmead Breast Cancer Institute, Westmead Hospital, Westmead, NSW 2145

Ductal carcinoma in situ (DCIS) refers to the non-invasive proliferation of neoplastic epithelial cells within the duct of the mammary gland. Myoepithelial cells (MEC) which harbour tumour suppressor functions surround the epithelial cells and confine the tumour in situ. However, malignancy-associated changes to the myoepithelial cells lead to impairment and progressive loss of the myoepithelial layer, which permits microinvasion and metastasis of the tumour cells resulting in invasive cancer.

Currently, the likelihood of recurrence or malignant progression of each DCIS case is unpredictable, thus surgical intervention often followed by radiotherapy after diagnosis is the current standard of care resulting in substantial overtreatment. A prognostic test to identify those at a greater or lesser risk of DCIS progression or recurrence is necessary to allow tailored management of patients and reduce burden on the health care system.

MEC-specific markers are routinely assessed in immunohistochemical (IHC) analysis of breast lesions to distinguish DCIS from invasive cancer, based on the presence of an intact myoepithelium. Emerging evidence has also revealed that the levels and distribution of myoepithelial markers become progressively altered in DCIS compared to normal myoepithelium, and that these changes are correlated with recurrence or progression to invasive disease.

We hypothesise that MECs play a critical role in maintaining the in situ phenotype of DCIS, and that DCIS MECs with phenotypes closest to normal will belong to low grade tissue and have a lower risk of disease recurrence and progression, whereas MECs with more abnormalities will be associated with a heightened risk of disease progression.

To address our hypothesis, we are assembling a cohort of low and high grade DCIS with extensive clinical follow-up in which to characterise expression of a panel of MEC markers by IHC. The study will identify critical markers of disease progression and recurrence in DCIS tissue.
Characterisation of prostate cancer prognosis by machine learning of 1,566 prostate proteomes generated by PCT-SWATH mass spectrometry

Rebecca Poulos1, Rohan Shah1, Tianan Guo2,3, Tiansheng Zhu2, Guobo Chen5, Jelena Ljubicic4, Peter Hains1, Natasha Lucas1, Yi Zhu2,3, Rutishauser Dorothea4, Rui Sun2, Hannes Roest3, George Rosenberger3, Janis Neumann6, Konstantina Charmpi6, Matteo Manica7, Marija Buljan3, Wenguang Shao3, Guan Ruan2, Niles Rupp4, Daniel SchIRMacher3, Pedrioli Patrick3, Maria Martinez7, AndreaS Beyer6,8, Roger Reddel1, Phil Robinson1, Peter Wild4,9, Ruedi Aebersold3,10, Qing Zhong1,4

* These authors contributed equally to this research.
1ProCan, Children's Medical Research Institute, University of Sydney, Westmead, New South Wales, Australia;
2Westlake University, Hangzhou, China;
3ETH Zurich, Zurich, Switzerland;
4University Hospital Zurich, Zurich, Switzerland;
5People's Hospital of Hangzhou Medical College, Hangzhou, China;
6CECAD, University of Cologne, Cologne, Germany;
7IBM, Zurich, Switzerland;
8CMMC, University of Cologne, Cologne, Germany;
9University Hospital Frankfurt, Frankfurt am Main, Germany;
10University of Zurich, Zurich, Switzerland

Aims: Patients diagnosed with prostate cancer at an intermediate Gleason score can harbour aggressive or non-aggressive disease. These disease subtypes cannot presently be accurately distinguished, resulting in under- or over-treatment of some patients. The aim of our study was to apply machine learning to proteomic data obtained from mass spectrometry to assess prostate cancer prognosis.

Methods: We performed quantitative proteomic analysis of 1,566 prostate tissue samples from 290 patients procured from the ProCOC (Prostate Cancer Outcomes Cohort). Small tissue samples were processed via pressure-cycling technology and the data were acquired by SWATH-MS (Sequential Window Acquisition of all Theoretical Mass Spectra-Mass Spectrometry). We analysed tumour tissues classified by two pathologists alongside matched benign tissue samples for each patient. The sample cohort was divided into 31 batches, and each batch comprised two control samples to evaluate technical reproducibility. Each sample was analysed twice in ProCan (Sydney) to ensure high reproducibility.

Results: Altogether, we quantified 2,800 SwissProt proteins (FDR<1%) in all of the samples, with an average missing value of 30%. We quantified 73% of proteins in over 50% of samples. We corrected for batch effects and imputed missing values using technical and biological replicates. This comprehensive dataset allowed us to apply machine learning methods to find protein signatures that can identify tumour and normal samples, and to assess Gleason scores. Using Random Forest, we were able to separate tumour and normal samples with an area under the curve of 0.92. Moreover, we applied a random survival forest model to tumour samples and identified the top 100 proteins that predict survival, using time to recurrence and censoring information.

Discussion: To our knowledge, this is the largest SWATH-based proteomic dataset generated to-date in human tissues (>1,500 proteomes). Our study demonstrates the feasibility of SWATH-MS for the proteomic analysis of prostate cancer prognosis.
The role of mechanosensing in determining the dissemination pattern of high grade glioma brain tumours

Jegathees T1,2, Prior V1,2, Grundy T1,2, Sarker F1,2 and O'Neill GM1,2
1Children's Cancer Research Unit, The Children's Hospital at Westmead, Sydney, NSW, Australia.
2Discipline of Child and Adolescent Health, University of Sydney, Australia.

High grade gliomas (HGG) are the deadliest and most frequently diagnosed brain tumours. A major limitation in treatment is the highly disseminating nature of HGG cells. It is now realised that mechanical properties of the extracellular matrix, measured as tissue elasticity (Young's modulus, E), is an independent cue for cancer cell migration and invasion.

The brain parenchyma is a mechanically soft tissue, with E values varying between 0.1-1 kPa. We have previously reported that molecular subclasses of HGGs exhibit rigidity-sensitive and rigidity-insensitive migration. Following these findings, the present project aims to determine if mechanosensitive phenotypes define the pattern of dissemination of multicellular tumour spheroids (MCTSs). MCTSs are composed of 3 distinct regions; the core, which comprises the quiescent mass of cells surrounding a necrotic focus, the halo, which contains proliferative cells in the process of escaping the hypoxic core, and the periphery, which comprises single cells which have escaped the tumour bulk.

We hypothesised that mechanosensitive HGG cells will demonstrate a greater halo-to-core area ratio with increased substrate stiffness whilst mechanoinsensitive are hypothesised to have a constant halo-to-core area ratio regardless of substrate rigidity. To test these hypotheses, MCTSs of primary patient-derived HGG cells with pre-established mechanosensitive phenotypes were cultured on mechanically tuneable polyacrylamide hydrogels, mimicking the range of physiological tissue rigidities.

Bright-field time-lapse images were then captured over a period of 48 hours, 6 images per hour. In a pilot study, we adapted and tested a previously published automated image analysis program that segments the images into halo and core regions. We are now poised for high throughput analysis of the MCTSs under different conditions to rigorously test the association between mechanical phenotypes and dissemination pattern.

This study will be the first to test the role of HGG mechanosensing to direct the pattern of dissemination of primary patient-derived HGG MCTSs.
Ex vivo expanded PRAME-specific T lymphocytes for tumour immunotherapy exhibit an extended cytokine profile

Koon Lee1,2, Janine Street1, Leighton Clancy1,4, David Gottlieb1, 2, 3, Kenneth Micklethwaite1-4, Emily Blyth1-4,

1 Westmead institute for Medical Research, University of Sydney
2 University of Sydney
3 Department of Haematology, Westmead Hospital
4 Sydney Cellular Therapies Laboratory, Westmead

**Aim:** Adoptive immunotherapy with ex vivo expanded donor-derived tumour-specific T-cells may improve disease control following allogeneic haematopoietic stem cell transplant. We previously demonstrated high expression of preferentially expressed antigen in melanoma (PRAME) in primary AML samples. Here we sought to develop GMP compliant manufacturing methods for PRAME specific T-cells from normal donors for use in adoptive immunotherapy.

**Method:** Peripheral blood mononuclear cells (PBMCs) or G-CSF primed haemopoietic progenitor cells (HPC) from healthy donors were pulsed with PRAME 15-mer overlapping peptide mix. After 16 hours, enrichment of activated cells was performed using immunomagnetic beads. Cells were co-cultured with irradiated activation marker-negative fraction and supplemented with IL-2, IL-7 and IL-15. Cultures were restimulated with antigen-pulsed autologous cells after 10 days and subsequently weekly. Multiparameter flow cytometry was performed to assess cellular phenotype and cytokine response following antigen re-exposure. 3 cultures were selected for detailed phenotypic and functional analysis with mass cytometry by time of flight (CyToF).

**Results:** PRAME stimulated cultures (n=10) had mean expansion of 38000-fold at day 25. 9/10 cultures showed PRAME specificity by TNF-α production (mean 14% of CD4+ cells) of which 8 cultures also demonstrated interferon-γ production (mean 7% of CD4+ cells). Mean CD3+ percentage was 96% with CD4:CD8 ratio of 4.9:1. Central and effector memory cells were 23% and 72% respectively, with 23% T cells expressing PD1. CyToF showed 39% of CD4+ cells had Th1 phenotype (CXCR3+ CCR4- CCR6- Tbet+) with <1% exhibiting Th2 phenotype (CXCR3- CCR4+ CCR6+ GATA3+) and an extended spectrum of cytokine production including IL-2, IL-4, IL-8, IL-13 and GM-CSF (2%, 6%, 8%, 4%, 11% of all cells respectively).

**Conclusion:** PRAME-specific T-cells for adoptive tumour immunotherapy were successfully enriched from healthy donor PBMC and G-CSF mobilised HPC. The final T cell product exhibited a Th1 phenotype and produced multiple cytokines.
Utility of Dual Staining Of PD-L1 with TTF1 or p40 in Non-Small Cell Lung Carcinoma

S. Nair1, W. Varikatt1, K. Tan1, D. Daneshwar1, J. Armes1
1. Tissue pathology & Diagnostic Oncology, ICPMR, Westmead Hospital, NSW, Australia.

AIM: The study aimed to determine whether the accuracy of PD-L1 expression scoring in non-small cell lung cancer was increased when simultaneously identifying the tumour cells by dual IHC staining of PD-L1 with TTF1 or p40 in adenocarcinoma and squamous cell carcinoma of lung, respectively, in comparison with PD-L1 staining alone.

METHODOLOGY: Twenty five cases of lung adenocarcinoma and squamous cell carcinoma each were retrieved from the Tissue Pathology archives. The H & E stained slides were assessed for tumor adequacy. Initially PD-L1 IHC was performed and the percentage of tumor cells showing membranous staining counted. On further serial section the percentage of tumor cells highlighted by TTF1 & p40 and concurrently showing PD-L1 membranous staining were estimated and tabulated. The IHC results from the two separate sections from each case were compared.

RESULT: Preliminary data indicates that the dual staining with PD-L1 and TTF1 or p40 is easier to interpret as to which cells express PD-L1, giving more accurate expression of PD-L1 in the tumor cell population, and excluding false positive interpretation due to PD-L1 expression on tumor infiltrating immune cells.

DISCUSSION: PD-L1 membrane expression is heterogeneous within tumors and may be seen more frequently in areas of tumor with higher levels of tumor-infiltrating lymphocytes.

IHC interpretation is complicated by the fact that immune cells, including lymphocytes also may express PD-L1, which renders scoring of PD-L1 confined to tumor cells difficult and time consuming. In this study, we assessed the utility of dual staining of PD-L1 (which has membranous expression) with either TTF1 or p40, since both of these proteins are expressed in the nucleus in either adenocarcinomas or squamous cell carcinomas of lungs, respectively. TTF1 or p40 highlighted the tumor cell nuclei with a red chromogen and PD-L1 was highlighted by a brown chromogen. This enabled easier identification of PD-L1 positive tumor cells among background immune cells.
A Novel Predictive Tool for Heavy Axillary Nodal Involvement in Sentinel Node Positive Breast Cancer

Jayatilleke IN1, Elder E1,2, Sherman K 2,3, Kilby C3, Azimir A2, Kabir M2, Mahajan H2

Affiliations:
1. The University of Sydney
2. The Westmead Breast Cancer Institute
3. Macquarie University

Background: The sentinel lymph node (SLN) is the only site of metastasis in up to 70% of early breast cancer patients who progress onto a completion axillary lymph node dissection (cALND). There is an emerging body of evidence that demonstrates the safety of avoiding cALND in minimally involved axillae. However, a subset of patients with extensive nodal involvement (defined in this work as 4 or more axillary lymph nodes) will still benefit from cALND to achieve optimal local control.

Aim: We developed a novel clinical calculator which predicts the likelihood of heavy axillary nodal involvement, which is also the first such calculator derived from an Australian population.

Method: All female patients with positive SLNs in the time period 1999-2013 were identified from the Westmead Breast Cancer Institute database. Male patients and those undergoing neoadjuvant therapy were excluded. Primary tumour and nodal characteristics were gathered from pathology reports. Chi-squared and t-tests identified significant clinical variables associated with heavy nodal involvement, and subsequent multivariate logistic regression, and backwards elimination was used to develop a predictive model (P<0.05). Accuracy of the model was determined by the area under the receiver operator characteristic curve (AUC).

Results: After applying the selection criteria to the 2,776 patients who had sentinel lymph node biopsy within the study period, 453 qualified for the study development dataset. Based on the data from this subset, the largest invasive tumour size (odds ratio (OR) 1.03, 95% confidence interval (CI) 1.02-1.05, P=<0.0005) and total sentinel node metastasis size (OR 1.11, 95% CI: 1.08-1.14, P=<0.0005) were the two significant variables predicting heavy nodal involvement. The AUC was 0.85.

Discussion: The very high predictive value of this novel tool is comparable to international models.

We hope this tool will provide a decisional aid for clinicians to risk stratify sentinel node positive patients who are likely to benefit from further cALND.
Pathological factors in positive circumferential margins in rectal cancers

J A Pasch1, E MacDermid1, L B Pasch2, C Premaratne1, K Fok3, K Kotecha2, T El Khoury3, W Barto1

1. Nepean Hospital 2. Western Sydney University 3. Westmead Hospital

**Purpos:** Positive circumferential resection margin (CRM) status is associated with local disease recurrence and reduced survival in rectal cancer. Despite preoperative staging with MRI, improvements in surgical technique and neoadjuvant therapies patients remain at risk of positive CRM.

**Methodology:** Rectal cancers were identified from a histopathology database of colorectal resections performed at five Western Sydney Hospitals from 2010 to 2016. Univariate analysis was performed using Student T-test and Mann-Whitney U test to determine pathological factors related to CRM. Multivariate analysis was undertaken using binary logistic regression to determine significance and adjusted odds ratios. 5-year survival was calculated using Kaplan-Meier analysis.

**Results:** A total of 502 rectal cancer patients were identified comprising 68 (13.6%) with involved CRM. Each group had a similar distribution of age, gender and use of neoadjuvant radiotherapy. Positive CRM tumours comprised T3 and T4 disease of which 52.9% received neoadjuvant radiotherapy. Positive CRM was significantly associated with tumour size, circumferential and perforated tumours on univariate analysis. Multivariate analysis identified abdomino-perineal resection (OR 4.85; p<0.001), T4 stage (OR 8.135; p<0.001), perineural (OR 3.92; p<0.001) and vascular invasion (OR 2.56; p=0.01) as independent risk factors for positive tumour margins. Five-year survival was significantly worse for CRM positive patients (31% vs 70% p < 0.001).

**Conclusion:** CRM status reflects not only technical success but also aggressive disease phenotypes which direct requirements for adjuvant therapy. The true rate of positive CRM is double that estimated in recently published studies based on voluntary national Colorectal Society data submission. Further work is needed to examine reasons for omission of neoadjuvant radiotherapy in some of our at-risk patients, and any effect this has had on the incidence of positive CRMs.

Strengthening MDT Performance in Cancer Services in Western Sydney One Year On

Authors: Dr Lynleigh Evans, Mr Brendan Donovan, Prof Paul Harnett

Aim: To strengthen the performance of multidisciplinary team meetings (MDMs) through the utilisation of annual assessment and educational tools to engage teams in performance improvement strategies.

Methods: The study protocol comprises a survey to evaluate MDM members’ perceptions of their team’s performance before the implementation of the program and annually thereafter; and a tumour program maturity matrix designed as a self-assessment tool showing five levels of maturity across 20 domains. Each MDM used the matrix to collectively assess its performance and identify priority areas for improvement.

Results: The first survey has been completed and the second survey is nearing completion. 129 member surveys from 12 MDMs were completed in the first round and 104 responses have been received so far from the second survey. Overall improvement has been impressive with 19 of 25 (76%) questions with positive/negative answers showing improvement and a mean improvement of over 30%. Some questions such as “does the team have Terms of Reference” showed marked improvement (16% to 43%). All the teams completed the matrix in year one with results confirming that there was marked variation in performance between teams. Feedback showed, however, that the method of delivery needed to be simplified and digitised. This is now being embarked upon.

Discussion: This study fills a gap in the literature by describing a means of improving performance from an organisational perspective. It differs from others in that it targets all tumour streams within the organisation and provides a framework by which MDMs can determine areas for improvement, while allowing considerable flexibility in the activities each team chooses to address.

The MDM survey and maturity matrix provide an excellent means not only for teams to identify their strengths and weaknesses but also for management to review its performance against standardised criteria and to identify priority areas for improvement and further support.

Affiliations: Sydney West – Translation Cancer Research Centre, This program is supported by the NSW Cancer Institute.
Analysis of paired primary and metastatic ovarian cancer samples using mass spectrometry

Shah R1,* Espersen M1,2, Tully B1, Mahboob S1, Xavier D1, Lucas N1, Robinson P1, Reddel R1, Zhong Q1, Balleine R1, Defazio A2,3

*These authors contributed equally to this research.
1ProCan, Children’s Medical Research Institute, The University of Sydney, Westmead, New South Wales, Australia;
2Centre for Cancer Research, The Westmead Institute for Medical Research, Sydney, New South Wales, Australia;
3Department of Gynaecological Oncology, Westmead Hospital

Aims: Due to biological heterogeneity, measurement variability and high dimensionality, a single tissue sample may be insufficient to accurately measure the protein content of a cancer, and multiple tissue samples may be required. This preliminary study aims to determine how many replicate samples are required from an ovarian high grade serous carcinoma, to reliably characterise the tissue proteome.

Methods: We performed protein quantification using replicate samples from 11 patients, using SWATH-MS. Between two to five cores were taken per tumour, and each core was divided into 4 – 11 sections, resulting in 431 sections, each run on two machines at ProCan. Approximate composition was recorded for the top section of each core. We validated this data using T-SNE visualisation and random forest classification. For each tumour, we assumed that the observed proteomic data were fully representative of the sample proteome. We then assessed the performance of different cluster–sampling strategies according to their coefficients of variance.

Results: We found that any two sections could be used to identify the source tumour with 100% accuracy. We found that a patient’s primary and metastasis tumours were more similar to each other than to primary or metastatic samples from another person.

Discussion: Our results suggest that taking two sections from two different cores is a reasonable strategy for ovarian cancer tissue sampling. We also determined the value of fine grained tissue composition estimates to guide interpretation of proteome variation in a complex cancer sample. Without such estimates, it can be impossible to distinguish tumour heterogeneity from changes in the composition of the sections.
Mutation profiling and molecular analysis in the INOVATe Study: Increasing ovarian cancer patient participation in molecularly targeted clinical trials

Hodgkinson V1,2,3, Bouantoun N1,3, Provan P1,2,3, Harnett P1,2,3, Friedlander M4,5, Balleine R2,6, Bowtell D7,8,10, Samimi G9,10, Brand A2,3, Hacker N11, Marsh D2,12, Beale P2,13, Boros J1,3, Chiew Y-E1,3, Gao B5,14, Jamieson A3, Kennedy C1,3, Lei Y1, Mapagu C1,2,3, Mirochnik O15, Nevins N1,2,3,22, Pianova S1,2, Sayer R3, Sharma R2,15,16, Stenlake A3, Srirangan D1, Tan K15, Baron-Hay S17,18, Diakos C17, Gard G2,17,18, Hogg R2,17,20, Maidens J17, Nevell D17,19, Phillips K2,12, Pillai U2,12, Valmadre S17,18,21, Ashrafy A22, Shannon J22, Stevanovic A22, Pattnaik S10, Farrell R11, Rao A11, Robertson G11, Webber K11, Norris C11, Newton E11, Rutovitz J20, Marx G20, High H20 and deFazio A1,2,3 for the INOVATe Investigators

1Westmead Institute for Medical Research, 2University of Sydney, 3Westmead Hospital, 4Prince of Wales Hospital, 5University of New South Wales, 6Children's Medical Research Institute, 7Peter MacCallum Cancer Centre, 8University of Melbourne, 9National Institutes of Health, 10Garvan Institute of Medical Research, 11Royal Hospital for Women, 12Kolling Institute of Medical Research, 13Chris O'Brien Lifehouse, 14Blacktown Hospital, 15Pathology West ICPMR Westmead, 16University of Western Sydney, 17Royal North Shore Hospital, 18North Shore Private Hospital, 19Pathology North, 20Sydney Adventist Hospital, 21Mater Hospital, 22Nepean Hospital

Background: Precision medicine or ‘the right treatment for the right patient at the right time’ requires a deep understanding of factors that drive the malignant phenotype and underlie treatment response and resistance in individual cancer subtypes. INOVATe (Individualised Ovarian Cancer Treatment through Integration of Genomic Pathology into Multidisciplinary Care) is a NSW-wide program which aims to establish the processes required to implement a personalised approach to the management of women with ovarian cancer.

Aim: Our aim is to establish the mechanisms to screen for patients suitable for inclusion in marker-driven clinical trials.

Methods: All women >18 years of age with epithelial ovarian cancer are eligible for multi-platform, molecular tumour profiling, including next generation sequencing and whole genome copy number analysis. Testing is performed at diagnosis and relapse (if relevant). Results are used to identify patients that are eligible for biomarker-based clinical trials at disease progression.

Results: At June 2018, 250 eligible patients have included for molecular analysis. Results include mutations identified in BRCA1 (n=10), BRCA2 (n=8) and/or identification of Homologous Recombination Deficiency (high HRD score, n=13) which would predict response to PARP inhibitors; mutations in KRAS (n=11), BRAF (n=6) which may predict response to MEK/BRAF inhibitors; CCNE1 gain/amplification (n=15) indicating potential eligibility for a CCNE1-targetted trial; and high expression of Mesothelin to identify patients for an early phase trial. An online portal for the visualisation and analysis of molecular results has been established to enhance the multidisciplinary team’s ability to interpret molecular profiling data.

Discussion: The INOVATe study will provide a framework for integration of molecular pathology into current models of multidisciplinary care, including the implementation of precision medicine for women with ovarian cancer. This model may also be applicable to patients with other cancer types.
Targeted axillary dissection in patients with clinically node positive breast cancer after neoadjuvant systemic therapy.

Kanesalingam K, Graham S, Meybodi F, Elder E, Brennan M, French J
Westmead Breast Cancer Institute

Over the last decade neoadjuvant systemic therapy (NAST) has gained considerable therapeutic importance and has been extended to include breast cancer patients with node positive disease.

However, it may no longer be necessary to commit all these patients to axillary dissection to stage the axilla accurately after completing NAST. Targeted axillary dissection (TAD) is a new technique where the marked positive node is excised along with the sentinel nodes and its response to chemotherapy is assessed.

The aim of this study was to determine the feasibility of marking positive axillary nodes with a clip and removing the clipped node after neoadjuvant treatment. We also assessed the concordance of the sentinel node with the clipped node. We retrospectively evaluated 20 clinically node positive patients who underwent NAST. The overall identification rate of the clipped node was 85%.

The rate was 80% in patients who did not have the clipped node localised preoperatively. The clipped node was not retrieved as the sentinel node in 12% of patients.

TAD is a feasible option and with every new technique there is a learning curve. With the increasingly experience globally and the refinement in marking and localisation techniques, the accuracy of performing TAD will continue to improve.
People with inherited high cholesterol have inflammatory changes in their blood cells and statin treatment does not resolve these changes

Helen Williams1,2, Habib Francis1,2, Rekha Marimuthu1,2, Heather Medbury1,2, Stephen Li3

1. Department of Surgery, Westmead Hospital, NSW 2. Westmead Clinical School, University of Sydney, NSW 3. ICPMR, Westmead Hospital, NSW.

Background: Cardiovascular disease (CVD) claims the life of 1 in 3 Australians, and high cholesterol is a major risk factor. Monocytes, a type of white blood cell, are important contributors to the formation of the atherosclerotic plaques in CVD. How these cells are altered in inherited high cholesterol (known as familial hypercholesterolemia [FH]) is yet to be understood.

Aims of Study: To investigate if monocytes are more inflammatory in people with FH, and whether lowering their cholesterol improves this.

Methods: Blood was collected from people with FH who were either taking (n=11) or not taking (n=7) lipid-lowering medication, and healthy controls (n=8). Presence of CVD risk factors was obtained by questionnaire.

Cholesterol levels were analysed by standard methods. Different subsets of monocytes were quantified and monocyte subset expression of inflammatory (CD86) and anti-inflammatory (CD163) markers determined by whole blood flow cytometry.

Results: In people with FH who were untreated, cholesterol and LDL-C levels were high whereas those taking statin medication had reduced cholesterol and LDL-C levels. No differences in monocyte subset proportions were seen between FH groups and controls.

The levels of the inflammatory marker, CD86, was higher on monocytes in people with FH, and this was true for both treated and untreated groups. Interestingly, correlations between cholesterol levels and CD86/CD163 existed, with a strong negative correlation seen for HDL-C and a weaker positive correlation for LDL-C in treated FH patients.

Discussion: FH is associated with subtle inflammatory changes in the monocytes. However, as these were present in both treated and untreated patients, lowering cholesterol appears to be insufficient to correct these changes. The strong negative correlation between HDL and CD86/CD163 indicates that increasing HDL could be beneficial as a form of anti-inflammatory targeting.
Prognostic Impact of Atrial Fibrillation in Hypertrophic Cardiomyopathy: A Systematic Review and Meta-analysis

Patricia Alphonse, BSc(BioMed)/MBBS,1 Sohaib Virk BMed/MD,1 Jhonna Collins BMSc/MSc,2 Saurabh Kumar BSc(Med)/MBBS, PhD.1,3

1Department of Cardiology, Westmead Hospital; 2Nepean Clinical school; 3Westmead Applied Research Centre, University of Sydney, Sydney, Australia.

Introduction: Atrial fibrillation (AF) is common in hypertrophic cardiomyopathy (HCM). This systematic review and meta-analysis sought to (a) prevalence of AF; (b) examine the impact of incident AF on subsequent clinical outcomes of thromboembolism, heart failure, sudden death in HCM.

Methods: Search of all major databases (Pubmed, MEDLINE, Embase, Cochrane, Scopus, Web of Science) was performed up to March 2018 using the search terms “atrial fibrillation” AND/OR “hypertrophic cardiomyopathy” in the title or abstract. Studies with data on AF incidence, prevalence and/or outcomes with AF were included. 45/1367 unique citations met the inclusion criteria.

Results: Using random-effects modelling, the estimated pooled prevalence of AF amongst 20,363 (32 studies) was 19.9%. 15,444 patients from 28 studies were included in analysis of outcome data. Mean age was 51±3 years (37% female), mean LVEF 69%. Over a median follow up duration of 6.9 years (range 2.8 to 11.7 years), AF, compared to sinus rhythm (SR) was associated with significantly increased risk of thromboembolism (Relative risk [RR] 7; 95% CI, 4.6-10.7; I² = 57%), heart failure (RR 2.75; 95% CI, 1.63-4.64; I² = 82%), sudden death (RR 1.7; 95% CI, 1.3-2.3; I² = 0%) and all-cause mortality (RR 2.50; 95% CI, 1.83-3.40; I² = 69%).

Conclusion: AF is highly prevalent in patients with HCM. The presence of AF is associated with major adverse clinical outcomes. These findings suggest that both, aggressive screening and treatment of AF, is likely to have major prognostic impact on outcomes in HCM.
Persistent reductions in Global Longitudinal Strain late after Radiotherapy in chemotherapy naive left-sided breast cancer patients

Siddharth Trivedi, Preeti Choudhary, Queenie Lo, Hari Sritharan, Vikneswary Batumalai, Geoff Delaney, Liza Thomas

Department of Cardiology, Westmead Hospital, Sydney, Australia
Department of Cardiology, Liverpool Hospital, Sydney, Australia
Cancer Therapy Centre, Liverpool Hospital, Sydney, Australia

Background: More than 80% of breast cancer patients receive radiotherapy. However, radiotherapy can lead to cardiotoxicity, which develops over time, making diagnosis difficult. Two-dimensional speckle tracking strain echocardiography (STE) is a new technique that detects subclinical cardiac alterations prior to development of overt cardiac dysfunction. We have previously described subclinical cardiac alterations, detected by STE, in left ventricular (LV) function immediately following radiotherapy in breast cancer.

Hypothesis: Subclinical myocardial changes in LV function consequent to radiotherapy cardiotoxicity, observed early, persist in the longer-term.

Methods: 40 chemotherapy naive women with left-sided breast cancer treated with surgery and adjuvant breast radiotherapy were prospectively recruited from two tertiary hospitals (April 2009 to November 2012). Transthoracic echocardiography was performed at baseline (pre-radiotherapy), 6 weeks post-radiotherapy, and 12 months post-radiotherapy (long-term).

Results: An increase in LV end diastolic and end systolic volumes was seen from baseline, consistent with persistent LV remodelling; however, due to the increase in both systolic and diastolic volumes, over time, no change in LV ejection fraction (EF) was observed. Global longitudinal strain (GLS) and S’ velocity remained significantly lower at 12 months post-radiotherapy (Figure 1). GLS dropped by >10% in 16 patients and by >20% in 4 patients compared to baseline.

Conclusions: Subclinical cardiac dysfunction using strain analysis was evident early after radiotherapy and persists late after completion of radiotherapy, while conventional indices such as LVEF show no significant change. GLS shows persistent reduction even at 12 months following radiotherapy, and this may be of particular importance in breast cancer patients receiving concomitant chemotherapy. Long-term prospective studies are required to determine if reductions in strain post-radiotherapy are associated with future adverse cardiovascular events. Figure 1. Changes in echocardiographic parameters over time (EDV – end diastolic volume, ESV – end systolic volume, EF – ejection fraction, GLS – global longitudinal strain).
Alterations in Myocardial Mechanics and Tachycardia Mediated Cardiomyopathy

Siddharth Trivedi, Liza Thomas, Saurabh Kumar

Department of Cardiology, Westmead Hospital, Sydney, Australia

Background: Idiopathic ventricular arrhythmias may evolve into tachycardia-induced cardiomyopathy. Speckle tracking echocardiography (STE) can detect subclinical changes in ventricular myocardial mechanical function before development of overt cardiomyopathy with decreased left ventricular (LV) ejection fraction (EF).

Hypothesis: We hypothesised that patients without overt electrical and structural abnormalities and otherwise “idiopathic ventricular arrhythmias” may have subclinical changes in myocardial mechanics that may help explain predilection toward tachycardia-induced cardiomyopathy.

Methods: STE was performed in 20 consecutive patients with idiopathic ventricular arrhythmias (no structural heart disease by cardiac magnetic resonance imaging; Group A) prior to undergoing catheter ablation/high-density voltage mapping and compared to 20 age/gender matched controls (Group B).

Results: Baseline characteristics were similar for age (A vs. B: 40±18y vs 42±15y) and LVEF (54±6% vs. 59±8, p=0.10). Right Ventricular (RV) global longitudinal strain (GLS) was similar between the two groups (A vs. B: -20.9±4.8% vs. -23.6±2.8%, p=0.15) and all patients had normal RV voltage. LV GLS and LV circumferential strain were significantly impaired in Group A vs. B (-16.9±3.3% vs. -19.2±1.7%; p=0.03 and -18.7±2.9% vs. -22.3±2.1%, p=0.006 respectively).

Conclusion: Alterations in myocardial mechanics, even remote from the site of arrhythmia, in patients without overt electrical and structural abnormalities may help explain why tachycardia-induced cardiomyopathy develops with otherwise “idiopathic” ventricular arrhythmias. Further study is needed to examine if these changes are reversible with ablation, and if alterations in STE can differentiate early forms of primary cardiomyopathy from reversible tachycardia-induced cardiomyopathy.
ABSTRACT 34

2D and 3D Transthoracic Echocardiography vs. Gated Heart Pool Scanning in Assessing Right Ventricular Function post ST Elevation Myocardial Infarction

D Selvakumar, P Brown, J. Natividad, AR Denniss, C. Saunders, L Thomas

Background: Quantification of right ventricular function (RVF) after ST elevation myocardial infarction (STEMI) may help in assessing prognosis and arrhythmic risk. Volumetric analysis of the right ventricle (RV) by transthoracic echocardiogram (TTE) is challenging given its complex crescentic shape, an issue which is less likely to influence gated heart pool scans (GHPS).

Aim: To compare RVF assessed by 2D and 3D TTE with GHPS in STEMI patients.

Methods: STEMI patients who underwent GHPS and 2D/3D echocardiography were prospectively enrolled. RV ejection fraction (RVEF) by area-length method and Fractional Area Change (FAC) were measured on 2D TTE. 3D RVEF was then calculated and the 3 parameters were compared to RVEF from GHPS using Pearson’s correlation and Bland Altman analysis.

Results: 87 patients were enrolled however 3D data was only obtained in 48 patients due to poor visualisation or endocardial definition. The mean age of patients was 62 years with the majority (73%) of patients being male. The mean RVEF measured by 2D TTE, 3D TTE and GHPS were 47 ± 8, 45.5 ± 7, and 46.6 ± 8 respectively. The mean FAC was 33.6 ± 7. RVEF measured by 3D TTE had the best correlation with GHPS (0.87), with 2D TTE and FAC correlating poorly (0.31, 0.21). Bland Altman analysis showed no systematic bias between 3D TTE and GHPS.

Conclusion: 3D imaging improves the diagnostic accuracy of TTE and provides RVEF measurements comparable to GHPS. Increasing operator experience will overcome initial technical difficulties and improve the diagnostic yield.
Coronary Arteritis in IgG4-related Disease presenting as Acute ST Segment Elevation Myocardial infarction

D Selvakumar, J Jiang, L Berglund, M Cooper

Introduction: Immunoglobulin G4-related disease (IgG4-RD) is a systemic disorder, characterised by infiltration of affected tissue by predominantly IgG4-positive lymphplasmacytic cells. Though multiple organ systems may be affected, involvement of the coronary arteries is extremely rare.

Case Description: A 69-year-old male with a history of IgG4-RD with hepatic and pulmonary involvement, presented with a 2-day history of chest pain. High sensitivity Troponin I was significantly elevated and an electrocardiogram demonstrated inferior Q waves with 1mm of ST-segment elevation, concerning for a completed transmural inferior infarction. Of note, his inflammatory markers and serum IgG4 were significantly elevated. Coronary angiography demonstrated a large (21x12mm) aneurysmal segment of the mid left circumflex artery with an occluded distal branch.

No intervention was performed and he was commenced on warfarin to reduce the risk of recurrent aneurysmal thrombosis. Computerised tomographic angiography (CTA) demonstrated inflammatory soft tissue thickening at the site of the circumflex aneurysm. A fluorodeoxyglucose-positron emission tomographic scan also demonstrated uptake at the aneurysmal site. Treatment of the peri-coronary inflammation secondary to IgG4-RD was initiated with escalation of his regular immunosuppression to a tapering course of high dose prednisone and mycophenolate therapy.

Follow-up outpatient examination demonstrated reduction in serum IgG4 and inflammatory markers, with progress CTA demonstrating resolution of the inflammatory arterial changes. He is planned for surgical bypass and ligation of the aneurysmal segment.

Comments: We present a rare case of coronary vasculitis in the setting of IgG4-RD with excellent sero- and radiological response to immunosuppression.
Utility of TAPSE and RV S’ velocity in identifying abnormal right ventricular systolic function following ST elevation myocardial infarction

D Selvakumar, P Brown, J. Natividad, AR Denniss, C. Saunders, L Thomas

**Background:** Tricuspid annular plane systolic excursion (TAPSE) and RV S’ velocity are echocardiographic parameters that are commonly utilised in clinical practice to assess right ventricular systolic function (RVSF). However, both techniques assume the function of a single segment represents that of the entire right ventricle (RV). This may not be reliable in conditions such as ischaemic heart disease in which regional wall motion abnormalities may exist.

**Aim:** To assess the utility of TAPSE and RV S’ in identifying patients with abnormal global RVSF post ST elevation myocardial infarction (STEMI).

**Methods:** STEMI patients who underwent GHPS and 2D transthoracic echocardiography (TTE) were prospectively enrolled; TAPSE and RVS’ were measured from TTE. Abnormal RVSF was defined as TAPSE < 16 mm and RVS’ < 10 cm/s. GHPS RV ejection fraction (RVEF) was used as the criterion standard for RVSF, with abnormal RVSF defined as RVEF < 40%.

**Results:** The mean age of the 73 enrolled patients was 60.74 years with the majority (74%) being male. In 28% of patients, the right coronary artery was identified as the culprit vessel. The mean TAPSE, RVS’ and GHPS RVEF values were 20 mm, 13 cm/s and 41% respectively. The test characteristics of TAPSE and RVS’ for abnormal RVSF were as follows: sensitivity = 36% and 59%, specificity = 98% and 93%, positive predictive value: 56% and 71%, negative predictive value = 86% and 92%.

**Conclusion:** In the post-STEMI population, RVS’ and TAPSE are highly specific but lack sensitivity in estimation of global RVSF.
Strain analysis identifies sub clinical LV dysfunction in patients who have had a bone marrow transplant

Tejas Deshmukh1, Lucy Geraghty1, Shanthosh Sivapathan1, Megan Hogg2, Paula Brown1, Louis Do2, Shyam Panicker2, Mikhail Altman1, David Gottlieb2, Liza Thomas1
1Department of Cardiology, Westmead Hospital
2Department of Haematology, Westmead Hospital

Background: The role of echocardiography in identification of cardiac dysfunction following chemotherapy after a bone marrow transplant is a new and emerging field. We hypothesised that strain imaging would be able to identify sub-clinical LV dysfunction, thereby allowing early identification of patients at risk of developing overt cardiac failure.

Methods: 25 patients post a bone marrow transplant with echocardiographic surveillance (Nov 2016 - Oct 2017) were evaluated and compared to age and gender matched controls from a departmental database. 11 BMT patients received myeloablative conditioning while the rest received reduced intensity conditioning. The mean time from BMT to TTE was 8.4±4.4 years. Strain analysis was performed using offline software (EchoPac). LVEF was measured by Simpsons biplane method.

Results: There was a significant reduction in global longitudinal strain (GLS) between the BMT and control group (-17.8±-3.4 vs. -21.6±-2.7%, p=0.001). Furthermore, this was reflected in a significant reduction in endocardial strain (-20±-3.9 vs. -24.4±-2.9%, p=0.001) and epicardial strain (-15.9±-3 vs. -19.1±-2.5%, p=0.001). There was no significant difference in LVEF between BMT and control groups (61±7 vs. 63±8%, p=0.32). A subgroup analysis looking at global longitudinal strain with a LVEF < 60% revealed a significant reduction within the BMT group (-15.9±-3 vs. -20.2±-1.6, p=0.01) but not the control group (-20.2±-1.6 vs. -20.3±-2.2, p=1.0).

Conclusion: Despite the relatively small number of patients analysed, this study highlights the utility of using GLS and multi-layer strain, in the identification of patients with sub-clinical LV dysfunction after exposure to chemotherapy for bone marrow transplant.
Additive effect of mediastinal radiotherapy on subclinical cardiac dysfunction in bone marrow transplant patients

Tejas Deshmukh1, Lucy Geraghty1, Shanthosh Sivapathan1, Megan Hogg2, Paula Brown1, Louis Do2, Shyam Panicker2, Mikhail Altman1, David Gottlieb2, Liza Thomas1

1Department of Cardiology, Westmead Hospital
2Department of Haematology, Westmead Hospital

Background: Strain analysis is a novel non-invasive modality to evaluate subclinical LV dysfunction in patients who have received chemotherapy. There is evidence that adjuvant radiotherapy (RTx) has an additive effect on LV dysfunction. We hypothesised that mediastinal RT results in a further reduction in global longitudinal strain (GLS) in patients who have received a bone marrow transplant (BMT).

Methods: 24 patients who had received a bone marrow transplant (15 with BMT alone and 9 who had BMT and RTx) were compared with age and gender matched healthy controls from a departmental database. Multi-layer strain analysis was performed using offline software (Echo Pac). LVEF was measured using Simpsons biplane method.

Results: There was no difference in epicardial strain (-17±-2.4 vs. -15±-2.8%, p=0.10) and a trend towards reduced mid myocardial strain (-19±-2.7 vs. -16.7±-3 %, p=0.06) between the BMT and BMT+RTx groups. However, endocardial strain was significantly lower in the BMT +RTx group (-21.4±-3 vs. -18.7±-3.3, p=0.046). There was significant reduction in multi-layer strain when BMT and BMT+RTx groups were compared to controls, with no significant difference in LVEF between the BMT, BMT+RTx and controls (62.7±7.5 vs. 58.2±3.8 vs. 63.5±8.4, p=0.19). An increase in LV filling pressures with increased E/e’ was noted in the BMT+RTx compared with the BMT group (9.6±1.6 vs. 8.2±2.8, p=0.02) and versus controls (9.6±1.6 vs. 7±2.2, p=0.003).

Discussion: Despite small numbers, there appears to be a signal towards reduced endocardial strain in patients who have received BMT+RTx with associated alteration in LV diastolic metrics despite preserved LVEF.
Safety and efficacy of programmed ventricular stimulation in the early phase of post ST elevation myocardial infarction (STEMI)

Tejas Deshmukh1, Sarah Zaman1, Arun Narayan1, Pramesh Kvoor1, Saurabh Kumar1
1Department of Cardiology, Westmead Hospital

Background: Risk stratification of patients who have had a STEMI remains controversial. The safety of programmed ventricular stimulation (PVS) in the acute phase after STEMI remains unclear.

Hypothesis: We propose that PVS post STEMI is a safe and efficacious procedure.

Methods: Patients who present to Westmead Hospital with a STEMI have a day 3 gated heart blood pool scan to assess their LVEF. If their LVEF is ≤40%, they undergo PVS with up to 4 extrastimuli to assess for ventricular tachycardia (VT) inducibility. A defibrillator is implanted if VT is inducible.

Results: Between 2011 and 2017, 379 patients had a PVS post STEMI. 119 had inducible VT (cycle length ≥200 ms, for ≥10s), 126 had inducible ventricular fibrillation (VF) and 134 had no inducible arrhythmia. There were no acute (within 7 days) adverse events during/after PVS such as in-stent thrombosis, cardiogenic shock or hemodynamically unstable arrhythmias. Baseline characteristics and follow up duration between VT, VF and no arrhythmia groups were similar. Mean follow up was 2011 days post STEMI. There were 35 deaths on follow up with 15 in the VT group, 8 in the VF group and 12 in the normal group. There was no significant difference in survival between the 3 groups (log rank p = 0.394).

Conclusion: This study highlights that PVS post STEMI is safe, even when VF is induced and terminated. PVS performed early after STEMI allows for both efficacious and efficient risk stratification with long term reduction in risk of death in a vulnerable population (LVEF≤40% with inducible VT) to the rate similar to patients in whom no arrhythmia or VF is inducible.
Multi-layer strain as a useful technique to identify Cardiac Amyloidosis

Authors:
Sivapathan, S1,2., Boyd, A.C3., Deshmukh, T1,2., Kowk, F1., M., Altman1, Richards, D3., Stewart, G1,2., Denniss, A.R1,2., Thomas, L1,2.

Affiliation:
1 – Westmead Hospital
2 – The University of Sydney
3 – Westmead Private Hospital

Background: Cardiac involvement from AL amyloidosis portends a poor prognosis. Strain analysis by echocardiography (TTE) has demonstrated a reduction in strain with specific apical sparing pattern. However, this has not been validated in a different patient cohort. Additionally, little is known about the utility of multi-layer strain in cardiac amyloidosis.

Aims: To validate the apical sparing pattern and evaluate the utility of multi-layer strain in AL amyloidosis.

Methods: TTE with 2-D strain was performed on 60 AL amyloidosis patients (Aug 2008 – Jun 2016) and compared with 60 healthy controls. Global multi-layer strain was obtained from the epicardial, mid-myocardial and endocardial layers from apical LAX, 4 and 2-chamber views. Additionally, segmental strain was measured as an average of six basal, six mid and six apical segments from mid-myocardial longitudinal strain.

Results: Longitudinal strain was reduced in the epicardial (-14.3±-4.2 vs. -17.7±-2.4%, p=0.001), mid–myocardial (-16.2±-4.7 vs. -20.0±-2.6%, p=0.001) and endocardial layers (-18.6±-5.4 vs. -22.6±-3.1%, p=0.001) in amyloid patients. Segmental strain analysis confirmed significant reduction in basal (-11.1±-3.8 vs. -17.4±-1.9, p=0.001) and mid (-14.7±-4.4 vs -19.5±-2.0) LV segments with no difference in apical strain (-22.3±-7.3± vs –23.2±-4.3, p=0.1). ROC analysis showed an optimal cut-off of -15.9% for basal longitudinal strain could differentiate cardiac amyloid from controls with a sensitivity of 98%, specificity of 78%, AUC 0.96.

Conclusion: Multi-layer strain demonstrated myocardial involvement in all layers in cardiac amyloidosis, with preserved apical strain in the amyloid group. Furthermore, reduced basal longitudinal strain was the most sensitive and specific strain parameter for the diagnosis of cardiac amyloid in our cohort of patients.
Relative apical sparing using longitudinal strain to diagnose cardiac amyloidosis

Authors:
Sivapathan, S1,2,., Boyd, A.C3,., Deshmukh, T1,2,., Kowk, F1,., M., Altman1, Richards, D3,., Stewart, G1,2,., Denniss, A.R1,2,., Thomas, L1,2.

Affiliation:
1 – Westmead Hospital
2 – The University of Sydney
3 – Westmead Private Hospital

Background: Cardiac involvement portends poor prognosis in AL amyloidosis. Strain analysis has demonstrated a relative ‘apical sparing’ pattern as a sensitive and specific method in identifying cardiac involvement in AL amyloid patients from other patients with increased LV wall thickness.

Aims: To evaluate the value of “apical sparing” pattern in AL amyloid patients with mildly increased wall thickness in amyloid patients and in healthy controls.

Methods: 2D longitudinal strain (LS) measurements were performed on 60 AL amyloid patients with normal-mild LVH (wall thickness < 12mm) and 60 healthy controls. Segmental strain values were measured as an average of six basal, mid and apical segments. Apical sparing was calculated as: Average apical LS / (Average basal LS + Average mid LS). ROC analysis was performed to compare diagnostic accuracy in 60 AL amyloid patients with normal-mild LV wall thickness versus 60 healthy controls.

Results: The ROC analysis showed that when relative apical sparing formula was applied in AL patients with normal-mild left ventricular wall thickness versus healthy controls the AUC was 0.83. A cut-off of 1.0 was not sensitivity or specific in identifying patients with amyloidosis. However, for the same population, the ROC analysis for average basal segmental strain had an AUC of 0.96 using a cut-off of -15.9% with a sensitivity of 98% and specificity of 78%.

Conclusion: Average basal segmental strain may be a more sensitive discriminator for amyloid patients with normal to mild LV wall thickness than the application of relative apical sparing formula.
EXPLORING THE CARDIOPROTECTIVE EFFECTS OF GHRELIN VIA GENE THERAPY

George Ghossein1, Cindy Kok1, Sindhu Igoor1, Rhys Skelton1, Renuka Rao1, Melad Farraha1, James Chong1,2,3, Eddy Kizana1,2,3
1 Centre for Heart Research, The Westmead Institute for Medical Research, The University of Sydney
2 Sydney Medical School, The University of Sydney
3 Department of Cardiology, Westmead Hospital, Sydney

Aims of the Study: Ghrelin is commonly known as the 'hunger hormone' due to its role in stimulating food intake within the human body. Recent evidence, however, suggests that the roles of ghrelin extend far beyond regulating hunger. Rather, ghrelin affects many organs within the body including the heart where it may be protective. Hence, we aim to investigate the ability of ghrelin to protect against a common type of cardiac injury, hydrogen peroxide injury, within an in vitro model.

Methods: Lentiviral vectors encoding ghrelin and LacZ (control) were constructed and functionally validated. H9c2 cells, a rat myoblast cell line derived from the heart, were transduced and collected 4 days later. qPCR and Western blot analysis were then conducted to confirm ghrelin mRNA and protein overexpression, respectively. In ongoing work, H9c2 cells will be transduced with ghrelin followed by application of an in vitro model of hydrogen peroxide injury. Various assays and markers will be utilised to investigate possible mechanisms of protection.

Results: We will present qPCR and western blot data demonstrating that H9c2 cells transduced with a lentiviral vector encoding ghrelin have increased ghrelin mRNA and protein expression. We will also present data to show that ghrelin protects against hydrogen peroxide injury through mechanisms such as decreased apoptosis, increased autophagy and increased mitochondrial function.

Discussion/Conclusion: We are currently functionally validating that a lentiviral vector encoding ghrelin causes ghrelin mRNA and protein expression. We will then utilise this vector to overexpress ghrelin in H9c2 cells before applying an in vitro model of hydrogen peroxide injury. Eventually, we will explore other common cardiac injuries such as ischemia/reperfusion injury and drug induced cardiotoxicity. We will also repeat this study in induced pluripotent stem cell derived cardiomyocytes which will have greater relevance for human biology since they are a human cardiomyocyte cell line.
A strategy to protect the heart against doxorubicin induced cardiotoxicity

Cindy Kok1, George Ghossein1, Renuka Rao1, Sindhu Igoor1, Rhys Skelton1, James Chong1,2, Eddy Kizana1,2
1Center for Heart Research, The Westmead Institute for Medical Research, The University of Sydney
2Department of Cardiology, Westmead Hospital

Doxorubicin is an anti-cancer drug used in treating a variety of malignancies. However, its major adverse effect is cardiotoxicity, which is dose dependent and can be either acute or chronic. Doxorubicin causes injury by DNA damage, formation of free reactive oxygen radicals and induction of apoptosis.

Aim: Our aim is to induce expression of the multiple drug transporter gene (MRP1) in cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CM), to determine whether the increased efflux transporter activity will allow cells to effectively remove doxorubicin.

Methods: To determine the dose of lentiviral vector required for efficient gene delivery to iPSC-CM, a GFP vector (LV.GFP) was used to transduce cells at various MOIs. Having determined the optimal dose, we then generated a lentivirus vector for inducing expression of MRP1 (LV.MRP1) and validated its function in iPSC-CM by qPCR and western blot. The activity of the MRP1 was also tested, by using an efflux assay to detect the amount of dye exported from the cell by the transporter. A LacZ vector (LV.LacZ) was included as a control.

Results: Using LV.GFP, we found that an MOI of 20 was sufficient for transduction of >90% of cells. We also showed that cells transduced with LV.MRP1 exhibited significantly elevated levels of MRP1 mRNA by qPCR, and protein by western blot. This protein was also functional, as demonstrated by the efflux assay, showing reduction of dye sequestering in cells overexpressing MRP1.

Discussion: We have optimised the conditions for gene delivery to human iPSC-CM in vitro. We have also shown that we can successfully over-express MRP1 protein in iPSC-CM, with functional transporter activity. The next step is to determine the dose of doxorubicin which induces cell toxicity, and then to assess the protective effect of MRP1 in those cells.
Monocyte reprogramming with increased cardiovascular disease risk

Corinne Mack1,4, Sravanthi Naralashetty1,4, Rana Baraz1, Stephen Li2,4, Desmarini Desmarini3, Julianne Djordjevic3,4, Heather Medbury1,4

1Vascular Biology Research Centre, Department of Surgery, Westmead Hospital; 2ICPMR, Westmead Hospital; 3Centre for Infectious Diseases & Microbiology, Westmead Institute for Medical Research; 4University of Sydney

Background: Atherosclerosis, the underlying condition of cardiovascular disease (CVD), is associated with biomedical risk factors including high levels of low-density lipoprotein (LDL) and oxidized LDL (oxLDL). Individuals with perturbed levels of these risk factors have pro-inflammatory monocytes, which may promote progression of the atherosclerotic plaque. The exact mechanism by which these risk factors influence monocyte phenotype remains unclear; however, we propose that the cells are functionally reprogrammed, via an initial step involving changes in cellular metabolism.

Aims: To determine the impact of CVD biomedical risk factors on monocyte metabolic profile, including an assessment of surface receptors that the risk factors may interact with to promote metabolic changes.

Methods: The human monocytic cell line THP-1 was cultured with clinically low, normal and high concentrations of LDL and oxLDL. Flow cytometry was used to assess monocyte expression of surface receptors including Toll-like receptors (TLR2), scavenger receptors (CD36) and LDL-receptor. The Seahorse Extracellular Flux Analyser was used to assess the glycolytic rate and fuel source of monocyte metabolism. Further experiments will investigate additional risk factors and the metabolic pathways in more detail.

Results: After 24-hours exposure to LDL, CD36 expression increased, however this was not observed with oxLDL. LDL-receptor expression decreased after exposure of the cells to either risk factor. oxLDL also induced a decrease in oxidative phosphorylation and increase in basal glycolytic rate in monocytes, with a dose-dependent shift to glycolysis.

Discussion: These preliminary results require repetition to confirm whether LDL and oxLDL do impact monocyte surface receptor expression. However, they suggest that oxLDL can impact monocyte metabolism, an initial step key to cellular reprogramming. Determining the specific effects of each risk factor will enable identification of unique treatment targets, depending on an individual's particular profile of CVD biomedical risk factors, to interfere with monocyte functional reprogramming towards a pro-disease state.
Engraftment and migration potential of induced pluripotent stem cell-derived cardiomyocytes in a rodent model of heart failure

Foulis CL1,2, Igor S1, Skelton RJP1,2, Rashid F1, Clayton ZE1,2, Chong JJH1,2,3
1 Centre for Heart Research, Westmead Institute for Medical Research
2 The University of Sydney School of Medicine
3 Department of Cardiology, Westmead Hospital

Rationale and Aims: The discovery of stem cell therapies, and their potential to regenerate the damaged myocardium, have sparked immense scientific and public interest. This work, aims to investigate the potential for human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to engraft and migrate within the native myocardium, using a rodent model of heart failure.

Methods: Human iPSC lines expressing either a tdTomato or GFP fluorescent reporter, were differentiated to cardiomyocytes, using an optimised small molecule protocol. The viability and purity of the cardiomyocyte populations were determined by flow cytometry, using a fixable viability stain and anti-human cardiac Troponin T antibody, respectively. To test iPSC-CM engraftment, 5x10^6 iPSC-CMs were transplanted into healthy rodent hearts via intra-myocardial injection. Hearts were collected on days 1, 4, 7 and 14 post-transplantation. Engraftment was assessed by immunohistochemistry, using anti-RFP (tdTomato) and anti-GFP antibodies. To investigate iPSC-CM migration and functional efficacy, we will simultaneously deliver tdTomato and GFP cardiomyocytes to the rodent heart following ischaemia/reperfusion surgery (I/R), a model of heart failure. iPSC-CMs or vehicle control will be transplanted 5 days following I/R. Echocardiography will be performed at baseline, on day 0 and on day 14, to assess the functional benefits of iPSC-CM therapy. Hearts will be collected 14 days post-transplantation for histological analyses.

Results and Discussion: tdTomato iPSCs were successfully differentiated to cardiomyocytes, producing a population of 82.5% purity with 76.1% viability. tdTomato expression has been confirmed through immunocytochemistry of iPSCs, and immunohistochemistry on rat heart sections. We propose that the GFP iPSCs will follow a similar trend, differentiating into cardiomyocyte populations with high purity and viability. Simultaneous delivery of tdTomato and GFP iPSC-CMs will further the understanding of the in vivo behaviours of these cells, and may provide a foundation to tackle current obstacles faced in cardiac regenerative medicine.
Alterations in left atrial structural and functional parameters associated with cardioembolic stroke

Authors: Ferkh, Aaisha; O'keefe, Emily; Evans, Andrew; Duma, Stephen; Duggins, Andrew; Brown, Paula; Sivapathan, Shanthosh; Thigalingam, Aravinda; Altman, Mikhail; Chong, James; Denniss, Alan Robert; Kizana, Eddy; Thomas, Liza

BACKGROUND: Cardioembolism contributes to ischaemic cerebral events; left atrial (LA) parameters may identify an ‘atrial cardiomyopathy’ in such patients.

AIMS: To determine LA structure and function on transthoracic echocardiogram (TTE) in ischaemic strokes.

METHODS: We prospectively recruited ischemic stroke patients (March-May 2017) and TTEs were performed. Strokes were classified by TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria into cardioembolic and other aetiologies. LA size, volumetric functional parameters and 2D strain were evaluated.

RESULTS: 57 patients (34 male (59.6%), mean age 72yrs) were identified. Ischemic cerebral events were classified as cardioembolic (N=14, 25%), and other causes (N=43, 75%). LAVI_{max}, and LAVI_{min}, were increased (54 ± 9.1 vs 33 ± 24 and 36 ± 22 vs 15 ± 6.1 ml/m² respectively, p<0.001), while LAEF, LAEI and LAFI were reduced in the cardioembolic group versus other causes (36 ± 21 vs 57 ± 10%, 76 ± 72 vs 148 ± 62% and 0.18 ± 0.18 vs 0.37 ± 0.13 respectively, p<0.001). LA reservoir and contractile strain were also reduced in the cardioembolic group (13 ± 10 vs 25 ± 6.5%, p<0.001 and 10 ± 7.7 vs 15 ± 5.1%, p=0.048) as was late diastolic strain-rate (0.70 ± 0.46 vs 1.20 ± 0.44 s⁻¹, p<0.005). E/E’ was elevated in the cardioembolic group (15 ± 5.9 vs 10 ± 3.9, p=0.002), suggesting elevated LV filling pressures in this group.

CONCLUSIONS: Evaluation of LA enlargement and dysfunction in patients at risk of stroke may be beneficial with therapeutic decisions such as anticoagulation.
Electrophysiologic substrate of ventricular arrhythmias in ventricular noncompaction: a systematic review of the literature

Ashwin Bhaskaran, MBBS1; Saurabh Kumar, BSc(Med)/MBBS, PhD1,2

1Department of Cardiology, Westmead Hospital, NSW Australia; 2Westmead Applied Research Centre, The University of Sydney, NSW Australia.

Aim: ventricular noncompaction (VNC) is a form of structural heart disease prone to ventricular arrhythmias (VAs) and sudden cardiac death. The noncompacted myocardium has long been presumed to be the VA substrate; however, growing evidence suggests otherwise. The aim of this study was to characterise the electrophysiologic VA substrate in VNC.

Methods: a systematic search of the literature was conducted to gather data from case reports, case series and observational studies.

Results: a total of 64 cases of VA in VNC were included from 39 studies. Median age at presentation was 39. 35% of symptomatic patients presented with syncope. The most common areas for left ventricular noncompaction (LVNC) were the apex (76%) and lateral wall (68%). 9 patients had right ventricular noncompaction (RVNC). Median left ventricular ejection fraction was 44.5%. Only 1 of 34 patients who underwent MRI had late gadolinium enhancement.

There were 17 polymorphic ventricular tachycardias (VTs) and 9 premature ventricular complexes (PVCs). 51% (28/55) of the monomorphic VTs were RBBB morphology and 49% (27/55) were LBBB, of which 56% did not have RVNC. 21 patients had RV substrate on voltage/activation mapping (5/21 tricuspid annulus, 8/21 RV outflow tract and 9/21 RV apex), of which 67% (14/21) did not have RVNC. 6 patients had epicardial substrate, whilst 3 had pathogenic mutations (2/3 RYR2; 1/3 CASQ).

Failure of anti-arrhythmics occurred in 15 cases, with a median of 2 failed agents (5/15 sodium channel blockers; 9/15 beta-blockers; 8/15 class III agents). Radiofrequency ablation was performed in 28 cases, with acute non-inducibility achieved in 82% (23/28) with a median of 1 procedure.

Conclusion: VA substrate in VNC appears more heterogeneous than the originally thought with a notable proportion of arrhythmias arising from structurally normal myocardium. Further research is required to understand the underlying pathophysiology of VA in VNC.
Title: Impact of Contact Force Sensing Technology on Safety and Efficacy of Radiofrequency Ablation for Atrial Fibrillation

Authors: Virk, Sohaib1; Kumar, Saurabh1,2
Affiliations: 1Department of Cardiology, Westmead Hospital, Sydney, Australia; 2Westmead Applied Research Centre, University of Sydney, Sydney, Australia

Aims: Radiofrequency ablation (RFA) is widely practiced for symptomatic, drug-resistant atrial fibrillation (AF). However, recurrence rates following RFA remain a major concern, and have been attributed to sub-optimal lesion formation. Contact force (CF) sensing catheters provide real-time feedback of the force applied between the catheter tip and targeted cardiac tissue during RFA, and may improve quality of lesions created. We sought to examine the impact of this technology on the safety and efficacy of AF ablation.

Methods: Electronic databases were systematically searched for controlled studies comparing outcomes of RFA for AF performed with and without CF guidance. The pre-specified primary endpoint was 1-year freedom from atrial fibrillation. Secondary endpoints included procedure duration, radiation exposure and incidence of major complications.

Results: Nine randomised controlled trials (RCTs) involving 903 patients, and 25 observational studies involving 3702 patients, were included. Overall, CF guidance was associated with reduced total procedure duration (mean difference [MD] 15.33 minutes; 95% confidence intervals [CI] 6.98–23.68), RFA duration (MD 3.07 minutes; 95% CI, 0.29–5.84), fluoroscopy duration (MD 5.72 minutes; 95% CI, 2.51–8.92) and radiation exposure (standardised mean difference 0.38; 95% CI, 0.18–0.59). Freedom from AF was more common with use of CF guidance (relative risk 1.10; 95% CI, 1.02–1.18). There was no difference in incidence of major complications or cardiac tamponade. In subgroup analysis of RCTs, there was no longer any difference between CF and non-CF guided RFA with regards to procedure duration, radiation exposure or freedom from AF.

Conclusions: Although CF guidance is associated with increased freedom from AF and improved procedural parameters in observational data, pooled analysis of RCTs demonstrates no significant differences. These findings suggest the perceived benefits of CF guidance are largely driven by confounders, and widespread adoption of CF sensing technology is not supported by current evidence.
Catheter ablation versus medical therapy for atrial fibrillation in patients with heart failure: a meta-analysis of randomised controlled trials

Authors: Virk, Sohaib1; Kumar, Saurabh1,2
Affiliations: 1Department of Cardiology, Westmead Hospital, Sydney, Australia; 2Westmead Applied Research Centre, University of Sydney, Sydney, Australia

Aims: The presence of atrial fibrillation (AF) in heart failure (HF) is associated with increased morbidity and mortality. We sought to examine the impact of catheter ablation for AF, compared with optimal medical therapy, on clinical outcomes.

Methods: Electronic databases were systematically searched for randomised controlled trials (RCTs) comparing catheter ablation versus medical therapy (rate and/or rhythm control) in patients with AF and HF. The pre-specified primary endpoint was change in left ventricular ejection fraction (LVEF). Secondary endpoints included 6-minute walk test (6MWT) distance, quality of life (QoL), major procedural complications and mid-term survival.

Results: Six RCTs (n=772) were included, with follow-up duration ranging from 6 to 60 months. Patients who underwent catheter ablation demonstrated significantly greater improvement in LVEF (mean difference [MD] 5.67%; 95% CI, 3.01–8.33) and 6MWT distance (MD 25.12 metres; 95% CI, 0.59–49.65). Catheter ablation was also superior in improving quality of life, with greater reduction in Minnesota Living with Heart Failure questionnaire score (MD 9.03; 95% CI, 2.48–15.59). There was no peri-procedural mortality. Pooled incidence of major peri-procedural complications was 8.2% (95% CI, 3.7–17.2%). In the 3 RCTs (n=618) with minimum 1-year follow-up, catheter ablation demonstrated significantly reduced mid-term mortality (relative risk 0.52; 95% CI, 0.36–0.77; I²=0%; p=0.001).

Conclusions: Compared to medical therapy, catheter ablation in patients with AF and HF provides greater improvement in LVEF, functional status and QoL, as well as increased mid-term survival. Further studies with increased follow-up duration are required to assess long-term outcomes.
Cream based treatment for cutaneous melanoma deposits at Westmead Hospital

Chan L1, Fernandez-Penas Pablo1,2
Department of Dermatology, Westmead Hospital, Westmead, New South Wales
Department of Dermatology, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

Background: In-transit melanoma (ITM) is seen in up to 15% of all melanoma patients and presents a management challenge. Current treatments include repeated surgeries, laser ablation, intra-lesional injection or radiation; all with modest efficacy. Diphenylcyclopropenone (DPCP) cream has gained popularity as a new, minimally invasive and patient controlled topical treatment of ITM.

Objectives: To report the Westmead hospital dermatology experience of using DPCP in managing ITM.

Method: A prospective cohort study on the outcome of seven patients with ITM/satellite lesions managed at Westmead Hospital Dermatology Clinic from 1st May 2017 – 1st February 2018. All patients were treated with topical DPCP cream at a dedicated dermatology clinic in accordance to a standardised protocol.

Result: All seven patients who were treated where enrolled. The overall response rate was as follows: complete response 29%, partial response 29% and progressive disease 29%. One patient was lost to follow up due to hospitalisation elsewhere. The time to complete response was 2 and 6 months for the complete responders. Both patients had small volume macular melanoma deposits on lower legs. For those with partial response, the average treatment duration until improvement was 4 months. In terms of recurrence, one of the complete responders developed a recurrence in 2 months’ time.

Conclusion: Preliminary data from the Westmead Hospital experience of DPCP in the management of ITM shows similar response and recurrence rates to the Queensland group’s published result. However, our data demonstrated faster complete response times (4 months vs 10.5 months). DPCP was well-tolerated in our group. Greater patient numbers will provide valuable insight into questions surrounding dose variation depending on the site of the lesions and ability for DPCP to be combined with systemic immunotherapy to improve patient outcomes.
Using Dapsone for Acneiform eruption secondary to epidermal growth factor inhibitors

Chan L1, Fernandez-Penas Pablo1,2

Department of Dermatology, Westmead Hospital, Westmead, New South Wales
Department of Dermatology, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

Background/Aim: The last decade has borne witness to the explosion of biologic anti-cancer therapy which target specific molecules involved in tumour growth and survival. The epidermal growth factor receptor (EGFR) has become a key target as it is expressed by 30-100% of solid tumours. Two classes of EGFR inhibitors have both been consistently associated with severe acne-like skin eruption. This eruption can affect the face, neck and upper torso and is seen in 60-90% of patients with concurrent psychosocial morbidity. Our oncodermatology department has been researching new medications to combat this problem.

Results: (illustrated through a case study):
A 61-year old male with recently diagnosed metastatic lung cancer with adrenal and bone metastases was commenced on oral erlotinib. Four weeks later, he developed an erythematous pustulopapular eruption involving the face, scalp, and back. This was initially managed with Betamethasone valerate 0.02% ointment and doxycycline 100mg daily. Three weeks later, his eruption deteriorated and was commenced on dapsone 25mg daily for two weeks and then increased to 50mg per day. After four weeks of receiving dapsone, the patient’s skin score improved dramatically. We have approximately 15 patients who are currently receiving the same medication with good effect.

Discussion: Whilst the pathogenesis of EGFR related acneiform eruptions is not fully elucidated, undesired neutrophil recruitment and activation appears to play a key role. This formed the scientific basis for choosing oral dapsone. Dapsone is an anti-microbial agent with known anti-inflammatory properties and it is an approved acne treatment. Through our experiences at Westmead Hospital, we have discovered a new indication for Dapsone and it has helped our cancer patients remain on their cancer treatments.
A novel treatment for Epidermal growth factor receptor inhibitors induced paronychia

Chan L1, Fernandez-Penas Pablo1,2
Department of Dermatology, Westmead Hospital, Westmead, New South Wales
Department of Dermatology, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

Background: Epidermal growth factor receptor inhibitors (EGFRIs) are now considered first line treatment for EGFR-mutant advanced non-small cell lung cancer with a proven superiority in terms of response rate, delayed progression and improved quality of life over traditional platinum-based chemotherapy. The majority of side effects of EGFRIs are predominantly skin related (54-89%). Paronychia (inflammation around the nails) are one of the relatively common and difficult to manage side effects reducing the patient’s quality of life.

Aims: To report the successful treatment of severe EGFR induced paronychia with timolol topical gel 0.5% twice daily under occlusion for 4 weeks.

Method: All patients referred to our dermatology department with paronychia as an adverse effect of epidermal growth factor receptor inhibitor are screened for paronychia/periungual pseudo-PG. One patient was identified.

Result: A 57-year-old male with panitumumab induced mild paronychia affecting three fingernails was commenced on bleach baths initially. After two weeks, his paronychia and panitumumab induced papulopustular eruption worsened. The cutaneous adverse events resulted in temporary cessation of his panitumumab. He was then instructed to apply on timolol gel 0.5% twice per day under an occlusive dressing for two hours on each occasion for management of his paronychia. On review in one month, the erythema, swelling and exudative discharge from the fingernails had all resolved. There were no side effects reported. He continues to observe general measures for his nails and has been recommenced on panitumumab.

Conclusion: In this case study, we demonstrated that 0.5% timolol gel is a promising treatment for severe paronychia with minimal side effects. We will continue to prospectively monitor and report findings on the efficacy of this treatment at our institution.
Review of Reflectance Confocal Microscopy in the diagnosis of Cutaneous T-cell Lymphoma

Authors: Melhoranse Gouveia Bruna, Consuegra Germana, Wells Jillian, Fernandez-Penas Pablo

Affiliation: Department of Dermatology – Westmead Hospital

Background: Reflectance Confocal Microscopy (RCM) is a non-invasive technique that provides images with cellular level resolution. One of the greatest advantages of RCM is the dynamic information that can be acquired from real-time imaging of cutaneous lesions without some of the pitfalls associated with skin biopsies (such as pain and scars). In the last decade, a number of groups have described the use of RCM in cutaneous T-cell lymphomas (CTCL). This rare indolent cancer usually requires multiple biopsies for diagnosis because it can resemble other frequent conditions such as eczema and psoriasis.

To increase the diagnostic precision, RCM was described as a possible diagnostic method to determine the best area for biopsy, with the goal of decreasing false-negative pathology results.

Objective: This study aims to review the previously described RCM features for CTCL and propose the main characteristics seen with RCM in CTCL for application in clinical practice.

Methods: A systematic literature search was performed in eight electronic databases. The search was limited to publications on humans until May 15, 2018.

Results: Eighteen RCM features were collected in the eleven selected publications. A quantitative analysis was performed to establish the frequency of each reported feature. The most valuable ones were: interface dermatitis, spongiosis, atypical lymphocytes, epidermal disarray and vesicle-like structure (Pautrier's microabscess). Discussion: The systematic review herein helps to define the use of RCM in cutaneous T cell lymphoma. In the near future, RCM may have an application in the outpatient clinic in monitoring CTCL patients. This innovative imaging technique is likely to extend to other cutaneous diseases, including CTCL, not only during the initial clinical assessment but also while evaluating patients’ response to treatments.
Role of Hepatocyte Vitamin D Signalling in Liver Fibrosis

Keane, Jeremy T 1 2 , Liddle, Christopher 2 , Gunton, Jenny E 1 2
Centre for Diabetes, Obesity and Endocrinology, Westmead Institute for Medical Research, Westmead, NSW, Australia
Faculty of Medicine and Health, Sydney University, Westmead Hospital, Westmead, NSW, Australia

Objective: To investigate whether Vitamin D signalling in hepatocytes ameliorates liver fibrosis.

Methods & Results: Hepatocyte vitamin D receptor (h-VDR) knockout (KO) mice and their floxed controls (FC) were created. Mice were subjected to twice weekly intraperitoneal injections of thioacetamide (TAA) or saline for 10 weeks and subsequently sacrificed. The liver sections of mice from the h-VDR KO group demonstrated increased fibrosis staining and collagen compared to those of the FC group. qPCR results show an increase in mRNA levels of TNFa, and TIMP1, markers of hepatic inflammation, in the KO group when compared to the FC group, as well as increased mRNA levels of the macrophage marker F480, indicating that liver macrophage infiltration was increased by loss of h-VDR.

Conclusions: h-VDR may play a role in modulating chronic liver injury. These hepatoprotective effects may be of therapeutic use in human liver disease.

Lay description: Liver Vitamin D Receptor (VDR) may play a role in modulating chronic liver injury, as indicated by our TAA model. Mice lacking hepatocyte VDR, the receptor through which Vitamin D functions, had increased markers for liver fibrosis compared to those with a functioning VDR.
Title: Characterisation of an immunodeficient UCP1 knockout mouse model

Jennifer Chen1, Jenny E. Gunton1,2.
1Centre for Diabetes, Obesity & Endocrinology, The Westmead Institute for Medical Research (WIMR), Westmead, Sydney, NSW 2145, Australia
2Faculty of Medicine and Health, The University of Sydney, Westmead Hospital, Westmead, Sydney, NSW 2145, Australia

Background: Rising in prevalence each passing year, obesity is the result of heightened caloric intake that greatly exceeds energy expenditure. This leads to an excessive accumulation of fat which increases susceptibility of developing other health complications such as diabetes or cardiovascular disease. In brown adipose tissue (BAT), mitochondrial uncoupling protein 1 (UCP1) mediates non-shivering thermogenesis, consuming energy to generate heat. In mammals, induction of UCP1 activity can be achieved upon exposure to cold ambient temperatures, or by β3-adrenergic receptor agonists. Interestingly, adult humans have been found to possess some BAT deposits. Hence, UCP1 is a potential therapeutic target for increasing basal energy expenditure to modify body weight.

Aim: To examine the effect of mild cold stress on UCP1 knockout (UCP1Δ) and wildtype (WT) recombination activating gene 1 knockout (RAG1Δ) mice acclimated to thermoneutrality.

Methods: Immunodeficient BAT specific UCP1Δ- and WT RAG1Δ C57BL/6 mice were maintained at a temperature that is thermoneutral for rodents (29°C) for 1 week, before exposure to mild cold stress (18°C). We also induced UCP1 activity using an intraperitoneal injection of 0.5 mg/kg CL-316,243 (CL), a β3-adrenergic receptor agonist. Differences in energy expenditure between groups were assessed by indirect calorimetry using the Promethion metabolic cages system.

Results: Under mild cold stress, all mice exhibited a significant increase in energy expenditure. However, we found no significant differences in energy expenditure between groups. There was a ~35% increase in energy expenditure in WT RAG1Δ mice compared to their UCP1Δ- counterparts 1-hour post-CL injection.

Discussion: We demonstrate that UCP1 activation is one of the underlying mechanisms that mediate energy expenditure. It is likely that a lower ambient temperature is required to induce UCP1 activity. Further investigation exploring alternative methods of UCP1 activation using dietary supplements such as caffeine are underway.
Guiding stem cell differentiation by manipulating Mixl1 expression patterns

N Salehin1,2, J Studdert1, T Sibbritt1,2, P Osteil1,2, PPL Tam1,2
1Children's Medical Research Institute. Westmead NSW 2145 Australia
2Sydney Medical School, University of Sydney, Sydney NSW 2006 Australia

Gastrulation occurs early in mouse embryonic development and produces the three embryonic lineages. The gene Mixl1 is vital for gastrulation and its loss leads to a failure to gastrulate, loss of endoderm and embryonic lethality. In stem cell lines, the timing of endogenous Mixl1 appears to bias the endoderm propensity of the line.

Controlled activation of Mixl1 in inducible mouse epiblast-derived stem cells (mEpiSC) was used to determine the time dependence of Mixl1 activity. mEpiSCs were differentiated using an embryoid body protocol for 4 days and the expression of germ layer markers, particularly endoderm, was assessed using high throughput reverse transcription combined with quantitative polymerase chain reaction.

Using a Differentiation Score that collates multiple statistical tests, we determined Mixl1 activation on day 2 of differentiation was the best condition for endoderm formation.

This result mirrors the activation timing of endogenous Mixl1. This timing was used as the basis of further sequencing studies that explored the direct effects of Mixl1 on the genome and transcriptome, while accounting for the 3-dimensional folding of DNA.

Exploring the regions of the genome where Mixl1 is bound in this condition, we discovered that two markers of endoderm progenitor cells – Gsc and Lhx1 are directly bound and activated by Mixl1 providing a possible link between Mixl1 expression and cell fate during gastrulation.
Unveiling the roles of Activin and Nodal in stem cell differentiation

H Knowles1, N Salehin1, M Demuth1, P Osteil1,2, PPL Tam1,2

1Children’s Medical Research Institute. Westmead NSW 2145 Australia
2Sydney Medical School, University of Sydney, Sydney NSW 2006 Australia

During embryogenesis, gastrulation is a major event that results in the specification of embryonic cells into three progenitor layers; the ectoderm, mesoderm and endoderm. The formation of endoderm is reliant on strong signalling through the TGFB signalling family, of which both Activin and Nodal act to stimulate. In vivo, high Nodal concentrations in the mouse epiblast influence an endodermal cell outcome.

However, in vitro stem cell models of endoderm differentiation routinely use Activin in its place. In light of this disparity, this study aims to determine the influence of Activin and Nodal on endoderm differentiation in vitro by identifying differences in germ layer outcomes and TGFB signalling activation after inducing endoderm differentiation.

Epiblast stem cells (EpiSCs), a mouse gastrulation model, were tested to understand their requirements for in vitro endoderm differentiation. A reporter line of EpiSCs that fluoresce upon activation of endodermal gene markers FoxA2 and Sox17 was utilised. These cells were used to trial models of EpiSC endoderm differentiation by adjusting parameters such as ligand concentration and cell density. In order to assess the effectiveness of endoderm formation driven by various models, we performed flow cytometry sorting of positive populations and fluorescent imaging. An established model of differentiation will be treated with Activin or Nodal with samples collected on a timeline.

To examine the outcome of differentiation of the cells, the transcriptome of the EpiSCs will be assessed by analysing RNA expression level using qPCR on a germ layer specific gene set. This approach will unveil differences between Activin and Nodal induction of endoderm differences in vitro, an important piece of the puzzle in the endeavour of modelling in vivo cell specialisation.
Genomics, molecular diagnoses and genotype-phenotype insights in the Inherited Retinal Dystrophies

Benjamin Nash1,2,3,4*, Alan Ma1,3,4, Dale Wright2,4, Bruce Bennett2,4, John Grigg1,3,4, Robyn Jamieson1,3,4

*Email: Benjamin.Nash@health.nsw.gov.au
1 Eye Genetics Research Unit, Children's Medical Research Institute, Sydney Children's Hospital Network, Hawkesbury Rd, Westmead, NSW 2145 Australia
2 Sydney Genome Diagnostics, Sydney Children's Hospital Network, Hawkesbury Rd, Westmead, NSW 2145 Australia
3 Save Sight Institute, Macquarie St, Sydney, NSW 2000 Australia
4 Discipline of Genetic Medicine, Sydney Medical School, University of Sydney, Sydney, NSW 2000 Australia

Aims: The inherited retinal dystrophies (IRD) affect approximately 1/3500 people worldwide and are degenerative disorders of the retina affecting both rod and cone photoreceptors. Prioritising the >250 known causative disease genes is challenging. Until recently, there was no clinical diagnostic testing available for IRD in Australasia. This study applied next-generation sequencing (NGS) strategies to an IRD cohort to determine their relative clinical value in an Australian diagnostic laboratory setting.

Methods: In a cohort of 100 patients with familial or sporadic IRD, samples were processed following Illumina TruSight One clinical exome and the TruSeq Nano-DNA 30x whole genome protocols. Libraries generated were sequenced on the Illumina NextSeq 550 or HighSeq X Ten instruments. Variants detected in the 256 IRD genes of interest were filtered and prioritized in silico using population allele frequencies, conservation and pathogenicity prediction scores, before classification according to ACMG guidelines.

Results: Novel and previously reported mutations were identified in several genes including ABCA4, CACNA1F and RPRG, and also in syndromic genes BBS1, IFT140 and USH2A. Molecular diagnosis was achieved in 74/100 (74%) families allowing for improved patient management and recurrence risk information. This work has also led to novel genotype-phenotype correlations, including NMNAT1 mutations associating with cone and cone-rod dystrophy, while in other cases has facilitated a change in clinical diagnosis. WGS analysis in specific families has led to the identification of intronic variants in a novel vitreoretinopathy gene and other disease genes, which are predicted to affect natural gene splicing.

Conclusions: The application of our NGS strategy has been successful in identifying pathogenic variants in the IRDs, highlighting the clinical value of genomic technologies in highly heterogeneous disease. Integration with existing diagnostic NGS testing processes has resulted in our panel-based approach now being provided as the first clinical service for IRD molecular diagnostics in Australasia.
Role of FZD5 in microphthalmia, coloboma and WNT signalling

Steven S. Eamegdool, To Ha Loi, Anson Cheng, Robyn V. Jamieson
1. Eye Genetics Research Unit, Children's Medical Research Institute, University of Sydney, Sydney Children's Hospital Network, Save Sight Institute, Sydney, Australia
2. Discipline of Genetic Medicine, Children's Hospital at Westmead Clinical School, Sydney Medical School, University of Sydney, Sydney, Australia

Aim: Microphthalmia and coloboma (M/C) are congenital eye abnormalities leading to small eyes associated with failure of closure of the optic fissure. Incomplete penetrance and variable expression are among the factors hampering the disease gene identification process. Here we employed whole exome sequencing (WES) for affected members of an autosomal dominant M/C family. We identified a novel pathogenic variant in the Frizzled 5 (FZD5) gene, which encodes a receptor involved in the WNT signalling pathway, suggesting a role in development of the M/C phenotype.

Methods: In this study, we performed WES on four affected individuals from two generations in an Australian family with autosomal dominant M/C, where mutations in known disease genes were not previously identified. The human FZD5 gene, harbouring either the FZD5-WT or FZD5-mutated version, was cloned into a GFP-expressing vector, and transfected into Caco-2 and HEK293 cells. Subsequently, we performed immunostaining to visualize cellular FZD5 expression.

Results: Heterozygous variants shared by all four affected family members were selected for the analysis. Variants were filtered based on their population frequency, pathogenicity prediction and conservation scores. Subsequently we prioritised variants in previously investigated animal disease genes and pathways known to be involved in eye disease. The top candidate variant, a novel frameshift mutation, was in the FZD5 gene, which was confirmed by Sanger sequencing. Furthermore, FZD5-WT showed clear and even cell membrane localization in our cellular studies. The FZD5-mutated version showed weak and punctate localization around the cell membrane.

Conclusions: Our findings, based on a combined employment of exome-wide variant analysis, biological process gene ontology comparison, literature data and functional analyses, demonstrate the role of the FZD5 gene in eye morphogenesis and impact of its mutation on M/C pathogenesis.
Functional genomic and mouse model studies in characterisation of a novel retinal ciliopathy

Amin Sabri1, To Ha Loi1, Anson Cheng1, Maros Van Den Bergh1, Fidelle Karam1, Diana Jelovic1, John R. Grigg1,2, Robyn V. Jamieson1,3

1 Eye Genetics Research Unit, Children’s Medical Research Institute, The Children’s Hospital at Westmead, Save Sight Institute, University of Sydney, Sydney, NSW, 2145, Australia
2 Discipline of Ophthalmology, University of Sydney, Sydney, NSW, 2000, Australia
3 Disciplines of Genetic Medicine, and Child and Adolescent Health, University of Sydney, Sydney, NSW, 2145, Australia

Background: Inherited retinal dystrophies (IRDs) may be syndromic or non-syndromic, and many are caused by variants in genes affecting primary cilia structure or function of the photoreceptors, which are specialised sensory cilia. Through genomic analysis in a newly identified syndromic form of retinal dystrophy, we have identified a novel candidate kinase disease gene with a predicted role in centrosomal biology and ciliary function.

Methods: Cell-based, and animal model approaches were undertaken to understand the function of the novel disease gene. A CRISPR/Cas9 generated mouse model was investigated using electroretinography (ERG)and immunohistochemistry.

Results: Analyses in mouse retina showed expression of the protein in the connecting cilium region of the photoreceptors with a possible role in ciliary trafficking. Embryonic fibroblasts established from mutant mice showed decreased proportion of ciliated cells. ERG studies revealed significant decrease in scotopic and photopic responses in mice with the orthologous mouse mutation, and histology sections showed thinning of the retinal layers. Further immunohistochemistry studies confirmed aberration of connecting cilium proteins, Ift88 and centrin, in the mutant mouse photoreceptors.

Conclusion: Through functional genomic studies, we have shown that this newly identified candidate disease gene is critical for normal ciliary function in the photoreceptors. This study highlights the value of combined cellular and mouse model studies for detailed disease gene characterisation in the IRDs.
Successful in vivo editing of patient-derived primary human hepatocytes

Anais K. Amaya1, Samantha L. Ginn1, Sophia H.Y. Liao1, Cindy Zhu1, Michael Lee2, Hilda A. Pickett2, Claus V. Hallwirth1, Sharon C. Cunningham1, Grant J. Logan1, Kimberley Dilworth3, Leszek Lisowski3,4 and Ian E. Alexander1,5
1Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia; 2Telomere Length Regulation Group, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia; 3Translational Vectorology Group and Vector Genome Engineering Facility, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia; 4Military Institute of Hygiene and Epidemiology, Pulway, Poland and 5Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia

Rationale: The popularity of recombinant adeno-associated viral (rAAV) vectors based gene therapy targeting the liver has dramatically increased in recent years due to early signs of clinical success targeting indications such as Haemophilia A and B. The recent development of novel human hepatotropic capsids has markedly improved the efficiency with which human hepatocytes can be transduced, making the liver an increasingly promising target for more challenging therapeutic strategies such as gene repair/editing. Precise gene repair involves the correction of the genetic defect directly at the mutant locus, allowing retention of physiological expression under native endogenous control elements.

Aim of the study: To correct a disease-causing mutation in the ornithine transcarbamylase (OTC) gene in patient-derived primary human hepatocytes using AAV-CRISPR/Cas9-mediated genome editing.

Methods: Primary human hepatocytes can be xenografted into the FRG (Fah-/-, Rag2-/-, IL2rg-/-) mouse liver. In this animal model, human hepatocytes can be induced to repopulate the liver to create "humanised" mice. Using this model, we delivered rAAV-based editing reagents designed to cut the target locus and introduce a precise single nucleotide change by homology-directed repair (HDR). We quantified editing rates at the OTC locus by targeted deep sequencing.

Results and Discussion: We detected efficient CRISPR/Cas9-driven cutting at the native locus, with up to 70% of OTC alleles being modified after the treatment. The precise single nucleotide change was present in 11.4% to 16.6% of alleles. Moreover, correction of the mutation resulted in restoration of OTC enzymatic activity. In this study, we have demonstrated for the first time successful correction of a disease-causing mutation in patient-derived primary human hepatocytes in vivo. Furthermore, considering that the first gene editing trials in humans have already started, this strategy could potentially be adapted to validate gene editing vectors before clinical use, not only for OTC deficiency but also for most genetic liver diseases.
Evaluation of genome editing reagents for the correction of patient-derived human hepatocytes by homology-directed repair in vivo

Samantha L Ginn1, Anais K Amaya1, Sophia H Y Liao1, Erhua Zhu1, Sharon C Cunningham1, Claus V Hallwirth1, Szun S Tay1, Michael Lee2, Hilda A Pickett2, Kimberley Dilworth3, Leszek Lisowski3,4 and Ian E Alexander1,5
1Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia; 2Telomere Length Regulation Group, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia; 3Translational Vectorology Group and Vector Genome Engineering Facility, Children's Medical Research Institute, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia; 4Military Institute of Hygiene and Epidemiology, Pulway, Poland and 5Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Westmead, NSW, Australia

Rationale: The immense promise of liver-targeted gene therapy is in the early stages of realisation, with progress underpinned by advances in adeno-associated virus (AAV) vector technology and the use of humanised preclinical models. While contemporary human clinical trials have exploited relatively tractable gene addition approaches, exciting developments in genome editing technology offer the prospect locus-specific repair. Therapeutic efficacy has now been demonstrated in human clinical trials for haemophilia, making human hepatocytes a compelling target for both gene delivery and genome editing technologies. Access to patient cells, however, can be limited, making the assessment of gene therapy reagents that specifically target these cells difficult to model in vivo.

Aim: To introduce an exact copy of a patient-specific mutation into the murine liver using a novel recombinant viral vector system. This locus can then be used to functionally validate human-specific genome editing reagents to correct this mutation by homology-directed repair (HDR).

Methods and Results: We selected the human OTC gene as a clinically relevant target locus and delivered this “minigene” to the liver of newborn mice by intraperitoneal injection. Three weeks later, animals were treated with dual AAV vectors, one containing a donor template for HDR and a second vector expressing CRISPR-Cas9 and a single guide RNA. Using this approach, we have identified single guide RNAs and donor templates for effective target site cleavage and characterised the types of lesions observed at the cut site. Importantly, we have successfully corrected this patient-specific mutation introduced into the murine hepatocytes in vivo.

Conclusions: This “minigene” system provides a novel approach to functionally validate genome editing reagents, specifically single guide RNAs and AAV-based HDR donor templates, which can then be directly adapted for potential human application. We are currently extending the use of these reagents to correct patient-specific human hepatocytes in vivo with exciting results.
CRISPR-Cas9 gene editing to repair cells from patients with neurodevelopmental disease

Andre Grech1,2, Leszek Lisowski5,6; Ian Alexander4,7; Aaron Schindeler 3,4; Wendy Gold 1,4
Molecular Neurobiology Research Lab, Kids Research, Sydney Children's Hospitals Network, Westmead, Australia;
Applied Medical Sciences, Faculty of Medicine and Health, The University of Sydney, New South Wales, Australia;
Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead, New South Wales, Australia.
Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Australia
Translational Vectorology Group, Children's Medical Research Institute, The University of Sydney, Sydney, Australia
Military Institute of Hygiene and Epidemiology, The Biological Threats Identification and Countermeasure Centre, 24-100 Puławy, Poland.
Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, NSW Australia

Background: Neurodevelopmental and neurodegenerative disorders are genetic conditions with high morbidity and mortality. Many are clinically managed on a symptomatic level, and cannot cure the underlying genetic mutation. CRISPR-Cas9 gene editing is an emerging technology with the potential to yield enduring improvements in quality of life and survival. Our objective is to show proof-of-concept for its application in genetic diseases affecting the central nervous system (CNS).

Aims: (1) To customise and develop gene editing tools that will be suitable for a therapy for neurodevelopmental disease and; (2) To demonstrate that these tools have the capacity to correct existing mutations in patient cell lines.

Methods: Two patient cell lines with characterized mutations were obtained from hospital biobanks. Specific CRISPR-Cas9 gene editing approaches have been designed, and plasmid constructs are currently being produced. Plasmid vectors will be delivered to the patient cell lines by lipofection, and clonal cell lines selected and screened. Genetic rescue will be confirmed by DNA sequencing as well as Western blot analysis.

Results: Conditions for clonal Puromycin selection and lipofection (using a GFP control vector) have been optimized for the patient cell lines. CRISPR-Cas9 constructs have been designed and we have initiated the production of custom vectors using standard molecular cloning techniques.

Discussion: This project is on track to complete construct synthesis and produce gene edited patient cell lines. This will show proof-of-principle for the use of CRISPR/Cas9-mediated editing to correct a range of patient mutations at high frequency. This project synergises with other studies by our group developing high efficiency tools for in vivo delivery of vectors to the CNS.
New technologies for targeted gene editing in the central nervous system

Boyling, Alexandra1,2, Lee, Lucinda3,4, Lisowski, Leszek5,6, Alexander, Ian3,7, Schindeler, Aaron3,4, Gold, Wendy2,3
Applied Medical Sciences, Faculty of Medicine & Health, Sydney, New South Wales, Australia; Molecular Neurobiology Research Lab, Kids Research, Sydney Children's Hospitals Network, Westmead, Australia.
Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia.
Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead, New South Wales, Australia.
Translational Vectorology Group, Children's Medical Research Institute, The University of Sydney, Sydney, Australia.
Military Institute of Hygiene and Epidemiology, The Biological Threats Identification and Countermeasure Centre, 24-100 Puławy, Poland.
Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Westmead, NSW Australia

Background: Rett syndrome is a severe genetic neurological disorder primarily affecting females as a result of mutations in the X-linked Methyl CpG binding protein 2 (MECP2) gene. The disorder is characterised by profound physical and cognitive impairments and is currently incurable. Available therapies utilise a symptomatic approach, yielding minimal improvements on quality of life. CRISPR/Cas9 gene editing has the potential for rapid creation of mouse models of disease as well as eventually gene therapy rescue. However, methods for high efficiency delivery of vectors to the central nervous system (CNS) remains a barrier to these approaches.


Methods: Reporter constructs for viral expression of Cas9 and targeted RNA guides have been designed, and will be synthesised by the Vector Genome Engineering Facility (VGEF) at CMRI with AAV9 and AAV-PHP.eB capsids. These will be screened in Ai9 (Gt(Rosa)26Sortm9(CAG-tdTomato)Hze) reporter mice that express tdTomato in response to Cre recombinase expression. Finally, wild type C57BL6 mice will be transduced with custom CRISPR AAVs to inactivate MecP2 and we will measure MecP2 protein expression and phenotypic effects.

Results: Constructs, including cell specific-sequences for neuronal targeting, are currently being engineered into CRISPR/Cas9 plasmids via molecular cloning techniques. Viruses for testing efficiency of CNS delivery have been produced and are scheduled for animal trials within 4 weeks. Immunohisotochemistry techniques are being optimised for the detection of positively edited cells in ex vivo brains.

Discussion: This represents a critical proof-of-concept technology that has broad applications for the treatment of diseases of the CNS as well as generating experimental model systems.
Characterising genetic retinal disease in mouse models for applications of genetic therapy

Fidelle Chahine Karam1,2, To Ha Loi1,2, Amin Sabri2, Maros van den Bergh2, Anson Cheng1,2, Robyn Jamieson1, 2

1The University of Sydney, 2Eye Genetics Research Unit, Children’s Medical Research Institute, Westmead.

Introduction: Vision loss due to genetic disease takes a toll on the affected individuals, their families and society, with a financial burden of $9.85 billion p.a. in eye care. Treatment remains difficult due to the heterogenous nature of retinal disease and the experimental nature of gene therapy. The most common form of genetic retinal disease is retinitis pigmentosa, which is caused by a multitude of identified genes. PDE6B is one such gene, and is normally involved in the proper transduction of the visual signal in the retina.

As the mutation in this gene is a suitable candidate for future genetic therapy, its proper characterisation in a mouse model is critical to the success of the treatment.

Aims: The aim of this project is to characterise the onset and progression of retinal disease in Pde6b-/- mice.

Method: Electroretinography (ERG) was carried out to establish the functional output of the retina in Pde6b-/- mice at postnatal ages: 14 days, 21 days, 30 days and 2 months. Histological analysis was also done at these ages, as well as at 1 day and 7 days, to determine the time course of retinal degeneration.

Results: ERG analysis of retinal output suggests that by 2-month post-gestational age, the Pde6b-/- mice are functionally blind. Histology shows loss of photoreceptors and thinning of the outer nuclear layer of the retina at this age.

Discussion: These observations indicate that the mutation in Pde6b-/- mice causes retinal disease relatively early in the life of a mouse. Interventional therapy to treat this condition by correcting the genetic mutation needs to be carried out prior to this degeneration to prevent irreparable loss of photoreceptors.
Generating a toolbox for screening genetic variants associated with bone fragility disorders

O'Donohue, Alexandra1,2; Lee, Lucinda 1,3; Munns, Craig3,4; Little, David G.1,3; Biggin, Andrew3,4; Schindeler, Aaron1,3.
Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead, New South Wales, Australia;
The University of Sydney, Applied Medical Sciences, Faculty of Medicine & Health, Sydney, New South Wales, Australia;
The University of Sydney, Discipline of Paediatrics and Child Health, Faculty of Medicine & Health, Sydney, New South Wales, Australia;
Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, New South Wales, Australia.

Background: Genetic bone fragility disorders are associated with increased fracture rates and deformity, with osteogenesis imperfecta (OI) being the most common. However, a subset of individuals with genetic bone fragility fail to achieve a definitive genetic diagnosis. This impacts on genetic counselling, disease prognosis, symptom management, and the application of future genetic therapies. We hypothesize that cell models utilizing CRISPR gene editing can be used to confirm support or reject variants of uncertain significance (VUSs).

Aims: (1) To create a bank of human osteoblast cell lines with heterozygous and homozygous mutations in genes associated with bone fragility. (2) To assess these cell lines using a range of functional assays.

Methods: 23 patient VUSs were screened using the gnomAD and PolyPhen2 databases to prioritize mutations likely to be pathogenic and used to guide a priority list of candidate genes for CRISPR knockout. Base editing guides were generated using crispr.mit.edu and used to make CRISPR-Cas9 plasmids, which were confirmed by DNA sequencing.

Results: In silico analysis identified LRP5, BMP1, LEPRE1, PLOD2, FKBP10, and HSPG2 as high priority genes. CRISPR-Cas9 strategies were successfully designed and constructed to knockout function or base edit specific patient mutations. Constructs for COL1A1, COL1A2, and LRP5 were produced for transfection into hFOB1.19 cells. These cells were also screened for their sensitivity to puromycin selection.

Future Directions & Discussion: The CRISPR-Cas9 constructs will be introduced into human osteoblasts by transfection, placed under puromycin selection, and colonies selected and screened by sequencing. Gene modified clonal cell lines will be tested using a range of functional assays including ALP activity for bone differentiation, collagen SDS-page gels, and osteogenic gene expression by qPCR. Future work will also test in vivo xenograft assays to use gene modified cells to produce nodules of human bone. This system has significant potential for human diagnostic use.
Improving bone strength in a mouse model of Osteogenesis Imperfecta

Openshaw, Aimee1,2; Vasiljevski, Emily1,3; Little, David G.1,3; Munns, Craig3,4; Cheng, Teghan1,3; Schindeler, Aaron1,3.
Orthopaedic Research & Biotechnology, The Children’s Hospital at Westmead, New South Wales, Australia; Applied Medical Sciences, Faculty of Medicine & Health, Sydney, New South Wales, Australia; Discipline of Paediatrics & Child Health, Faculty of Medicine & Health, Sydney, New South Wales, Australia; Institute of Endocrinology and Diabetes, The Children’s Hospital at Westmead, New South Wales, Australia;

Background: Osteogenesis Imperfecta (OI) describes a group of genetic bone disorders that present in approximately 1:10,000 births. OI is characterised by bone fragility of varying severity. OI is widely treated with bisphosphonates, anti-resorptive drugs that increase bone mass. Preclinical studies suggest that the anabolic action of human growth hormone (hGH) can improve bone strength in OI, however this has not yet been examined in combination with existing treatments such as bisphosphonates.

Aims: To use an established preclinical mouse model of OI that recapitulates a patient collagen I mutation to (a) confirm hGH mediated improvements in bone strength in an alternative animal model to published studies; (b) test whether additive or synergistic effects can be achieved by combining hGH and the bisphosphonate zoledronic acid (ZA); and (c) testing secondary benefits of hGH including improved muscle mass.

Methods: A study is being carried out in n=80 mice of the Col1a2 c.610G>C line, half wild type (WT) and half heterozygous mutant (OI). For each genotype, n=10 mice receive [1] saline controls, [2] ZA, [3] hGH, and [4] ZA + hGH. Animals are dosed daily (6/7 days/week). The study lasts for 10 weeks with outcome measures including radiography, microCT analysis of bone volume and bone mineral density, bone mechanical strength, bone histology, and muscle wet weight and grip strength.

Results: Protocols for analysis including microCT and bone histology have been optimized. The dosing of groups [1] and [2] have been completed for WT and OI mice and groups [3] and [4] are underway.

Discussion & Translation: This study will be highly informative for clinical practice. In some hospitals hGH is prescribed off-label at high cost and uncertain efficacy. These data will allow hGH to be compared head-to-head with ZA for the first time, as well as examine the interaction between the two management approaches.
WGS in anterior segment dysgenesis and cataracts reveals structural variants in previously unsolved cases

Ma, Alan 1,2, Minoche, Andre E. 3, Grigg, John 1,4, Flaherty, Maree 5, Ho, Gladys 2,6, Amor, David J. 7, Cheng, Anson 1,2, Bennetts, Bruce 2,6, Cowley, Mark J. 3,8, Dinger, Marcel E. 3,8,9, Jamieson, Robyn V. 1,2,4 
1 Eye Genetics Research Unit, Sydney Children's Hospital Network, Children's Medical Research Institute, University of Sydney, Sydney, Australia
2 Discipline of Genetic Medicine, CHW Clinical School, Sydney Medical School, University of Sydney, Sydney, Australia
3 Kinghorn Centre for Clinical Genomics, Garvan Institute for Medical Research, Sydney, NSW, Australia
4 Save Sight Institute, University of Sydney, Sydney, Australia
5 Department of Ophthalmology, The Children's Hospital at Westmead, Sydney
6 Sydney Genome Diagnostics, Sydney Children's Hospital Network, Westmead, Sydney, Australia
7 Murdoch Children's Research Institute and University of Melbourne Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia
8 St Vincent's Clinical School, UNSW Sydney, Sydney, Australia
9 Genome.One, Sydney, Australia

Introduction: Disorders of the ocular anterior segment exhibit marked clinical and genetic heterogeneity. We have previously obtained a genetic diagnosis in a high proportion of patients utilising an NGS-based panel or whole exome sequencing (WES) approach and CGH microarray, finding answers in over 70% (33/46) of congenital cataract patients, and almost 40% (14/39) of patients with anterior segment abnormalities.

Methods: In this study we applied whole genome sequencing (WGS) to assist with 38 cases (13 congenital cataracts, 25 anterior segment abnormalities) unsolved after CGH microarray, and panel or WES. WGS utilized the Illumina HiSeq X Ten and a bioinformatics structural variant analysis pipeline, ClinSV, which allows confident detection of rare structural- and copy number variants, using evidence from split-reads, discordant pairs and depth-of-coverage.

Results: A causative variant was found in 3/38 patients, who harboured deletions too small to be seen on conventional microarray or capture-based sequencing. A novel 3.2kb deletion in the MIP gene was found in a patient with congenital cataracts and microphthalmia. A 1kb deletion in PAX6 was found in a family with autosomal dominant anterior segment dysgenesis. A 4kb deletion in the X-linked syndromal gene NHS was found in a male with congenital cataracts and coloboma.

Discussion: This study demonstrates an increased yield of genetic diagnoses in a cohort of patients with complex ocular phenotypes. While the yield of CGH microarray combined with panel/exome based testing is significant, WGS analysis revealed additional diagnoses of structural variants, providing new diagnostic and management information.

Ruebena Dawes1,2, Monkol Lek3 and Sandra T. Cooper1,2
1Kids Neuroscience Centre, Kids Research, Children's Hospital at Westmead, Sydney, New South Wales, Australia
2Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney
3Yale School of Medicine 333 Cedar Street New Haven CT

Background: The Exome Aggregation Consortium (ExAC) recently published a dataset cataloguing frequency of genetic variation in each protein-coding gene throughout 60,706 exomes [1]. The scale of the dataset enabled calculation of ‘observed versus predicted’ genetic variation, and thus scores of genetic constraint to missense or loss-of-function (LoF) variants. Additionally, gene editing technologies are enabling global and systematic creation of cell and animal knock-outs respectively for protein-coding genes [2-6].

Aim: With the explosion in genomic testing and novel gene discovery, this project investigates the utility of genetic constraint scores and model organism phenotypic data, as tools to prioritise potential novel disease genes.

Method: ExAC scores for 17,878 protein-coding genes were extracted, and a list of 3,185 Mendelian disease genes with clinically relevant phenotypes was compiled, appended with available inheritance and phenotype information. Genes essential for mammalian life were identified through mouse knockout phenotype data extracted from the Mouse Genome Database (MGD). Data from three CRISPR screening studies was aggregated to compile a list of cell-essential genes.

Results and Discussion: Mendelian disease genes show great diversity in levels of genetic constraint, correlating with their inheritance pattern; for example, LoF constraint is lowest in recessive genes, and highest in X-linked genes. Overall, genetic constraint is a poor predictor of whether a gene is likely to be disease-causing. Conversely, manifestation of a severe animal phenotype correlates strongly with being a disease gene. Results suggest murine phenotype information is a better tool for disease gene prioritisation than scores of genetic constraint. Integration of ExAC and mouse phenotype data identified a subset of genes likely to cause non-viable human phenotypes when dysfunctional; potentially relevant to recurrent death in utero. Further analysis of genes’ cell and mouse phenotypes, genetic constraint, gene function, orthologs, and protein-protein interactions will be undertaken to better understand their relationship to pathogenicity.

DYNAMIN STRUCTURE AND FUNCTION HINGING ON RYNGOS

David A. Cardoso1, Mohammed K. Abdel-Hamid2, Adam McCluskey2, and Phillip J. Robinson1

1Cell Signalling Unit, Children's Medical Research Institute, The University of Sydney, Westmead, NSW, Australia
2School of Environmental and Life Sciences, Faculty of Science, University of Newcastle, NSW, Australia

Dynamins are GTPase enzymes responsible for performing the final scission of invaginated plasma membrane prior to the completion of endocytosis. Pharmacological targeting of dynamin in relevant mouse models has been shown to provide therapeutic relief for ailments as diverse as chronic kidney disease and epilepsy. We have generated a series of small molecule modulators (Ryngos) which ‘lock’ dynamin into a ‘ring’ oligomeric state that structurally differs from the ‘helical’ state required for endocytosis.

However, these compounds exhibit different activities on enzyme activity in vitro (Ryngo-1: mixed-mode / Ryngo-3: stimulation). Due to their chemical similarity, it can be surmised that these pharmacological agents share a common binding pocket. This study aims to establish the binding site of Ryngos to allow for targeted drug design and dissection of dynamin residues responsible for inhibition or stimulation of activity. Advanced computer modelling was employed and predicted lead compounds; Ryngo-1-23 and Ryngo-3-32, independently localised to, and differentially interacted with Hinge 1, located between middle domain and bundle-signalling element of dynamin.

A partial overlap of implicated residues between Ryngo-1-23 and Ryngo-3-32 suggests drug binding to different sub-regions of Hinge 1 may be capable of imparting different actions (stimulation/inhibition) on dynamin activity in vitro. To validate this model, mutagenesis of implicated Hinge 1 residues was carried out and resultant mutants characterised.

Enzymatic assays largely support these predictions (i.e. single mutations specifically lost drug action) as well as highlight a broader role for Hinge 1 in dynamin characteristics (e.g. activity, oligomerisation). To account for allosteric effects of mutation, a chemically dissimilar dynamin-targeting compound (Dynole-34-2) was employed and revealed loss of Ryngo action to be specific to Hinge 1. The data supports the proposed model of these compounds differentially interacting with a flexible hinge within dynamin, an exceptionally rare binding site for such pharmacological agents.
The Role of MBOAT7 in Hepatic Inflammation: Implications for Therapy

Paul Sebastian Pirie1,2, Mohammed Eslam1,2, and Jacob George1,2,3

1 Storr Liver Centre, Westmead Institute for Medical Research
2 University of Sydney
3 Department of Medicine, Westmead Hospital

Aim: To determine the role of MBOAT7 in hepatic inflammation.

Background: Non-alcoholic fatty liver disease (NAFLD) afflicts more than one third of the western world, paralleling the global burden of obesity and metabolic syndrome. MBOAT7 is a phospholipid modifying enzyme highly expressed in both liver and macrophages. It belongs to a remodelling pathway that converts arachidonic acid (AA) to phosphatidylinositol (PI). With no definitive pharmacological treatment of NAFLD, we report a novel role of MBOAT7 in hepatic inflammation, through its regulation of toll-like receptors (TLR) and inflammasome activation.

Methods: Adeno-associated virus vectors were delivered to the murine macrophage cell line RAW264.7 to assess the effects of MBOAT7 overexpression and knockdown on toll-like receptor (TLR) function. Cells will be treated with several TLR agonists, and analysed for inflammatory markers. Other cell lines and human data may be employed to investigate inflammasome complexes. A diet-induced NAFLD mouse model will be used to determine the role of MBOAT7 on liver injury.

Results: qPCR will analyse RNA for gene induction of important molecules involved in inflammation, eicosanoid regulation, and monocyte recruitment. ELISA will measure cytokine production following TLR activation. Western blot will assess protein markers involved in NF-κB signalling. The results of these experiments will be presented. In the mouse model of NAFLD, markers for liver steatosis, inflammation, and fibrosis will be examined.

Discussion: Previous evidence has shown that MBOAT7 expression is downregulated in steatohepatitis, and in response to TLR activation, implicating a role as a negative mediator of TLR signalling. We propose that MBOAT7 confers anti-inflammatory properties in macrophages by regulating TLR-mediated activation of inflammasomes. Increased MBOAT7 activity may attenuate macrophage inflammatory responses by converting AA to PI shifting the inflammatory milieu towards resolution. Our study hopes to elucidate the physiological role of MBOAT7 in the setting of steatohepatitis, which remains poorly understood.
Methyl unlock: RARRES1 demethylation as a novel therapeutic epigenetic target for liver fibrosis

Ali Bayoumi, Jacob George, Mohammed Eslam Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, NSW, Australia

Background: More than 90% of the liver related morbidity and mortality is a consequence of liver fibrosis with no available anti-fibrotic drugs. Hence, there is unmet clinical need to identify drugs that specifically target liver fibrosis, irrespective of its etiology. Hepatic stellate cells (HSCs) are the principal cells responsible for the elaboration of excess matrix during liver fibrosis and thus is at the nexus of efforts to identify novel drug targets. Enhanced stellate cell survival maintains fibrosis progression, while fibrosis resolution requires HSCs apoptosis or reprogramming to a quiescent state. Epigenetic regulation, particularly DNA methylation, might be involved in this activity-switching process in HSCs. Recently, using a genome-wide DNA methylation array we identified RARRES1, a retinoid response gene, to be hypermethylated in advanced liver fibrosis.

Aim: Investigate the therapeutic potential of targeting RARRES1 as an anti-fibrotic agent, and its downstream effects.

Methods: RARRES1 mRNA, protein and methylation levels were analyzed in 19 different tissues, primary liver cells, patients with NAFLD and in multiple mouse models of liver fibrosis. Direct effects of RARRES1 on HSCs were studied in vitro.

Results: Liver and particularly HSCs were identified as a major RARRES1 expressing liver cell type. RARRES1 transcript in patients with fatty liver disease were significantly lower compared to matched healthy controls (p<0.0001). Consistently, RARRES1 expression was lower in three well-established models of mouse liver fibrosis (P<0.03, for all). In contrast, methylation levels were significantly higher. RARRES1 overexpression abrogated collagen-1 mRNA expression by 74% in response to TGF-β signaling, a major fibrotic signals (p <0.03).

Conclusion: Our studies implicate RARRES1 as a novel epigenetic regulator of liver fibrosis via modulation of responses to multiple growth factor targets that regulate fibrosis. Targeted demethylation of RARRES1 may be a potential therapeutic option for the treatment of liver fibrosis.
ROLE OF HSD17B13 IN NON-ALCOHOLIC FATTY LIVER DISEASE

Mayada Metwally, Jacob George, Mohammed Eslam
Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, NSW, Australia

Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder in Australia, affecting about one-third of the population. NAFLD is a leading cause for liver cancer and transplantation, with no approved treatments. HSD17B13 is a novel lipid droplet-associated protein, polymorphisms of which associate with serum levels of aminotransferases; its function however is largely of unknown, particularly in NAFLD. We explored the role of HSD17B13 in NAFLD.

Methods: HSD17B13 expression was analyzed in 19 different tissues, in primary liver cells, patients with NAFLD and in multiple mouse models of liver fibrosis.

Results: Liver and particularly hepatocytes were identified as a major site and source of HSD17B13. HSD17B13 mRNA and protein expression was much higher in the livers of individuals with steatosis or NASH, than in the livers of healthy individuals. (P<0.0001). Higher HSD17B13 levels were observed in the livers of individuals with NASH, compared to those with steatosis. Consistently, HSD17B13 expression was significantly higher in three established and accepted models of liver fibrosis in mice, namely mice fed with a diet deficient in methionine and choline (MCD diet), carbon tetrachloride (CCl4), bile duct ligation (BDL, 2 fold, P: <0.0001).

Conclusion: HSD17B13 is a novel-lipid droplet gene of unknown function and our data suggests that it is involved in NAFLD pathogenesis that may be a promising therapeutic target.
TARGETING GLUCOSE UTILIZATION AS A THERAPEUTIC APPROACH IN CHOLANGIOPATHIES

Afaf Elattar, Mahmoud Karimi Azardaryany, Mehdi Ramezani-Moghadam, Jacob George, Saeed Esmaili.

Storr Liver Centre, The Westmead Institute for medical research, Westmead Hospital, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia.

Introduction & Aim: Chronic liver injury can lead to cholangiopathies, the ductular reaction and biliary fibrosis. In response to liver damage, bipotential hepatic progenitor cells (HPCs) differentiate into cholangiocytes or hepatocytes. Moreover, activation of macrophages has been demonstrated to contribute to HPCs fate decisions. Our aim was to investigate the signaling pathways that contribute to HPCs proliferation and bile duct cell differentiation.

Methodology: Male C57BL6 mice were fed a normal chow or a diet containing 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC, 0.1%wt./wt.) to induce ductular proliferation. DDC diet fed mice were injected with a modulator of glucose utilization. Relative quantitative mRNA expression of inflammatory and progenitor cell markers was determined. The expression and localization of HPCs marker (SOX9) and a bile duct maker (CK19) was determined by immunofluorescence staining. In in vitro studies, we used MMNK1 and HUCCT-1 bile duct cell lines treated with and without the glucose modulator and cell proliferation was measured by BrdU assay.

Results: The presence of a ductular reaction was detected by H&E staining in the liver of mice fed the DDC diet. qPCR indicated higher expression of IL1b (2.8-fold, P<0.005), TNFα (1.2-fold, P<0.02) and HPCs markers SOX9 (1.04-fold, P<0.02), EPCAM (9.6-fold, P<0.00001), as well as bile duct cell markers CK19 (3.8-fold, P<0.00001) in DDC diet fed mice. Treating DDC fed mice with the glucose modulator led to downregulation of mRNA expression of IL-1b (30% P=0.04), TNFα (20% P<0.01), SOX-9(84% P<0.01), EPCAM (40%, P<0.01) and CK19 (44%, P<0.005). Immunostaining confirmed the reduction in SOX9 and CK19 levels in DDC diet fed mice treated with the glucose modulator. In agreement, treatment of both cell lines led to a significant reduction in cell proliferation.

Conclusion: Modulating glucose utilization regulates the proliferation of HPCs with a subsequent reduction in the ductular reaction, liver inflammation, and fibrosis.
Analysing recombinant antibodies from healthy controls and patients with central nervous system demyelinating disorders

Philomena Colagiuri1, Sudarshini Ramanathan1, Nese Sinmaz1, Fiona Tea1, Tina Nguyen1, Fiona Lee1, Vera Merheb1, Alicia Zou1, Joseph A. Lopez1, Deborah Lin1, Russel C. Dale1, Fabienne Brilot-Turville1

1Brain Autoimmunity Lab, Kids Neuroscience Centre, Kids Research, The Children’s Hospital at Westmead, Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney, Sydney, Australia

Background: Myelin oligodendrocyte protein (MOG) is a transmembrane protein expressed on the surface of myelin and oligodendrocytes in the central nervous system (CNS). This protein is a candidate target in autoimmune demyelinating conditions due to the extracellular location of its Ig-like domain which is accessible to antibody binding. Autoantibodies against MOG have been detected in a subset of patients with autoimmune demyelinating conditions, but little is known about the role of these antibodies in demyelination. Recombinant monoclonal antibodies (rAbs) from single B cells are valuable for investigating the specificity of antibodies and the diversity of the immunoglobulin (Ig) repertoire. They can also be used in pathogenic studies to determine anti-MOG antibody-mediated mechanisms of demyelination.

Aims: The aim of this study is to optimise the generation of human rAbs from healthy controls and, depending on availability, from patients with CNS demyelinating disorders. The immunoreactivity of control rAbs will be analysed against MOG to establish a threshold for MOG-specificity, from which the prevalence of anti-MOG antibodies in the Ig repertoire of demyelinating patients can be determined.

Methods: Plasmablasts are isolated from the serum of controls and patients by fluorescence-activated cell sorting (FACS), and the Ig heavy and light chain variable regions (VH and VL) that encode the antibody specificity are obtained and amplified by single cell RT-PCR. Cognate VH and VL DNA are cloned in eukaryotic expression vectors, sequenced and then co-transfected into human embryonic kidney cells to produce human rAbs with the same specificity that would occur in vivo. The concentration of rAbs is quantified by ELISA and the specificity of rAbs to MOG is assessed in a flow cytometry live cell-based assay.

Results: The single-cell cloning and expression of rAbs is currently being optimised and the results of the live cell-based assay will be presented.
Kupffer Cells Coordinate Interferon-lambda Driven Hepatic Inflammation

Scott A Read, Ratna Wijaya, Mehdi Ramezani-Moghadam, Christophe Macri, Ee Shan Pang, Jo Pooley, Christopher Liddle, David Booth, Meredith O’Keeffe, Jacob George, Golo Ahlenstiel

Interferon lambdas (IFN-λs) are antiviral cytokines that are a key component of barrier surface immunity. While they share signaling pathways with type I IFNs, they act primarily on the hepatic, gastrointestinal, and pulmonary epithelium, as well as select immune cells. IFN-λs demonstrate potent activity against numerous viral and non-viral pathogens but have also become associated with inflammation in viral (chronic hepatitis B and C) and non-viral (non-alcoholic fatty liver disease [NAFLD]) liver disease.

Despite this strong association, surprisingly little is known about how IFN-λ drives this response. We have demonstrated that monocytes are unresponsive to IFN-λs while monocyte derived macrophages (MDMs) and Kupffer cells are highly sensitive. Immune cells central to the innate (natural killer (NK) cell) and adaptive (B and T cells) immune responses are unresponsive to IFN-λs, suggesting that macrophages can coordinate local inflammation in response to chronic IFN-λ3 production.

Monocyte derived macrophages (MDMs) were generated from CD14+ monocytes using GM-CSF alone, or in combination with IFN-λ3 and Toll-like receptor ligands. IFN-λ3 potently activated MDMs, up-regulating chemokine, inflammatory cytokine, antigen presentation and co-stimulatory genes sets. Furthermore, incubation with IFN-λ3 exacerbated the inflammatory response to TLR ligands LPS (TLR4), polyI:C (TLR) and zymosan (TLR2). Functionally, macrophages differentiated in the presence of IFN-λ3 stimulated T, NK cell chemotaxis, increased NK cell cytotoxicity and stimulated apoptosis of hepatitis C virus (JFH-1 strain) infected hepatocytes. To examine the effect of IFN-λ in vivo, WT and IFNLR1-/- mice were injected with 50 µg of high molecular weight polyI: C or saline control, and livers were harvested after 4 h. Interestingly, hepatic infiltration of immune cells was significantly impaired in IFNLR1-/- mice following polyI: C treatment.

Together, our data suggests that Kupffer cells are key mediators of the hepatic IFN-λ response, stimulating immune cell chemotaxis and activation. These data provide relevant new mechanisms, and thus treatment options for chronic liver diseases.
Investigating the role of CD4+ T cells in D2R-mediated movement and psychiatric disorders

Deborah Lin1, Deepti Pilli1, Sudarshini Ramanathan1, Fiona Tea1, Fiona Lee1, Vera Merheb1, Alicia Zou1, Joseph A. Lopez1, Philomena Colagiuri1, Russell C. Dale2, Fabienne Brilot1

1Brain Autoimmunity Lab, Kids Neuroscience Centre, Kids Research, The Children's Hospital at Westmead, Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney
2Clinical Neuroimmunology, Kids Neuroscience Centre, Kids Research, The Children's Hospital at Westmead, Discipline of Child and Adolescent Health, Sydney Medical School, University of Sydney

**Aim:** To assess CD4+ T cell activation against and the functional immune response to the dopamine-2 receptor (D2R).

**Background:** Antibodies directed against CNS proteins have been identified in a subset of patients with movement and psychiatric disorders. Investigation into the involvement of B cells and their secreted antibodies have produced targeted immunotherapies with better clinical outcomes, while aberrant CD4+ T cell responses have also recently been implicated as key drivers in pathogenesis. As naïve B cells require activation by CD4+ T cells prior to antibody production, it is likely that both arms of the adaptive immune response are involved. Additionally, the dopaminergic system is central to the control of movement and behaviour, and D2R has been identified as an immune target in movement and psychiatric disorders. Given this, we hypothesized that patients with a putative autoimmune component possess autoreactive CD4+ T cells specific for D2R, and harbour serum antibodies directed against this protein.

**Methods:** (1) to detect antibodies against D2R, we performed flow-cytometry cell-based assays on human embryonic kidney cells (HEK293). These were transfected to express the surface protein and incubated with serum samples obtained from patients and controls (2) Whole blood was cultured with D2R peptide pools for 44 hours in a flow cytometry-based OX40 assay. As CD4+ T cells simultaneously express CD25 and CD134 (OX40) following interaction with their cognate antigens, we screened for activated D2R-specific T cells by measuring upregulation of these markers. Supernatants collected from these whole blood cultures were then analysed to determine the nature of the cytokine response using a LEGENDplex™ Human TH Cytokine Panel.

**Results:** the results of these experiments will be presented. Discussion: further characterization of the involvement of autoreactive CD4+ T cells will broaden our understanding of the autoimmune response in movement and psychiatric disorders and help develop novel therapies.
Immune cell subsets in human intestinal tissue are altered by Crohn’s disease

Chloe M Doyle, Jake W Rhodes, Martijn Gosselink, Anthony L Cunningham, Golo Ahlenstiel, Grahame Ctercteko, Scott N Byrne and Andrew N Harman

Centre for Virus Research, The Westmead Institute of Medical Research, Westmead NSW 2145

INTRODUCTION: Crohn’s Disease (CD) is a devastating chronic condition characterised by auto-inflammation in the gastrointestinal tract, which most commonly affects the terminal ileum and proximal colon. CD is still poorly understood and a key gap in our knowledge is the definition of immune cell subsets that drive recurrence.

AIMS: 1) To compare the subsets of key immune cells in healthy and Crohn’s affected ileum including: mononuclear phagocytes (MNP), innate lymphoid cells (ILCs), mucosal-associated invariant T (MAIT) cells and natural killer (NK) cells. 2) To determine the expression of functional receptors on mononuclear phagocytes in healthy and Crohn’s affected ileum.

METHODS: We obtained healthy and CD affected tissue from patients undergoing colorectal surgery at Westmead hospital. We investigated the immune cell subsets present in these tissues by flow cytometry after enzymatic tissue digestion. We then determined the differential expression of functional receptors on MNPs.

RESULTS: We optimised tissue digestion protocols for the isolation of immune cells from intestinal tissue. We next designed a multiparameter flow cytometry panel to identify and characterise intestinal MNPs. We defined MNPs subsets in healthy and CD affected tissue and found that the frequency of cells known to infiltrate inflamed tissue increased in Crohn’s affected tissue. We also investigated the expression of functional receptors on each MNP subset and found that chemokine receptors associated with leukocyte migration are expressed more highly on MNPs derived from Crohn’s tissue. Finally, we modified our tissue digestion protocols for the isolation of intestinal ILCs. Flow cytometry was again used to define and compare all currently known subsets of ILCs in Crohn’s affected tissue. We found that MAIT cells and ILC1 are reduced in Crohn’s affected tissue while NKp44+ ILC3s are increased. These results provide insight into the role of immune cells in CD and may offer new therapeutic targets for intervention.
Recapitulation of the adult HSC methylome in progeny cells: Implications for disease

Ong, Lawrence1,2, Parnell, Grant1, Stewart, Graeme1,2, Booth, David1  
1Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, The University of Sydney, New South Wales, Australia  
2Department of Clinical Immunology and Allergy, Westmead Hospital, Westmead, New South Wales, Australia

**Aims:** Differences in immune response between individuals is driven in part by epigenetic factors. To understand the contribution of DNA methylation to these differences, we have investigated the role of haematopoietic stem cell methylation in driving variation in daughter cell differentiation and state.

**Methods:** Haematopoietic stem cells (CD34+) and the cells derived from them, monocytes (CD14+) and natural killer cells (CD56+), were isolated from peripheral blood of 11 healthy individuals and subjected to modified reduced representation bisulfite sequencing. DNA methylation was profiled and compared at CpG islands.

**Results:** DNA methylation state is almost entirely recapitulated between progenitor and progeny cells. Fewer differences in DNA methylation were detected among these immune cells than between them and buccal mucosa. Cell subset differences in methylation occurred near genes important in cell lineage specific maturation and function. Methylation differences between individuals at specific CpG islands were generally small. The genes in cis with the CpG islands that varied most between individuals across subsets did not indicate specific biological processes.

**Conclusions:** Overall, this study suggests that the predominant DNA methylation setting in haematopoietic stem cells is transmitted to progeny cells, with differences between cell subsets and between individuals that are likely to be important in immune cell development and variation in response to pathogens, drug response and disease. Our findings provide a plausible mechanism by which genetic and environmental factors may contribute to the development of disease via unfavourable DNA methylation settings, particularly in haematopoietic stem cells.
Metabolic Profile of Peripheral Blood Mononuclear Cells in Patients with Low and High Risk Infections

Velma Herwanto1, Ya Wang1, Maryam Shojaei1, Kevin Lai2, Amith Shetty2, Benjamin Tang1,3, Anthony McLean3, David Booth1
1Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, Westmead, NSW
2Department of Emergency Medicine, Westmead Hospital, Westmead, NSW
3Department of Intensive Care Medicine, Nepean Hospital, Kingswood, NSW

Background: Sepsis (infection complicated by organ dysfunction) is associated with a dysregulated immune response. The mechanism of immune dysregulation is not well understood but impaired energy metabolism in circulating leukocytes is thought to play a role. Here, to gain mechanistic insight into immune dysregulation, we measured leukocyte metabolism in infected patients who were at risk of developing sepsis.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of healthy controls (n=7), low-risk (n = 5) and high-risk infection patients (n = 8). Low risk infection was defined as clinical suspicion of infection with a normal qSOFA score (qSOFA is a prediction index for the subsequent development of sepsis). High risk infection was defined as clinical suspicion of infection plus an increased qSOFA score. The PBMC metabolic profiles was measured by Agilent Seahorse XF analyser while total cellular reactive oxygen species (ROS) was measured by DCFDA Cellular ROS Detection Assay and quantified by flow cytometry.

Results and Discussion: The mitochondrial respiration in PBMC was significantly reduced in high risk infection patients as compared to healthy controls, especially in reserve respiratory capacity. There was also a trend towards reduced mitochondrial respiration in low risk infection patients as compared to healthy controls. Notably, this reduced mitochondrial respiration was not accompanied by a compensatory increase in glycolysis. No changes were detected in total ROS production across the three groups, thereby excluding oxidative stress as a potential cause of reduced mitochondrial respiration in PBMC.

Conclusion: The metabolic switch to glycolysis, a normal compensatory response to mitochondrial suppression in infection, was not observed in the circulating leukocytes of patients with a higher risk for sepsis. This novel finding reveals an unexpected level of complexity in the metabolic dysregulation of circulating leukocytes, thereby opening up a potential new avenue for investigation.
T Helper Cell Differentiation in Children with Food Allergy

Christopher Ong1, Dr. Peter Hsu1, Dr. Melanie Wong1
1Allergy and Immunology, Kids Research, Westmead Children's Hospital, University of Sydney

Food allergy in children is estimated to cost each child USD $4184 per year. Higher rates of social isolation, anxiety and depression have been linked with children with food allergy. Anaphylaxis is the most dangerous form of food allergy, with its incidence rapidly increasing in Western countries. T helper cells play a key part in the adaptive immune response to food allergy and are composed of several subsets. Our lab has recently established that children with food allergy have a distinct imbalance of T helper cell subsets. It is still unclear as to how this imbalance arises.

We hypothesise that there may be an intrinsic defect in the differentiation of naïve T cells into T helper cells, which causes T helper cell imbalance. We aim to establish the differentiation of naïve T cells into Th1, Th2, Th17, Treg and Tr1 subtypes in vitro and determine whether the phenotype and cytokine profile of these T helper cells may be altered in food allergy. To investigate this, Ficoll-Paque peripheral blood mononuclear cell separation was performed.

Twenty samples were collected; 10 from healthy children and 10 from children with food allergy. Cells will then be sorted for monocytes and naïve T cells. The naïve T cell fraction will be cultured in various T helper cell polarising conditions for 7 days, before phenotypic and cytokine analysis via flow cytometry.

Since antigen presenting cells are the main activator of naïve T cells, we hypothesise that they may also be defective in children with food allergy. Therefore, we aim to assess whether the cytokine profile of monocytes children with food allergy is defective in comparison to healthy controls. Sorted monocytes will undergo 4-hour LPS stimulation before their cytokine profile is assessed via flow cytometry. The results of these experiments will be presented at this symposium.
Effect of Vitamin D on Effector and Regulatory Immune Cell Populations in Children with Eczema

Cynthia Yau1, Peter Hsu1, Dianne Campbell1
1Allergy and Immunology, Kids Research, The Children's Hospital at Westmead, The University of Sydney

Background: The prevalence of eczema, or atopic dermatitis (AD), continues to rise, currently affecting around 20% of children worldwide. AD places a significant burden on children and their family, and a lack of understanding in the aetiology and disease mechanism of AD means there are no current cures or effective preventative measures. Immune dysregulation, potentially due to an imbalance of effector and regulatory cells may be a contributor in AD pathogenesis. Recently, studies have revealed an inverse correlation between serum vitamin D levels and AD severity in children. Since vitamin D can exert immunomodulatory effects, it has been proposed as a potential treatment for AD.

Aims: To investigate the phenotype and homing patterns of effector cells Th1, Th2, Th9, Th17 and ILC, and regulatory cells Treg, Tr1 and Breg in healthy and AD-affected children, and examine changes in AD-affected children before and after vitamin D supplementation treatment.

Methods: 26 children aged between 2-12 years diagnosed with moderate to severe AD were enrolled in a randomised, double-blinded, placebo-controlled trial. Daily administration of 1000IU of oral vitamin D was carried out for three months. Blood was collected before and after the treatment for comparison. 12 age-matched healthy controls were also included in the study. Surface and intracellular staining was performed on isolated peripheral blood mononuclear cells. Data was subsequently analysed using flow cytometry.

Results and Discussion: To date, cells from at least 20 blood samples have been stained and some data have been acquired. The results of these experiments will be presented. However, more samples will need to be processed before any significant conclusion may be drawn. Results from this study should help to clarify the immunological mechanisms of AD and assess whether vitamin D is a plausible alternative treatment option for children with AD.
Contemporary epidemiology of candidaemia and a risk prediction model for overall mortality: a prospective multicentre study


1 Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, New South Wales Health Pathology, Westmead Hospital, Westmead, Sydney, NSW.
2 The University of Sydney Marie Bashir Institute for Infectious Diseases and Biosecurity, Sydney, NSW and the Department of Infectious Diseases, Westmead Hospital, Westmead, NSW.
3 Department of Microbiology and Infectious Diseases, St. Vincent's Hospital, Sydney, NSW.
4 Eastern Health Clinical School, Monash University, Melbourne, Victoria
5 School of Mathematics and Statistics, University of NSW, Sydney, NSW.
6 Westmead Institute for Medical Research, Westmead, NSW.
7 Department of Infectious Diseases and Microbiology, Canberra Hospital, Australian National University Medical School, Canberra, ACT.
8 Department of Infectious Diseases, Royal Adelaide Hospital, Adelaide, SA.
9 Infection Management Services, Princess Alexandra Hospital, Brisbane, QLD.
10 Department of Intensive Care, Royal Melbourne Hospital, Melbourne, VIC.
11 National Mycology Reference Centre, SA Pathology, Adelaide, SA
12 National Centre for Clinical Excellence on Emerging Drugs of Concern (NCCRED), National Drug and Alcohol Research Centre (NDARC), University of New South Wales, Sydney, Australia
13 Department of Infectious Diseases, Royal Brisbane and Women's Hospital, School of Medicine, University of Queensland, Brisbane, QLD.
14 Department of Infectious Diseases and Microbiology, New South Wales Health Pathology, Royal Prince Alfred Hospital, Sydney, NSW.
15 Department of Infectious Diseases, Peter MacCallum Cancer Centre, National Centre for Infections in Cancer, Melbourne, VIC.

Objectives: To describe current epidemiology of candidaemia in Australia, analyse predictors of 30-day all-cause mortality, and develop and validate a mortality-based risk stratification score.

Methods: Adults with candidaemia were prospectively studied over 12 months (eight institutions). Clinical and laboratory variables at time of blood culture-positivity were analysed by multivariate analysis for association with 30-day all-cause mortality. A prediction score for mortality was examined by area under the receiver operator characteristic curve, with a historical data set used for validation.

Results: The median age of 133 patients with candidaemia was 62 years; 57 (43%) were female. Underlying haematologic malignancy was present in 20 (15%), and solid organ malignancy in 25 (19%) patients; 55 (41%) were in an intensive care unit (ICU). Non-albicans Candida spp. accounted for 58% of cases. A gastrointestinal or unknown source had a higher mortality than candidaemia with an intravascular or genitourinary source (p<0.01). All-cause 30-day mortality was 31%. A risk prediction score based on age >65 years, ICU admission, organ failure, preceding surgery within 30 days, haematological malignancy, source of candidaemia and antibiotic therapy for ≥10 days stratified patients into <20% or ≥20% predicted mortality. The model performed well when tested on a historical dataset (n=741).

Conclusions: Mortality from candidaemia remains high. A simple prediction score stratifying patients with candidaemia into <20% and ≥20% 30-day mortality is presented. This model uses information available at time of positive blood culture and is easy to implement via a paper-based questionnaire. Further validation of this model is warranted.
Comparison Study Between Hepatitis B Virus Genotypes C and D to Understand Liver Cancer

DISHEN (Corey) CHEN 1, 2, Anis Khan 1, 2, Enoch Tay 1, 2, Mark W Douglas 1, 2, Rifqiyah Nur Umami 1, 2, Sakthi Priya 1, 2.

1 STORR Liver Unit (STU), The Westmead Millennium Institute for Medical Research
2 The University of Sydney at Westmead Hospital

Aim: To compare the viral kinetics between genotype C and D and link the differences with interaction between SMC5/6 complex and HBxAg.

Background: There are total of 10 different HBV genotypes of which genotype C is highly associate with liver cancer and genotype D is more related to asymptomatic carrier. It is novel to discover the rationale behind such different clinical outcomes between the two genotypes. Firstly, the rationale will be addressed via viral kinetics comparison. Secondly, the interaction between HBx (viral transactivator protein) and SMC5/6 (host anti-viral protein complex) will be demonstrated in both genotypes to link it with the different clinical outcome between the two genotypes.

Methods: Viral expression clone of both HBV genotypes C and D were transfected into Huh7 cells. Transfected Huh7 cells were harvested at seven post-transfection time points (4hrs to 72hrs). Viral antigens levels were measured to compare the viral expression rate between the two genotypes. Intracellular HBV DNA level were also measured through qPCR to compare the viral genome replication rate between the two genotypes.

Result: HBV genotype D transfected Huh7 cells secrete more viral antigens (surface and "e" antigen) compare to HBV genotype C (P<0.05). This suggest that during the initial stage of viral infection, genotype D is able to express more viral protein in a faster pace compare to genotype C. Viral DNA is also higher in HBV genotype D.

Discussion & Conclusion: It is demonstrated that the HBV genotype D is able to express viral protein more effectively compare to genotype C. Based on the current result, it is proposed that due to the higher viral antigens expression in HBV genotype D, the host immune and inflammatory responses are triggered more effectively, which enhances the eradication of HBV particles to achieve asymptomatic carrier.
The oral microbiome beyond bacteria – what role do fungi play in childhood oral health?

Hanieh Salehi

**Background:** Dental caries (dental decay) is the most preventable chronic disease worldwide. Affecting 60-90% of school-aged children and nearly all adults worldwide. Caries has significant economic burden on our healthcare system and individuals. Caries can cause pain, reduction in quality of life, speech and learning problems and affect school performance in children.

Previous studies were mainly focused on bacteria role on dental caries. However, with the advancement in metagenomic and bioinformatics fields, it is crucial to look beyond just bacteria and investigate the whole oral microbiome in more detail, including the potential role of fungi in the caries initiation and progression.

**Aims:** of this study are firstly determine the diversity and composition of fungi in children with and without dental caries, using ITS region from rRNA of fungal species. And secondly to identify the relationship between fungi and bacteria in children with and without dental caries, by combining previously generated 16s rRNA data with ITS region data from fungi.

**Methods:** This study is take advantage of the current longitudinal study using classic twin cohort model form 400 individual's oral biofilm hard and soft tissue samples from 3-time points from across Australia. In this study various bioinformatic methods applied for sequence, statistical and data analysis, differential abundance, taxonomic and phylogenetic identification. Including Illumina Sequencing, DADA2 pipeline, UNITE and ISHAM-ITS database, R Phyloseq package, Qiime, Deseq2, DIABLO and Procrustes.

**Results:** The results of these study will be presented, however, is estimated that it will determine the overall diversity and abundance of species in oral microbiome and identify the relationship between fungi and bacteria in children with and without dental caries. This potentially improve our understanding of the pathogenesis of dental caries and may lead to improvement in prevention and detection methods in future.
“Herpes Simplex Virus Relay”- Characterising the role of novel CD11c expressing epidermal DCs during HSV infection

Jason J. Herbert, Naomi R. Truong, Konrad L. Feng, Hafsa Rana, Jacinta B. Smith, Kirstie M. Bertram, Andrew N. Harman and Anthony L. Cunningham (Westmead Institute for Medical Research – Centre for Virus Research & University of Sydney)

Introduction: Herpes simplex virus (HSV) causes oral and genital herpes. It affects approximately 70% of Australians and can cause serious complications. Langerhans cells (LCs) in the epidermis of skin are the first immune cell to interact with HSV and initiate an immune response by migrating to the dermis and establishing a virus “relay”. Recently, our lab has identified a novel epidermal dendritic cell (epiDC) in healthy human skin that is distinct from LCs, CD11c epiDCs. The role of CD11c epiDCs in HSV infection is currently unknown.

Aim: To compare the response of CD11c epiDCs to HSV infection with that of LCs, including levels of viral uptake, productive (replicative) infection and apoptosis.

Methodology: Epidermal cells were isolated from human abdominal skin, infected with HSV and analysed utilising flow cytometry for markers of infection and apoptosis. In other experiments, foreskin explants were topically infected with HSV and virus-cell interactions were examined in situ via immunofluorescence microscopy using a novel in situ hybridisation technique known as RNAscope.

Results: CD11c epiDCs took up significantly more HSV than LCs. A proportion of both cell types underwent apoptosis, which tended to be greater in LCs. The HSV immediate early protein ICP27 (a marker of productive infection) was expressed similarly in LCs and CD11c epiDCs. In foreskin explants, LCs and CD11c epiDCs were distributed throughout the epidermis. Preliminary data shows interactions of HSV with LCs and with CD11c epiDCs.

Discussion/future studies: The interactions of HSV with LCs and CD11c epiDCs will be quantified and levels of infection and apoptosis will be confirmed in the explants. Determining the role of CD11c epiDCs in response to HSV is essential to understand the complex pathways of HSV relay that occur during infection, resulting in an immune response. These studies may define potential DC targets for future vaccine adjuvants.
Identifying and Characterising T cell Subsets in Anogenital Tissues as Target Cells for HIV

Thomas O'Neil 1, 2, Kirstie Bertram 1, 2, Tony Cunningham 1, 2 & Andrew Harman 1, 2
1University of Sydney
2Westmead Institute for Medical Research

Introduction: While there have been great advances in the understanding of HIV pathogenesis, there are significant gaps in the literature with regards to transmission and early stages of infection. More recently, there have been large steps in advancing our understanding of tissue CD4 T cell subsets and their correct definition. It is therefore our aim to now correctly define the precise subsets of CD4 T cells that are present in the human anogenital tissues that form the portals of HIV entry.

Methods: Using discarded human anogenital tissues derived from surgery at surrounding hospitals, we will use optimised tissue digestion methods and report on these proportions in both the dermal and epidermal compartments using multi-parameter flow cytometry. Additionally, an optimised in situ hybridisation technique which utilises the flow cytometer will be used to identify infection among T cells isolated from these tissues following ex vivo infection with HIV.

Results: Dispase is an enzyme used to digest the basement membrane between the epidermis and the dermis, and is vital to this study. This enzyme is known to cleave cell surface receptors, including the distinguishing T cell subset markers CD4, CD8 and CD69. We have identified antibody clones that can be used after dispase treatment. Additional optimisation steps will be presented showing the validation of the use of antibodies that bind the transcription factors PU.1 and AHR, which define resting Th9 and Th22 subsets respectively. Preliminary reports of T cell proportions in anogenital tissues will be presented.

Discussion: This study will precisely define the subsets of CD4 T cells present in actual human tissues where transmission occurs, and specify which subsets are the first to become infected with the virus. This will be important in deepening our understanding of the mechanism of transmission and also in defining which subsets to target for vaccine design.
HIV and the Colorectal Mucosa - Investigating the Early Interactions of HIV with Mucosal Target Cells In Situ

Di Yuan1 2, Heeva Baharlou1, Toby Plasto1 2, Emma Wanicek3, Melissa Churchill3, Jacob Estes4, Anthony Cunningham2, Andrew Harman2

School of Medicine, Sydney University, Sydney, Australia
Centre for Virus Research, The Westmead Institute for Medical Research, Sydney, Australia
Human Biosciences, RMIT, Melbourne, Australia
Vaccine and Gene Therapy Institute, OR, USA

There is no vaccine for HIV. Antiretroviral therapy has helped reduce transmission rates, but alone is not enough to combat this significant global health issue. As such, we need to develop strategies to block transmission of the virus to complement current therapies. This requires an in depth understanding of early viral pathogenesis across the human anogenital tract, of which there is limited data.

In this study we infected human colorectal explant tissues and have performed an extensive analysis of HIV spread within the colorectal mucosa within minutes to hours post infection. To do this we have combined a new in situ hybridisation technology called RNAscope, with highly multiplexed microscopy to compare HIV uptake and transfer kinetics across multiple known HIV target cells including Dendritic Cells (DC), Macrophages and T cells, all in a single tissue section. Our results show that both DCs and CD4 T cells are able to take up HIV rapidly, within 30min post-infection, however macrophage involvement does not occur until 2h post-infection.

Furthermore, we have observed HIV in association with DC-T cell conjugates with the frequency of these contacts increasing with time. We devised several image analysis algorithms which show that in fact the majority of HIV resides within DC-T cell conjugates within the mucosa early post-infection. Although previously hypothesised, to our knowledge this is the first demonstration of DC involvement in early viral transfer to T cells within the mucosa.

We have also examined HIV entry into rectal lymphoid aggregates which are a known site of HIV latency, but unstudied in the context of HIV transmission. Our results show that within just 30min, HIV is able to enter both the T and B cell zones of these structures, associating with CD4 T cells and follicular DCs respectively, potentially indicating rapid seeding of the viral reservoir.
Delineating the role of cellular turnover rates in maintaining the latent HIV reservoir during effective antiretroviral therapy

Dai YH1, Morcilla V1, Horsburgh BA1, Fisher K1, Wright A1, Deeks SG2, Hunt PW2, Bacchus-Souffan C2, Palmer S1
1Centre for Virus Research, The Westmead Institute for Medical Research, The University of Sydney, Westmead, Australia; 2Department of Medicine, University of California San Francisco, San Francisco, USA

Aims: During antiretroviral therapy for HIV infection, genetically intact and replication-competent provirus persists in latently infected cells. These genetically intact proviruses will contribute to rebound viremia if antiretroviral therapy is terminated. Several approaches to eliminate this persistent reservoir of HIV-1 have been proposed. However, to succeed, a more thorough understanding of the cellular mechanisms that are primarily responsible for maintaining persistent replication-competent HIV-1 is required. Therefore, this study will investigate whether cellular turnover rates and half-lives affect the level of replication-competent proviruses within specific cells.

Methods: Using established labelling techniques, CD4+ T cell turnover rates were measured based on in vivo incorporation of deuterium into genomic DNA in sort purified HLADR- CD4+ T naïve (TN), stem cell memory (TSCM), central memory (TCM), transitional memory (TTM), effector memory (TEM), and effector (TEMRA) cells. These cells were sorted after oral administration of deuterated water to HIV-infected participants on effective antiretroviral therapy (n=4; 2 treated during acute and 2 treated during chronic infection). To investigate the level of replication-competent HIV within these cell subsets, we employed the FLIPS assay which uses LTR-specific primers to amplify proviruses at limiting dilution followed by next-generation sequencing. Proviruses were characterized as defective or genetically intact.

Results and Discussion: We predict that the proposed studies will show that more differentiated cells, such as TEM cells, contain the highest levels of intact proviruses and the rapid turnover rate of these cells contributes to the expansion of genetically intact proviruses. As a result, our study will deepen our understanding of the cellular mechanisms which contribute to and maintain the latent HIV reservoir.
High-throughput analysis of multidimensional microscopy to visualise HIV and its target cell interactions in situ

Nicolas Canete12, Heeva Baharlou12, Jasmine Yuan12, Anthony Cunningham12, Andrew Harman12 and Ellis Patrick23

School of Medicine, The University of Sydney, Sydney, NSW, Australia
The Westmead Institute for Medical Research, Sydney, NSW, Australia
School of Mathematics and Statistics, The University of Sydney, Sydney, NSW, Australia

Human Immunodeficiency Virus (HIV) is almost exclusively transmitted via sexual intercourse. Transmission via anal intercourse is 10-30 times more efficient than vaginal intercourse and the predominate mode of transmission in Australia.

However, due to technological limitations there are still major gaps in understanding of how HIV is transmitted across the anorectum. Microscopy is a powerful experimental technique that provides spatial information in the tissue environment, including cellular interactions, localisation, and morphology. The key HIV target cells are mononuclear phagocytes (dendritic cells and macrophages) and CD4 T lymphocytes.

In recent years these cell types have been shown to exist in multiple functionally distinct subsets that require multiple surface markers for accurate definition which has precluded the use of conventional fluorescence microscopy to study these cells in situ due its limited parameter definition. Furthermore, fluorescence microscopy is not sensitive enough to visualise single virion within tissue and multiple rounds of HIV replication are required meaning that the early HIV-target cell interactions have not been possible to establish in tissue.

Recently, multidimensional imaging methods such as cyclic immunofluorescence and imaging mass cytometry have been developed that allow multiple cellular markers to be identified, facilitating the analysis of diverse cellular phenotypes. However, there are currently no standard pipelines that are flexible enough to 1) handle the many exciting single-cell orientated hypotheses that are now possible with image data obtained through multidimensional microscopy or 2) overcome the many technical challenges associated with these imaging techniques.

Here we present a preliminary novel high-throughput pipeline which extracts single-cell data from multidimensional microscopy images. This pipeline will be presented in the context of HIV transmission across colorectal tissue.
Full-length sequencing of HIV proviruses in HIV-HBV co-infected individuals from Thailand

Xiao Qian Wang1,2, Bethany A. Horsburgh1,2, Katie Fisher1,2, Jennifer M. Zerbato3, Jennifer Audsley3, Anchalee Avihingsanon4, Julia Stout3, Sharon R. Lewin3,5, and Sarah Palmer1,2
1The Westmead Institute for Medical Research
2University of Sydney
3The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital
4HIV-NAT, Thai Red Cross AIDS Research Center (TRCARC)
5Department of Infectious Diseases, Alfred Hospital and Monash University

Background and Aims: HIV-hepatitis B virus (HBV) co-infected individuals experience higher rates of liver disease than mono-infected individuals. Previous studies have found that HIV co-infection can impact the natural course of HBV infection, but the reverse has not been confirmed. We aimed to determine the frequency of intact provirus in HIV-HBV co-infected individuals prior to ART initiation and whether this frequency was associated with any clinical parameters.

Methods: HIV-HBV co-infected individuals naïve to ART were recruited in Thailand as part of a prospective observational cohort study (n=39). Single near full-length HIV subtype AE proviruses were amplified using Full-Length Individual Proviral Sequencing (FLIPS) assay and sequenced using Next Generation Sequencing. Proviruses were then characterised as defective or genetically intact, and quantified to determine the proportion of infected cells.

Results: To date, a total of 522 HIV sequences have been sequenced and analysed from 17 individuals. When all sequences were pooled, 32.9% of HIV proviruses were genetically intact. These intact sequences were genetically unique and had genetic diversity ranging from 0.2 to 1.7%. Extremely large variation in the proportion of genetically intact HIV proviruses were observed between participants (7%-66%), which did not correlate to any clinical or laboratory parameters including plasma HBV DNA or HIV RNA, HBeAg status and ALT levels. The proportions of genetically intact provirus from these untreated participants were much greater than those found in a different cohort of participants on suppressive ART previously analysed by our laboratory (genetically intact: 1-10%).

Discussion: Genetically unique and intact HIV proviral sequences were commonly identified in untreated HIV-HBV co-infected participants. The frequency of intact virus was far higher than previous studies of on-therapy participants. Future work will focus on whether these findings are a result of HBV co-infection or whether intact virus is commonly found in all untreated HIV-infected individuals.
Mapping the egress pathway of HSV-1 by determining the interactome of viral envelope glycoprotein gE

Christopher E. Denes1, Eve Diefenbach2, Timothy P. Newsome3, Russell J. Diefenbach1,4

1Centre for Virus Research, The Westmead Institute for Medical Research, Westmead, New South Wales, Australia; 2Protein Core Facility, The Westmead Institute for Medical Research, Westmead, New South Wales, Australia; 3Molecular Bioscience, School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales, Australia; 4Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Macquarie Park, New South Wales, Australia

Rationale: Resistance of herpes simplex virus type-1 (HSV-1) to treatment antivirals is emerging. Viral surface glycoproteins are key for exit from the host cell during the viral lifecycle, with viral glycoprotein E (gE) necessary for exit and spread.

Aims: To define host-cell proteins that interact with HSV-1 gE during infection and take a subset of these proteins to further follow to identify potential novel antiviral targets.

Methods & Results: HeLa cells were infected for 24 hours with recombinant HSV-1 expressing gE-GFP or GFP alone. Protein lysates were processed by GFP-Trap affinity bead purification followed by limited on-bead trypsin digestion. Qualitative mass spectrometry was performed on each sample, with spectra compared to a total human or HSV-1 Strain 17 database. UniProt accession codes were then input for comparison to the online database at reactome.org to determine functionally enriched pathways against background. Adaptor protein complex subunits and a subset of Rab GTPases, proteins involved in vesicular trafficking, were discovered. To determine interaction domains, gE truncation mutants were designed for use in trans-complementation assays to begin to map gE interaction domains relevant for the newly discovered interactors. The locations of key trafficking motifs in the C-terminal tail of gE were assessed prior to this design, with secondary structure algorithms and cross-species sequence alignments used to confirm minimal disruption to protein folding and relevance of the motifs across the subfamily, respectively.

Discussion: HSV-1 gE interacts with a set of proteins involved in multiple vesicle-mediated transport pathways. This sheds new light on the egress pathway of HSV-1 from the host cell. The successful generation of gE truncation mutants for trans-complementation assays allows for the mapping of gE tail domains necessary for the protein-protein interactions involved in virus exit. Determining these sites may lead to the development of novel antiviral targets for future herpesvirus therapies.
An HIV-infected individual on suppressive ART with a massive expansion of effector memory T cells containing a defective provirus

Vincent Morcilla1, Marta Massanella2, Remi Fromentin2, Moti Ramgopal3, Lydie Trautmann4, Wei Wei Chiu5, David Looney5, Douglas D. Richman5, Sarah Palmer1, Nicolas Chomont2
1The Westmead Institute for Medical Research, The University of Sydney
2The Centre de Recherché du CHUM and Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montreal, Canada
3Midway Immunology and Research Centre, Fort Pierce, United States
4US Military HIV Research Program
5Centre for AIDS Research Molecular Biology Core, University of California San Diego

Background and Aims:
Cellular proliferation can contribute to the maintenance of the persistent HIV reservoir within individuals on antiretroviral therapy (ART). Here we report the unique case of an ART-treated individual with a massive expansion of a defective provirus.

Methods: Memory CD4+ T-cell subsets were sorted based on the expression of CD45RA, CD27 and CCR7. The frequency of memory cells harbouring integrated HIV DNA was quantified using Alu-PCR, and integrated proviruses were genetically analysed using single-genome sequencing targeting the HIV envelope (ENV) region. T-cell receptor clonality was analyzed in memory CD4+ T-cell subsets by high-throughput sequencing of the T-cell receptor (TCR) β-chain gene using CDR3 sequences. HIV integration sites were found by extracting and sonicating genomic DNA followed by ligation-mediated PCR and next-generation sequencing (NGS). To genetically characterise the provirus in effector memory T-cells, we used a modified full-length individual proviral sequencing (FLIPS) assay, which amplifies HIV provirus at limiting dilution followed by NGS.

Results: A high percentage (45%) of effector memory T-cells (EM) contained an integrated HIV genome. The majority of the ENV sequences were genetically identical in EM cells. One clonotype accounted for 60% of the TCR sequences of the EM cells. Chromosome 14 was the only integration site shared across all sorted memory CD4+ T-cell subsets and represented the highest frequency in EM cells. Using forward primers in chromosome 14 and reverse primers in the 3'LTR of HIV, we sequenced the expanded provirus in EM cells. The majority (83%) of these proviruses belonged to an identical sequence expansion which matched the ENV region from our earlier sequence analyses but lacked the 5'LTR-pol region.

Discussion: These results indicate that a massive expansion of a single defective provirus can occur in vivo. These proliferative events contribute to the weak association between DNA measurements and replication competence in some individuals.
The Genetic Traits of Full-Length HIV Sequenced from Memory T Cell Subsets

Bethany A. Horsburgh1, Bonnie Hiener1, Eunok Lee1, John-Sebastian Eden1,2, Timothy E. Schlub3, Susanne von Stockenstrom4, Jeffrey Milush5, Teri Liegler5, Elizabeth Sinclair5, Rebecca Hoh5, Remi Fromentin6, Nicholas Chomont6, Steven G. Deeks5, Frederick M Hecht5 and Sarah Palmer1

1. Centre for Virus Research, The Westmead Institute for Medical Research 2. Sydney Medical School, The University of Sydney 3. Sydney School of Public Health, Sydney Medical School, The University of Sydney 4. Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Karolinska University Hospital 5. Department of Medicine, University of California San Francisco 6. Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology, Université de Montréal

Aims: A thorough understanding of the distribution and genetic traits of replication-competent HIV will be needed to design eradication therapies. We used the Full-Length Individual Proviral Sequencing (FLIPS) assay to examine the traits of proviruses within memory CD4+ T cell subsets, and their contribution to the latent reservoir during ART.

Methods: Naïve, central (CM), transitional (TM) and effector (EM) memory CD4+ T cells, and CD45RA-HLA-DR+ and CD45RA-HLA-DR- CD4+ T cells, were sorted from the peripheral blood of six participants who initiated ART during either acute or chronic infection (n=3 each). FLIPS was used to obtain near-full-length HIV DNA sequences, using LTR-specific primers to amplify proviruses at limiting dilution followed by next-generation sequencing. Proviruses were characterized as defective or genetically-intact. Expansions of identical sequences (EIS) were determined as ≥2 identical proviral sequences.

Results: Of the 728 sequences isolated, 5% were considered intact. Intact provirus was found in all cell subsets except CM (0/125 sequences intact). The proportion of intact provirus was different across the cell subsets (EM>TM>CM and HLA-DR+>HLA-DR-; p=0.001). The frequency of cells infected with intact provirus was highest in HLA-DR+ memory T cells (48 vs <10 infected cells/million cells in HLA-DR+ vs all other subsets). Eighty-three percent of intact and defective sequences contributing to an EIS was 54% (12/35) and 46% (319/693) respectively. In one participant, 56 identical sequences contained a defective packaging signal but were intact in the coding region. Despite this, corresponding intracellular RNA was detected.

Discussion: Genetically intact and therefore likely replication-competent, CCR5-tropic HIV is enriched in cells expressing HLA-DR and EM cells. This indicates the latent HIV reservoir is established early and maintained by cell proliferation and differentiation. Defective proviruses can also produce viral transcripts, demonstrating that RNA quantification is not specific for cells containing intact HIV.
Characterising the responses of ‘bona-fide’ plasmacytoid and Axl+ Siglec6+ dendritic cells in initial HIV infection

Tong O1, Nasr N1, Cousins K1,2, Bertram K1, Harman AN1, Royle CM1, Gosselink MP 1,3 and Cunningham AL1

1Centre for Virus Research, The Westmead Institute for Medical Research, Westmead 2145, Australia, 2School of Medical Sciences, The University of Sydney, Camperdown 2050, Australia, 3Department of Surgery, Westmead Hospital, Westmead 2145, Australia

Aims: At mucosal sites of initial HIV infection, type I interferons (IFNs) such as IFN-α/β represent a potent first line of innate immunity, and are produced by a subset of dendritic cell (DC) called plasmacytoid dendritic cells (pDCs). However, previous reports examining pDC responses to HIV have included contaminating Axl+ Siglec6+ (AS)DCs, a recently described myeloid DC that expresses classical pDC markers. As such, the role that pDCs and ASDCs play during initial HIV infection, and how IFNs and other soluble factors produced by these DC subsets mediate these effects remains ambiguous.

Methods: Sorted ‘bona-fide’ pDCs and ASDCs were exposed to HIV-1BaL for 18 hours, upon which gene expression was assessed in cell lysates using the nCounter® Human Immunology Codeset on the Nanostring XT Analysis System. The presence of pDCs and ASDCs in human anogenital tissues was also assessed using flow cytometry.

Results: ‘Bona-fide’ pDCs and ASDCs had distinct gene expression profiles both in mock-infected controls, and following HIV exposure. IFNA/B was significantly induced in pDCs alone whilst ASDCs had higher costimulatory molecule expression. Chemokines involved in CD4+ T cell recruitment, namely CCL3-5, were predominantly upregulated in pDCs, but also in ASDCs. Proinflammatory chemokines and cytokines such as CCL22, TNF and IL32 were induced primarily in ASDCs. ‘Bona-fide’ pDCs and ASDCs could be identified in inflamed rectal samples (n=2), but were not present in healthy rectal tissue.

Discussion: Both ‘bona-fide’ pDCs and ASDCs have multifaceted responses to HIV – although pDCs remain the predominant IFN-producing cell, both cells produce proinflammatory mediators that may enhance infection in CD4+ T cells. Our data also demonstrates for the first time that both pDCs and ASDCs can be recruited to peripheral tissues, and are therefore likely to influence HIV infection in target cells and ultimately, HIV acquisition.
DEFINING THE SUBSETS OF MONONUCLEAR PHAGOCYTES PRESENT

Jake Rhodes, Kirstie Bertram, Rachel Botting, Heeva Baharlou, Hafsa Rana, Tony Cunningham and Andrew Harman
The Westmead Institute for Medical Research, Sydney, Australia

Multiple subsets of mononuclear phagocytes (MNP) are known to exist within human tissue which include; cDC1, cDC2, CD14+ monocyte-derived macrophages and autofluorescent macrophages. These are the first cells to interact with HIV which they detect via a repertoire of surface C-type lectin receptors (CLR) which is unique to each subset. Importantly these cells then transfer the virus to CD4 T cells in which the virus explosively replicates.

Due to difficulty of access to human tissue and technological limitations, surprisingly little is known about the very early events in HIV transmission and how HIV crosses mucosal surfaces. Identifying the initial HIV target cells will be essential for guiding the development of a vaccine and better PrEP regimens. Similarly defining the target cells for HIV latent HIV reservoir will aid in achieving a functional cure.

Although skin and intestinal MNP subsets are becoming increasingly well characterised, little human data is available concerning the specific subsets that inhabit the different anogenital tissues, particularly in the rectum and anus. Therefore, we have gained access to all the human anogenital tissues that pathogens encounter during sexual transmission (labia, vagina, glans penis, foreskin, urethra, anus and rectum) as well as other tissues for comparison (abdomen, colon, ileum and jejunum).

Using 18-parameter flow cytometry we have determined which specific subsets of MNPs are present within each tissue and also determined their relative abundance. We observe considerable tissue specific differences in proportions of each subset present; macrophage like cells predominate in mucosal tissue whereas cDC2 predominate in skin. We have also gone on to thoroughly characterise how efficiently each of these subsets transfers HIV to its target CD4 T cells.

We have just recently started to optimise a CyTOF panel of up to 40 parameters to try and define the MNP subsets within inflamed tissue. This is of importance as inflammation is known to increase the transmission of sexually transmitted pathogens, such as HIV, up to ten times and these cells may be a major contributing factor to this increase in transmission.
The role of apoptosis in Herpes Simplex Virus infections

Jacinta B. Smith, Naomi R. Truong, Konrad L. Feng, Andrew N. Harman and Anthony L. Cunningham
(Westmead Institute for Medical Research – Centre for Virus Research & University of Sydney)

INTRODUCTION: Herpes Simplex Virus (HSV) is a viral infection that causes cold sores and genital herpes. HSV affects over 3.7 billion people worldwide with significant incidence rates and complications. HSV currently has no vaccine and all human vaccine trials thus far have been unsuccessful. Previous work from our lab found that when HSV infected Langerhans cells (LCs) in the epidermis of the skin, they matured and migrated into the dermis and underwent apoptosis before interacting with dermal dendritic cells (dDCs).

AIMS: To identify the apoptotic receptors and signals HSV-infected LCs and dDCs utilise in their interactions, to determine any differences in receptor expression between distinct dDC subsets, and to block identified apoptotic receptors as confirmation of their importance in the interaction between HSV-infected LCs and dDCs.

METHODOLOGY: To obtain dDCs, an optimised procedure for isolating human immune cells from abdominal skin was utilised. The expression of apoptotic receptors was determined by RNA-seq followed by flow cytometry. To confirm which receptors were involved in interactions, blocking antibodies were utilised and interactions analysed by flow cytometry and immunofluorescence microscopy.

RESULTS: Variable expression of apoptotic receptors was observed; however, the expression of each receptor was consistent across resting dDC subsets. CD36, a scavenger receptor, was constitutively expressed on the surface of all dDCs. In preliminary experiments, blocking CD36 and related ligands resulted in a decreased percentage of interactions between apoptotic cells and healthy bystanders.

FURTHER STUDY/DISCUSSION: The relative importance of CD36 involvement in recognition of HSV-infected cells needs to be determined. Other apoptotic receptors also need to be investigated to determine if they play a role as the interactions progress between infected and bystander cells. Knowledge of the receptors involved in this pathway is crucial in understanding the pathway to CD4/CD8 T cell activation, which is critical in response to HSV infection.
Bacteriophages as adjuvant therapy to knock down the bacteraemic burden in severe Staphylococcal sepsis

A Petrovic Fabijan1, Josephine Ho1,2, S Maddocks1,2,3, RC Lin1,3,4, J Iredell1,2,3
1Westmead Institute for Medical Research, Centre for Infectious Diseases and Microbiology, 2Westmead Hospital, ICPMR, 3School of Medicine, University of Sydney, Sydney, Australia, 4School of Medical Sciences, University of New South Wales, NSW, Australia

Background: Intravenous bacteriophage therapy for severe Staphylococcus aureus infection was first reviewed in 1931. This described good outcomes after an initial inflammatory response to the bacteriophage infusion, but clinical experience with modern GMP-quality preparations are lacking. Here, we describe our own initial experience (n = 4) with intravenous bacteriophage therapy for critically ill patients with severe staphylococcal infections.

Materials/Methods: AB-SA01, a 3-phage specific combination developed as an off-the-shelf GMP product for Staphylococcus aureus infections, was manufactured under GMP conditions by AmpliPhi Biosciences. Two Infectious Diseases specialist opinions were obtained to ensure that there was a significant risk of death or severe adverse outcome despite optimal conventional therapy and that adjunctive bacteriophage therapy was indicated. Bloodstream isolates were tested for susceptibility to AB-SA01 prior to seeking informed consent. Daily intravenous administration of 50 mL saline containing 3x10^9 plaque-forming units of AB-SA01 was then carried out respectively for 14 days, in combination with optimal antibiotic therapy. Bacterial quantification of patient blood samples before and after phage administration was carried out.

Results: Four critically ill patients were treated between August and December 2017. Bacteriophage therapy was well tolerated with no post-infusion reactions or treatment-related adverse outcomes. Bacteriophage elimination from the bloodstream was evident within hours. Bacterial elimination with evident control of infection was demonstrated in 3 patients, all of whom were well at final clinical census (day 90).

Conclusions: AB-SA01 bacteriophage therapy may be a safe intravenous option for adjunctive therapy of severe staphylococcal infection. The small number of cases prevent any conclusions regarding efficacy but the burden of staphylococcal DNA in the blood was clearly knocked down by bacteriophage infusion in the two successful cases in which it was detectable.

We would like to acknowledge AmpliPhi BioSciences for supply of phage, clinical staff Drs T Gilbey and I Sandrandura.
**Mycobiome of Bondi Beach sand – a clinical connection?**

Darcii Terre1,2,3, Laszlo Irinyi2, Vanessa Marcelino2, Belinda Chapman3, Wieland Meyer2

1Sydney School of Science, The University of Sydney, Sydney, NSW, Australia; 2Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney - Westmead Clinical School, Faculty of Medicine and Health, Westmead Institute for Medical Research, Westmead, New South Wales, Australia; 3Quantal Bioscience Pty Ltd, Oatlands, NSW, Australia

Clinically significant fungi such as *Trichophyton*, *Microsporum*, *Cladosporium*, *Epidermophyton* and *Candida* have been found residing in beach sand worldwide. Many factors influence the fungal load in beach sand, with the frequency and level of human interaction being a significant contributor.

This study assesses the impact of seasonality and level of human activity on the recovery of medically relevant fungal species from six locations at Bondi beach (Sydney). Beach sand was collected from six areas of Bondi beach, with five samples taken at each location and made into a composite. For traditional sequence-based identification, isolates were cultured on three agar media and DNA extracted using the phenol-chloroform extraction method.

The ITS1–ITS4 primer set was used to amplify the primary fungal barcode (ITS1/2) and the Al33F–Al33R or Al34F-Al34R primer sets were used to amplify the secondary barcode (TEF1α). All PCR products were sequenced in both the forward and reverse directions by Sanger sequencing. For metabarcoding analyses, total genomic DNA was extracted from the sand samples. Fragments were amplified with the primers ITS1F and ITS2 targeting the ITS1 region and the amplicon was sequenced on the MiSeq® platform, Illumina. Reads were processed using the UNOISE algorithm to obtain error-corrected (denoised) sequences.

Each denoised sequence was considered an Operational Taxonomic Unit (OTU). Taxonomy was predicted for the OTU sequences using the SINTAX algorithm in usearch v10.0. Both saprophytic and medically relevant fungal species were identified by traditional culturing methods and metabarcoding, with species belonging to the genera *Mucor*, *Rhodotorula*, *Cladosporium*, *Alternaria*, *Aspergillus* and *Candida*. Metabarcoding produced more species level identification than traditional culturing methods.

This is the first study of the mycobiome of beach sand in Australia. Many medically relevant species have been identified, a large proportion of remaining unidentified OTUs highlights the importance to urgently update the reference databases.
Hepatitis B vaccination or infection induces novel antigen-specific human memory natural killer cells

Ratna S. Wijaya1,2, Scott A. Read1,3, Stephen Schibeci1, Mahmoud K. Azardaryany1, David van der Poorten5, Rita Lin5, Jacob George1,5, Mark W. Douglas1,5,6, Golo Ahlenstiel1,3,4

1 Storr Liver Centre, The Westmead Institute for Medical Research, The University of Sydney, Westmead, NSW 2145
2 Faculty of Medicine, Pelita Harapan University, Tangerang, Indonesia
3 Blacktown Medical School, Western Sydney University, Blacktown, NSW 2148
4 Blacktown Hospital, Blacktown, NSW 2148
5 The Westmead Hospital, University of Sydney, NSW, Australia.
6 Centre for Infectious Diseases and Microbiology, Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney at Westmead Hospital, Westmead NSW 2145.

Immunological memory is considered to be the domain of B and T lymphocytes. This view has recently been challenged by the identification of antigen-specific memory natural killer (NK) cell in mice and primate studies.

However, to date it is largely unknown whether antigen-specific memory NK cell develop in humans, particularly after hepatitis B virus (HBV) vaccination and infection. Here we demonstrate, that total and purified NK cells from peripheral blood from vaccinated subjects show higher degranulation against hepatitis B surface antigen-pulsed dendritic cells (HBsAg-pulsed DCs) compared to unvaccinated subjects (6.13% vs 0.22%; 4.07% vs 0.31%, p<0.01, respectively).

NK cell from vaccinated subjects efficiently lysed HBsAg-pulsed DCs compared to DCs pulsed with non-vaccinated hepatitis B core antigen (HBcAg) (10.06% vs 4.05%, p<0.05) or TNF-α (10.06 vs 4.79%, p<0.05). NK cells lysed HBsAg-pulsed DCs in an NKG2D-dependent manner. Further, in response to HBsAg-pulsed DCs, NK cells from vaccinated subjects proliferated more actively than unvaccinated subjects (5.45 vs 0.57%, p<0.01).

Finally, NK cell from chronic HBV infection patients exhibited greater cytotoxic immune response against HBcAg-pulsed DCs compared to unvaccinated (4.95 vs 0.37%, p<0.01) and vaccinated (4.95 vs 0.56%, p<0.01) subjects. In summary, our data suggests that both HBV vaccination and HBV infection can induce NK cell memory in humans. Further study is required to elucidate their role in primary HBV prevention and disease progression in chronic hepatitis B.
Synergetic effects of antibiotics against multidrug resistant Gram-negatives

H. Chen1, A. Ginn1, 2, 3, J. Iredell1, 3
1The University of Sydney, Sydney, Australia; 2Antimicrobial Resistance Reference Laboratory, Centre for Infectious Diseases and Microbiology Laboratory Services, NSW Health Pathology, Westmead; 3Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, Westmead.

Antibiotic resistance is a major global threat to human health and a number of pathogens are now resistant to many of the available therapeutic options. Multidrug resistant Enterobacteriaceae are of particular concern as these resistances are generally attributable to mobile (acquired) genes. In addition to this, inappropriate use of antibiotics has been identified in more than a fifth of all prescriptions issued in recent years, contributing to selection of multidrug resistance.

As a result of the increase in multidrug resistance, many therapeutic options previously abandoned due to toxicity have now become the last line of defence against this urgent threat. We aimed to investigate less toxic, or lower concentration, therapeutic options particularly in the context of antibiotic combinations.

The Klebsiella pneumoniae Carbapenemase (blaKPC), a carbapenem resistance gene, is carried on mobile plasmids, confers resistance to all β-lactam antibiotics and is typically found in isolates that have resistance to a number of other antibiotic classes. High mortality rates (>50%) have frequently been associated with blaKPC, which can have the effect of driving inappropriate antibiotic usage but antibiotic synergy (the use of antibiotic combinations) has been suggested as a more effective therapeutic approach. We assessed combinations of antibiotics against K. pneumoniae carrying this gene to determine the combination with the lowest expected toxicity and lowest concentration required to inhibit bacterial growth.

A number of combinations were identified as synergetic against the majority of blaKPC but no combination was effective against all isolates tested. This confirms that the optimal approach to treatment of a multidrug resistant pathogens may not the selection of a single agent, but rather the careful selection of combinations designed to defeat that pathogen’s specific resistance mechanisms and genes.
Improving fungal diagnosis via dual fungal DNA barcoding

Minh Thuy Vi Hoang1, Laszlo Irinyi1, Krystyna Maszewska1, Sharon Chen1,2, Tania Sorrell1 and Wieland Meyer1
1Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney - Westmead Clinical School, Faculty of Medicine and Health, Westmead Institute for Medical Research, Westmead, New South Wales, Australia; 2Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, New South Wales Health Pathology, Westmead Hospital, Westmead, NSW, Australia

Sequencing of short species-specific signatures, DNA barcodes, is the gold standard of fungal identification. The internal transcribed spacer (ITS) region has been established as the primary fungal DNA barcode and the translational elongation factor 1α (TEF1α) as the secondary fungal DNA barcode. The ISHAM Barcoding Database has been established to contain quality controlled ITS and TEF1α sequences, enabling identification of most human pathogenic fungi via the dual barcoding system. This study aimed to extend the reference database and to compare the resolution power and accuracy between ITS and TEF1α for the identification of pathogenic fungi by investigating the existence of barcoding gaps between species.

Primary and secondary fungal DNA barcodes were generated and submitted to the ISHAM Barcoding Database. Four genera were selected for this study; Aspergillus, Candida, Cryptococcus and Scedosporium.

The genetic distances between the strains were calculated using the Kimura 2-parameter model, compared for intra- and inter-species genetic distances and were graphed against their frequency. Barcoding gaps were determined to be present if there was no overlap between the intra- and inter-species distances. Aspergillus sp. demonstrated a barcoding gap for both ITS and TEF1α indicating accurate identification using both regions.

Candida sp. and Cryptococcus sp. did not have barcoding gaps for either region, with TEF1α having less of an overlap, increasing the confidence in DNA barcode identification. Scedosporium sp. did not generate a barcoding gap for the ITS region, but TEF1α did, as such TEF1α has allowed accurate identification that was not possible with single region DNA barcoding.

The secondary fungal DNA barcode has been demonstrated to be more accurate and has a higher resolution than the primary barcode. The addition of TEF1α as a secondary DNA fungal barcode improves the accuracy of DNA barcoding to the identification of the agents of mycoses in a clinical setting.
Establishment and optimization of a combined MLST scheme for Pneumocystis jirovecii

Lana Pasic1, Laszlo Irinyi1, Sharon Chen1,2, Tania Sorrell1 and Wieland Meyer1

1Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney-Westmead Clinical School, Faculty of Medicine and Health, Westmead Institute for Medical Research, Westmead, New South Wales, Australia; 2Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, New South Wales Health Pathology, Westmead Hospital, Westmead, NSW, Australia

Pneumocystis jirovecii is a unique unicellular fungus that most commonly causes Pneumocystis Pneumonia an opportunistic infection with high morbidity and mortality. Multilocus sequence typing (MLST) is currently considered the preferred approach for the analysis of genetic diversity of P. jirovecii, however, there is still no consensus MLST scheme. Aims of the herein presented study were: (A) Comparison of all MLST schemes currently used worldwide and establishing the discriminatory power for each locus using Hunter-index. (B) Establishing a MLST scheme combining the loci with the highest amplification efficiency and discriminatory power. (C) Analysing various factors influencing MLST genotyping: source, effectiveness of sample site of source, extraction and amplification techniques. (D) Applying the combined MLST scheme to explore the genetic diversity, disease burden, and establish the epidemiological relatedness of global strains. A literature review was performed to select the most appropriate loci, the discriminatory power for each locus was determined. P. jirovecii isolates collected from sputum, BAL and pharyngeal swab were used to test several DNA extraction methods to establish the optimum conditions for maximum DNA yield.

The discriminatory power of the 8 most common loci: mt26s, 26S rDNA, ITS1, β-TUB, SOD, CYB, DHPS, and DHFR, was determined to enable the selection of the most appropriate locus. The DNA extraction was optimised by adapting the Norgen DNA extraction kit, with the addition of a liquid nitrogen grinding step, resulting in a reliable high yield of DNA. This study highlights the importance of an effective DNA extraction method to minimise yield lost during DNA preparation for MLST analysis. Additionally, it determined a new MLST scheme with the highest discriminatory power, for P. jirovecii, intended to become the globally accepted consensus MLST scheme for this important human pathogen, to improve genotyping consistency; facilitating worldwide collaborations and the establishment of a global database.
Exploiting virulence-promoting non-protein kinases as antifungal drug targets

Sophie Lev1,2,3, Desmarini Desmarini1,2,3, Tania C Sorrell1,2,3, Philip Thompson4 Jacqui Matthews5 and Julianne T Djordjevic1,2,3

1Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, 176 Hawkesbury road, Westmead; 2Sydney Medical School-Westmead, The University of Sydney, Westmead; 3Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney.4Monash Institute for Medical Research, Parkville Vic; 5School of Life and Environmental Sciences, The University of Sydney.

Invasive fungal disease (IFD) poses a serious threat to human health, especially in patients immunocompromised by HIV infection or blood cancer therapy, and in organ transplant recipients. IFD affects 300 million people and causes 1.6 million deaths annually. Current therapies are toxic, sub-optimally effective or poorly absorbed and resistance is emerging.

Although novel therapies are needed urgently no new drug classes have been introduced into clinical medicine since the echinocandins in 1986. Using the genetically tractable and major fungal pathogen, Cryptococcus neoformans, as a model we showed that the inositol polyphosphate kinase (IPK), Arg1, is critically linked to virulence and IFD and potentially serves as a novel antifungal drug target. Arg1 is the first of a series of IPKs, which act sequentially to convert IP3 to IP7, a key metabolite promoting stress tolerance, metabolic adaptation and fungal dissemination.

Arg1 also conveys IP7–independent functions, including capsule production, high temperature growth, cell wall organization, and normal N-linked mannosylation of cell wall proteins. Work is presented which provides proof-of-principle that compounds can be developed that block fungal Arg1 (and hence prevent the synthesis of Arg1 products) but not mammalian IP3 kinases, and hence are selective for fungi. We also present evidence that a key role of Arg1 products is to promote fungal virulence by regulating gene expression via 2 distinct mechanisms.
OUTBREAK OF ST5 METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) WITH fusC MEDIATED FUSIDIC ACID RESISTANCE IN A CAPTIVE MEERKAT (SURICATA SURICATTA) POPULATION

Marc Ramsperger1,2, Cecilia Li1,2, Paul Thompson3, Larry Vogelnest3, Frances Hulst3, Gabrielle Tobias3, Matthew O’Sullivan1,2

1Centre for Infectious Diseases and Microbiology – Public Health, Westmead Hospital, Australia, 2The University of Sydney, Australia, 3Taronga Conservation Society Australia

Aim: We investigated the molecular epidemiology of S. aureus isolates from an outbreak of skin infections, affecting ten out of a group of eighteen captive meerkats from January to May 2018.

Methods: 19-target binary typing (bt) was performed by multiplex PCR and Reverse Line Blot Hybridization Assay (mPCR/RLB). Whole genome sequencing (WGS) using the Illumina NextSeq was employed to investigate the relationship between isolates and to reconstruct transmission pathways.

Results: All ten meerkats were infected with a fusidic acid resistant (FA-R) MRSA strain belonging to bt132352. WGS showed the strain belonged to ST5. Comparative genomics indicated this strain was closely related to a published human isolate NZAK3 and harboured the immune evasion cluster (IEC), associated with human host adaptation. Four core genome SNPs separated the sequenced ST5-MRSA isolates, consistent with a single continual outbreak rather than multiple introductions.

Conclusion: This study confirmed a clonal outbreak of fusidic acid-resistant ST5-MRSA amongst a group of captive meerkats. The outbreak strain was found to be closely related to NZAK3, an isolate representative of a clone that has recently emerged in New Zealand, thought to be driven by high levels of non-prescription topical fusidic acid use in that country.

The presence of the IEC suggests an introduction into the group from human contact, but this remains to be confirmed. Individual case treatment with oral trimethoprim-sulfamethoxazole was not successful in terminating the outbreak: group treatment of all 18 animals is now underway.
**Functional Diversity of Toxin-Antitoxin Systems in Antibiotic Resistance Plasmids in Enterobacteriaceae**

Alma Wu1, Muhammad Kamruzzaman1, Jon Iredell1,2

1Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, The University of Sydney, Westmead, New South Wales, Australia

2Westmead Hospital, Westmead, New South Wales, Australia

**Introduction:** The rise of transmissible antibiotic resistance (AbR) in the bacterial family Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, is of major concern. In these bacteria, resistance is mainly spread by self-transmissible extrachromosomal DNA elements called plasmids, which commonly contain toxin-antitoxin systems (TAS). TAS ensure the stable maintenance of plasmids in bacteria by killing plasmid free cells, and contribute to the spread of AbR in and within species. This study aimed to define the TAS present in plasmids found in K. pneumoniae, and to compare the functions of several common TAS in various Enterobacteriaceae species.

**Methods:** TAS in plasmids found in K. pneumoniae were determined bioinformatically using TA Finder, and the promoter regions of TAS from different species compared. Promoter-GFP reporter systems were used to determine promoter strengths in normal growth conditions, as well as expression changes during environmental stress. Cell survival and plasmid stability assays were also used to determine TAS roles in stress response and plasmid maintenance respectively.

**Results:** A total of fifteen different TAS were identified among 306 K. pneumoniae plasmids, with two (ccdAB and pemIK) also being common in other Enterobacteriaceae plasmids. The promoters of these two TAS taken from various species and plasmids types showed variation in strength depending on the strain it was present in rather than the species. Neither plasmid borne ccdAB nor pemIK responded to nutrient limitation or antibiotic stress, nor did they confer any survival advantage under antibiotic stress.

**Discussion:** The prevalence of common TAS varies between species and/or plasmid types, with some being relatively specific. TAS common to multiple plasmids vary in promoter sequence, and their expression varies between host strains, not species. Plasmid borne ccdAB and pemIK are likely to be specialised plasmid maintenance systems, and do not play a role in bacterial stress response.
Hajj-associated risk of importation and transmission of antimicrobial resistant enteric infections – implications for Australia

Abd El Ghany M1,2., Yasir M3., Azhar EI3,4., and Hill-Cawthorne GA2,5.

1 The Westmead Institute for Medical Research, The University of Sydney, Sydney, Australia.
2 The Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, Australia.
3 Special Infectious Agents Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia.
4 Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.
5 School of Public Health, The University of Sydney, Sydney, Australia.

Background: Hajj, the annual Muslim pilgrimage to Mekkah, Saudi Arabia, is a recurrent mass gathering event that attracts over 2.5 million pilgrims from around the globe. Hajj has previously been associated with an increased risk of airborne, foodborne, bloodborne and zoonotic infections. Recent studies from our and other groups have raised concerns that Hajj might be a focal point for the acquisition, emergence and global dissemination of antimicrobial resistant (AMR) infections. While Australia is geographically distant from Mekkah, it is still likely that the pilgrimage has a significant impact on the status of public health in Australia through the introduction of AMR bacteria, carried in the gut of Australian pilgrims (~5000 Hajj pilgrims a year) upon their return from the pilgrimage.

Aims: We aim to identify the clinical and environmental factors that impact the emergence, dissemination and dynamicity of AMR enteric bacteria in Hajj settings. We will evaluate the potential roles of preventive measures in reducing the importation of AMR bacteria by pilgrims upon return to their home countries.

Methods: We have standardised a reproducible culture-free metagenomic approach that uses Illumina technology to directly characterise the aetiologic agents associated with Hajj-diarrhoeal infections and the AMR elements occurring in different environments, including the human gut and sewage samples.

Results and discussion: This is the first large-scale study to identify pathogens associated with diarrhoeal infections during Hajj. Foodborne bacteria, including Escherichia coli, Salmonella spp. and Shigella spp., were the most common aetiologic agents identified in 600 faecal samples collected from symptomatic pilgrims from 40 countries. Of particular concern were the presence of ESBLs (primarily blaCTX-M-15) and carbapenemases (e.g. blaNDM) in 40% of Salmonella and E. coli-positive samples. Further findings from our ongoing research, including human gut microbiome structural and functional variations associated with the acquisition of AMR enteric infections, will be discussed.
We created and verified a Staphylococcus argenteus mass spectrum profile (MSP) library for use with the Bruker MALDI-TOF mass spectrometer for routine, rapid identification of S. argenteus in a clinical diagnostic setting.

MALDI-TOF spectra from a reference panel of 8 S. argenteus isolates were generated and used to create a S. argenteus MSP database. 24 diverse S. argenteus isolates (confirmed by whole genome sequencing) and 32 S. aureus isolates were used to test the performance of the database.

Spectra obtained from the 8 S. argenteus reference isolates revealed the absence of peak m/z 5525, which was present in S. aureus. When simultaneously interrogating the standard MALDI spectral database (which does not contain S. argenteus) with the S. argenteus database, all 24 S. argenteus isolates were correctly identified, with identity scores between 1.99 - 2.46 (> 2.00 indicating high confidence identification, 1.70 - 1.99 indicating low confidence identification). All S. aureus isolates were identified as S. aureus using the combined databases.

When interrogating the standard database only, S. argenteus isolates were all misidentified as S. aureus with identity scores between 1.81 - 2.11.

The rapid identification and differentiation of S. argenteus and S. aureus isolates using the MALDI-TOF MS is suitable for routine diagnostic use. The addition of the S. argenteus MSP library to the MALDI spectral database will now be used to prospectively identify S. argenteus, and will allow studies to accurately determine the incidence and disease associations of this organism in our population.
High rates of IPD caused by serotype 3 in children less than 5 years old age in Australia, despite high vaccine uptake.

Shahin Oftadeh1, Rebecca Rocket2, Robin Gilmour3, Vitali Sintchenko2, 4 and Lyn Gilbert2, 4
1NSW Pneumococcal Reference Laboratory, Institute of Clinical Pathology and Medical Research - Pathology West, Westmead Hospital, Westmead, 2145, Australia
2Centre for Infectious Diseases and Microbiology - Public Health, Westmead Hospital, Westmead, 2145, Australia
3Communicable Diseases Branch, NSW Health, Sydney, Australia
4Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Camperdown, 2050, Australia

In Australia, the PCV7 was introduced to the routine vaccination schedule, in 2005, for children (at 2, 4 and 6 months) and was replaced with PCV13 in 2011. Although the numbers of most vaccine serotypes have dramatically decreased among invasive pneumococcal disease (IPD) isolates, vaccine failure cases have been noted and are predominately due to serotype 3 and 19A.

In this study serotype 3 IPD cases in young Australian children was investigated using IPD surveillance data.

Prior to the introduction of PCV13, serotype 3 caused 3% (24/812) of IPD cases in Australian children less than 5 years of age. Between 2012 and 2015 the proportion of serotype 3 isolates has increased to 9% (58/653) and, to date, in 2017, it has been responsible for 14% (18/132) of IPD cases and now is the most prevalent IPD-causing serotype. More concerning is that almost half (48%, 37/76) of the serotype 3 IPD cases are from fully vaccinated children.

The low immunogenicity of serotype 3 antigen in PCV13 might explain to some extent the increasing incidence of serotype 3 IPD in Australia and elsewhere. However, our preliminary data highlight the role of diversity in the polysaccharide capsule antigen of Streptococcus pneumoniae, as the main surface antigen and the target of the currently used pneumococcal vaccines, in limiting vaccine efficacy. Our limited understanding of this diversity creates a major obstacle in eliminating pneumococcal disease.

Further research is warranted to monitor diversity and evolution of the serotype 3 capsular genes and antigens in order to improve and maintain vaccine efficacy.
Mosquitoes in urban wetlands: are there conflicts between biodiversity and public health?

Hanford, Jayne K.1,3, Webb, Cameron E.2,3, Hochuli, Dieter F.1
1 School of Life and Environmental Sciences, The University of Sydney, Sydney
2 Medical Entomology, NSW Health Pathology, Level 3 ICPMR, Westmead Hospital, Westmead
3 Marie Bashir Institute of Infectious Diseases and Biosecurity, The University of Sydney, Sydney

Aims & Study Rationale: Constructed wetlands are increasingly incorporated into urban areas to improve water quality and conserve biodiversity. However, these wetlands are often perceived as a source of nuisance-biting and pathogen-transmitting mosquitoes, undermining community support for wetlands and potentially increasing risks to public health. Our aims were to determine if the aquatic biodiversity and surrounding land use of urban wetlands influenced mosquito-related public health risks.

Methods: Mosquitoes, aquatic macroinvertebrates and physical habitat variables were sampled on two occasions through summer and autumn at 24 wetland sites in the greater Sydney region. Aquatic macroinvertebrates were used as an indicator of aquatic health and biodiversity and GIS data were used to determine the percentage of urban land use surrounding each wetland.

Results: Mosquito diversity and abundance were highly variable among sites. We found that constructed wetlands supported multiple mosquito species that are known nuisance-biting pests and vectors of mosquito-borne pathogens. Two widespread and abundant species, Coquilletidia linealis and Mansonia uniformis, showed positive relationships with aquatic macroinvertebrate richness, while Culex annulirostris and Culex quinquefasciatus showed positive relationships with surrounding urbanisation. These results suggest that while some mosquito species of pest and public health significance respond to wetland biotic traits, others respond to broader landscape traits.

Conclusion: There is a need for species-specific approaches in assessing and mitigating mosquito-related public health risks associated with urban wetlands. Greater understanding of relationships between wetland biodiversity and mosquito-related public health risks will enhance the value of constructed urban wetlands and avoid conflicts with health risks posed by mosquitoes.
Antibiotic resistance genes in the microbiome of wild birds

Vanessa R. Marcelinoa,b,c, Michelle Willed, Aeron C. Hurtd, Daniel González-Acuñae, Marcel Klaassenf, John-Sebastian Edena,b,c, Mang Shiac, Jonathan R. Iredellb, Tania C. Sorrellab, Edward C. Holmesac

aMarie Bashir Institute for Infectious Diseases and Biosecurity, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia.
bWestmead Institute for Medical Research, Westmead, NSW 2145, Australia.
cSchool of Life & Environmental Sciences, Charles Perkins Centre, The University of Sydney, Sydney, NSW 2006, Australia.
dWHO Collaborating Centre for Reference and Research on Influenza, at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC 3000, Australia.
eLaboratorio de Zoología y Vida Silvestre, Facultad de Ciencias Veterinarias, Universidad de Concepción, Concepción 3349001, Chile.
fCentre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, VIC 3216, Australia.

Our arms race against pathogens is challenged by the spread of bacteria that are resistant to antibiotic treatment. Environmental pollution with human waste carrying antibiotic resistant bacteria and antibiotics in sublethal doses favors the development and persistence of antibiotic resistance in natural habitats and wildlife.

Using bulk RNA-sequencing (meta-transcriptomics) we assessed the functionally active resistance genes in the microbiome of wild birds. We found clinically relevant resistance genes in birds from all localities, including in metropolitan regions in Australia and in supposedly pristine habitats in Antarctica.

There was a strong link between the carriage of resistant bacteria by birds and the impact of human activities on natural habitats. Notably, ducks living at a wastewater treatment plant harboured the highest resistance gene load, including genes typically associated with multidrug resistance plasmids. Our results suggest that human waste contributes to the spread of antibiotic resistance genes into the wild.
Huntington Disease: What is the profile of symptoms and signs at earliest clinical motor onset for the commoner CAG repeat lengths in the ENROLL HD study?

Florence Chang1 Elizabeth McCusker1,2 Therese Alting1 Jillian McMillan1 Clement Loy1,2,3,4 Huntington Disease Service Neurology Dept Westmead Hospital1, Medical School2 and School of Public Health3 University of Sydney and Garvan Institute4

Aim to examine the profile of symptoms, signs and function for Huntington disease (HD) with CAG repeats 42-44 and Total motor score (TMS) 5-10 in the ENROLL data set.

Background: Huntington Disease is caused by a variably expanded (CAG) n repeat in the Huntingtin gene. Westmead is a site for the multinational ENROLL HD study. Interventions at earliest stage disease could deliver the best outcomes. We reviewed the profile at the earliest clinical stage, including symptoms, functional measures, TMS components and cognitive/behavioral items as a baseline for trials and to determine reliability of participant report and functional measures.

Methods: Analyze ENROLL data set for the commonest CAG 42-44 and early motor score. We examined participant and rater report, signs, cog measures (MMSE, SDMT, Stroop word), depression, suicidality, apathy and functional changes compared to 103 genotype negative individuals with Mamm-Whitney U test, SPSS 23.

Results: Of 3773 participants, 2868 (76%) had CAG 42-44. Of these 274(9.6%) had TMS 5-10. Of this group 129 (41.7%) female/145 (52.9%) male, median age 45 yrs. (range19-63); 118 (43%) paternal,150 (55.4%) maternal inheritance. 'Yes' was recorded for the question 'Have motor symptoms compatible with HD ever been part of the participant's medical history' for 169 (61%). Only 19.71% of participants reported the initial manifestation was motor, compared with the clinician (rater), 36.09%.

The rater reported more initial behavioral manifestations. There was no significant functional impact at this stage.

Conclusion: Only 20% of participants with early motor signs reported motor onset, despite a recording that 'motor symptoms compatible with HD was part of the medical history'. UHDRS (Unified Huntington Disease Rating Scale) and ENROLL items may not capture the profile of early disease when interventions, e.g. Huntingtin (HTT) lowering could be effective. Self-reported symptoms are unreliable. Some signs are without symptoms or functional impact.
Understanding the emergency cell survival mechanism of membrane repair

Claudia E Reed1,2, Frances Lemckert1,2 & Sandra T Cooper1,2
Kids Neuroscience Centre, Kids Research, Children's Hospital at Westmead, Sydney, New South Wales, Australia
Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, New South Wales, Australia

Background: Emergency cell membrane repair is a fundamental biological process that remains largely undescribed. Defects in skeletal muscle membrane repair are present in a rare group of muscular dystrophies caused by mutations in the dysferlin gene known as dysferlinopathies. Dysferlinopathies are characterised by late-onset and progressive atrophy of skeletal muscle, eventually resulting in permanent disability. There is no cure or definitive treatment. Through the study of dysferlinopathies, our lab has previously characterised key proteins involved in muscle membrane repair as well as identified potential therapeutic targets. Calpains are a family of calcium-activated proteases involved in many cell processes including remodelling of the cytoskeleton during membrane repair. They have recently become the subject of intense research as pharmaceutical targets for neurodegenerative disorders, with enormous potential to be used in neuromuscular diseases such as dysferlinopathy. Calpain-1 and calpain-2 have been shown to functionally modify the dysferlin protein. We believe the resulting C-terminal product “mini-dysferlin” is the key facilitator for transport and fusion of new membrane vesicles in the repair of muscle membrane injuries. Further, we hypothesise that membrane repair defect is the mechanistic pathology underpinning dysferlinopathy.

Aims: In mice without the capacity to form mini-dysferlin, we aimed to study the ability for skeletal muscle cells to repair membrane injuries and recruit dysferlin protein to sites of membrane injury. We also aimed to study the onset and progression of a muscular dystrophy in these mice.

Methods: Primary myoblast cell cultures were derived from mice without the capacity to form mini-dysferlin and wild-type controls. Membrane injuries to these cells were induced in the presence of fluorescent markers to assess membrane repair and the recruitment of dysferlin. Histological experiments studied the clinical hallmarks of muscular dystrophy including fibre size, fibrosis and fat deposition in skeletal muscle. Results will be prepared for presentation in August.
Generating a global knockout of PYROXD1 in mice

Authors: Madison Gonenale, Frances Evesson & Frances Lemckert
Affiliation: Kids Research – Institute of Neuroscience and Muscular Research

Background: Mutations to PYROXD1, coding an oxidoreductase, cause congenital myopathy. Patients have reduced PYROXD1 protein and/or enzymatic activity. Global knockout of Pyroxd1 is early-embryonic lethal in mice and Pyroxd1 protein expression is essential for cell survival. PYROXD1 has enzymatic activity and our current focus is on finding its substrate. This will not only mean uncovering an important player in cell survival, but may also give insight into the cause of congenital myopathy.

Aims: This project aims to create a late-embryonic PYROXD1 global knockout mouse by using tamoxifen-inducible Cre recombination. Cre-recombinase excises a target sequence flanked by LoxP sites. To knockout PYROXD1 in our mice, exon5 is flanked by LoxP sites creating a targetable allele. Cre is carried by the PFLP mice as a transgene, with expression regulated by a modified estrogen-2 receptor (ERT2). Activation of Cre-recombinase relies on tamoxifen binding to ERT2, giving us temporal control over when PYROXD1 expression is knocked out.

Initially we are optimising tamoxifen dosage using mouse embryos heterozygous for the targetable allele, enabling us to avoid embryonic lethality. For the second stage, the best tamoxifen dosage is given to mice homozygous for the targetable allele. Tissues of these mice will be studied for the effect of PYROXD1 loss.

Methods: Pregnant females were injected once with tamoxifen on embryonic day 15 (ED15) or twice on ED14 and ED15. Pups were harvested on either ED18 or day 21 as neonates. Skeletal muscle and liver were taken and PCR used to determine degree of recombination.

Results: PCR results demonstrate Cre-positive muscle and liver tissue show recombination whilst Cre-negative tissue show no recombination.

Discussion: Cre-recombination of the PFLP allele was successfully achieved with tamoxifen injection. We are working to quantify the amount of tissue recombination, which has not been done in other Cre-recombination knockouts.
How to signal to a synapse for more neurotransmitter release

Mason J. Burns, Jesse R. Wark, Mark E. Graham

Synapse Proteomics, Children’s Medical Research Institute, The University of Sydney, 214 Hawkesbury Road, Westmead, NSW, 2145.

The brain is comprised of neurons which are connected by a network of 100 trillion synapses. Each of these synapses have a presynaptic terminal, which releases neurotransmitter and is detected by postsynaptic receptors. The ability of the synapse to remodel in response to stimulation, is known as synaptic plasticity and results in an increase or decrease in strength. This remodelling underlies learning and memory.

Mutations which disrupt biomolecular components that regulate plasticity have been associated with neurological diseases. There is a lack of knowledge on how electrical activity signals to biomolecules to change their function, thereby altering the level of neurotransmitter. We are tracking the signalling pathway with the aim of discovering how neurotransmitter levels are altered. The signal begins with electrical activity.

When the electrical signal reaches the presynaptic terminal, it causes a change in electrical potential, which triggers Ca2+ influx. Neurotransmitter filled synaptic vesicles are released upon Ca2+ influx, but the level of vesicle release is regulated by vesicle docking proteins. One such docking protein is Rab3-interacting molecule 1 (RIM1), which is associated with schizophrenia and autism. Ca2+ influx also activates protein kinases, which are enzymes that add phosphate to proteins to change their function. We hypothesise that RIM1 is phosphorylated downstream of the Ca2+ influx as a signal to increase neurotransmitter release and this occurs by optimising RIM1-protein interactions.

We will determine how the phosphorylation of RIM1 changes how it interacts with other proteins involved in regulating vesicle release. This will be done using a combination of powerful tools. Genetic manipulation has allowed the mimicry of protein phosphorylation and this will be combined with mass spectrometry to rapidly identify phosphorylation-dependent interactions. The elucidation of a clear mechanism of altering neurotransmitter release will allow a greater understanding of the biology underlying plasticity and related neurological diseases.
Functional Properties of Single Motor Units Recorded from Human Medial Pterygoid Muscle during Jaw Movement

Chen Hui1,2, Whittle Terry2, Murray Greg2, Klineberg Iven2*

1 Oral Restorative Sciences, Faculty of Dentistry, University of Sydney, Level 3, Westmead Center for Oral Health
2 Jaw Function and Orofacial Pain Research Unit, Faculty of Dentistry, University of Sydney, Level 2, Westmead Center for Oral Health
*Corresponding Author:
Tel: +61-2-98457734; Fax:+61-2-96332893; Email: iven.klineberg@sydney.edu.au

Background: Understanding jaw muscle function is essential for the description of normal function as well as how the jaw motor system accommodates to perturbations such as chronic pain and prosthodontic interventions. In contrast to the extensive knowledge of the functional properties of the masseter and temporalis muscles, the medial pterygoid (MPT) muscle has been much less studied.

Aims: To identify the functional properties of single motor units (SMUs) recorded from the MPT muscle during standardized jaw movement tasks.

Methods: Intramuscular electrodes were placed in the right MPT muscle of 20 participants (10 female and 10 male; average age: 25 yrs) who performed standardized jaw tasks: rest, open-close, ipsilateral (right), contralateral (left), and protrusive jaw movement. All tasks were performed with teeth apart. A CT scan was performed to verify the placement of the electrodes within the MPT, and the MPT was divided into 4 divisions in the horizontal plane.

Results: Increased MPT activity was noted in the return phase of the ipsilateral task, throughout the contralateral and protrusive tasks, and in the closing phase of the open-close task in most participants. EMG activity was noted in 8 participants during the rest task. The thresholds of 46 SMUs could be identified in relation to displacement. Thresholds increased significantly (p<0.0001) with increases of movement velocity during the outgoing phase of the contralateral task and the return phase of the ipsilateral task. Significant differences were found in SMU thresholds among the 4 divisions during the outgoing phases of contralateral (p<0.0001) and protrusive (p=0.0003) movement.

Conclusion: The evidence suggests that the MPT muscle is a complex muscle involved in the fine control of low forces as required for the stabilization of vertical mandibular position throughout horizontal jaw movements. The differences in SMU thresholds among the 4 divisions provide evidence for functional heterogeneity within MPT.
Tertiary level management of severe paediatric obesity: Interventions must focus on younger children and address attrition rates

K Chisholm 1,2, S Alexander 2
Nutrition and Dietetics, The Children’s Hospital at Westmead, Sydney
Weight management Services, The Children’s Hospital at Westmead, Sydney

Background: Overweight and obesity remains high in Australian children with 1 in 4 school-aged children and 1 in 5 preschoolers affected, thus it is imperative we determine optimum weight management interventions. Data from tertiary level treatment programs can help inform patient and service characteristics most likely to yield successful outcomes.

Aim: As part of ongoing service improvement, we evaluated data from our NSW tertiary paediatric multi-disciplinary weight management services clinic, CHOOSE (Children’s Hospital Overweight and Obesity Services) Health clinic, to determine potential identifiable criteria predictive of greater weight loss results.

Method: A retrospective audit with analysis of data (demographics and anthropometry) collected on 480 children ages 2-16 years from 2012-2016 attending CHOOSE Health clinic. Clients attended three parent-only workshops, then nine frequent and tailored individual sessions with the team’s health professionals. Mixed models were used to assess the statistical changes from the base line using SAS version 9.

Results: 80% of the children had morbid obesity (International Obesity Task Force IOTF). Mean baseline Body Mass Index z-score (BMI z-score) and Waist-to-Height ratio (WHtR) were 2.7 (range 1.2 – 6.4) and 0.78 (range 0.49-1.0) respectively. Only 60% of families attended the initial appointment after workshops. For multiple attenders, there was a significant (p<0.0001) mean change in BMI z-score from visit 2 to last visit being greatest in those ≤ 6 years of age. With increasing number of visits, there was a mean reduction in BMI z-score of 0.04 units (95% CI 0.04 to 0.051).

Conclusion: Tertiary level care is effective in management of childhood obesity with significant reductions in BMI z-score. There was a high level of morbid obesity particularly in the younger age group however, this cohort responds well to treatment. Lifestyle interventions should target attrition rates and younger children.
Ulna length for weight status assessment in children

Ms. Henderson Joanne1,2 (Joanne.henderson@health.nsw.gov.au)
Associate Professor Garnett Sarah3,4 (sarah.garnett@health.nsw.gov.au)
Dr. Alexander Shirley1,5 (Shirley.Alexander@health.nsw.gov.au)
Dr. Van Dam Pieter2 (pieter.vandam@utas.edu.au)

1 Weight Management Service, The Children’s Hospital at Westmead, Westmead, Australia.
2Department of Medicine, University of Tasmania, Hobart, Australia.
3Institute of Endocrinology and Diabetes, The Children’s Hospital at Westmead, Westmead, Australia.
4Discipline Paediatrics and Child Health, The University of Sydney, Sydney, Australia.
5Auburn School, The University of Notre Dame, Sydney, Australia.

Aims: Assessing weight status is challenging in children when no clinical alternative to standing height measurement is available. Height from ulna length can be calculated from reliable and reproducible linear regression formula however, its effect on weight status has not been explored.

The study aimed to ascertain if Body Mass Index (weight (kg)/height (m²)), determined by a calculation of height from ulna length, was reliable in identifying overweight and obese children.

Methods: In this exploratory study of 20 participants aged 2-16 years, weight status from the control, BMI from standing height was compared to BMI with height determined from ulna length, using two different methods with a disposable paper measuring tape.

Results: Intra and inter reliability was high on both ulna measures with intraclass correlation (0.99). Both techniques provided similar results, respectively between mean difference in height (0.055m and 0.051m) and BMI (-1.65kg/m² and -1.51kg/m²) all P≤0.001. In assessment of weight status for overweight and obese, there was 100% agreement between weight status determined by standing height and weight status determined by ulna length.

Discussions: Ulna length provides a convenient simple alternative method of height measurement, viable for use in assessing weight status for overweight and obesity in children. The method can be reproduced in most clinical areas and with those who have physical limitations or infectious risk where a standing height cannot be obtained. It should not be used to replace standing height when available, but can be used to reduce barriers to assessment of weight status on children, in health care facilities.

It is recognised that the findings of this exploratory study prove a reliable method for assessment of weight status in children for overweight and obesity in the absence of standing height, however limited numbers suggest the need for larger studies.
Does the General Movements Assessment predict neurodevelopment following neonatal surgery?

Cathryn Crowle1,2, Claire Galea3, Karen Walker1,2,3, Iona Novak1,3, Nadia Badawi1,2,3

1Children’s Hospital Westmead, Sydney, Australia
2University of Sydney, Sydney, Australia
3Cerebral Palsy Alliance, Sydney, Australia

Aims: Predictive assessment tools are vital to identify infants at risk of neurodevelopmental disability in order to intervene early and improve outcomes. Neonates who undergo major surgery are known to be at risk of poor neurodevelopment, however there is a paucity of data on the use of the General Movements Assessment (GMA) with this population. The objective of this study was to investigate the predictive ability of the GMA for neurodevelopmental outcomes at one year of age for infants following surgery in the neonatal period.

Method: 278 infants who had undergone cardiac surgery (n=149, 54%), non-cardiac surgery (n= 123, 44%), or both surgeries (n=6, 2%) were assessed using the GMA at a mean age of 12 weeks (SD 1.6 weeks). Videos were independently rated by three clinicians, two blinded to clinical details. Multidisciplinary follow-up was at one year of age.

Results: At three months, 248 (89%) infants had normal fidgety movements, twenty five (9%) had absent fidgety, and five had abnormal fidgety. Of those with normal fidgety movements none had a diagnosis of CP at 12 months of age, however as expected in the surgical population, a large proportion (n=109, 44%) demonstrated motor delay. There was a significant difference in outcomes of infants who had absent fidgety movements on all subtests of the BSID-III (p<0.05) for both parametric and non-parametric analysis. For prediction of CP there was 100% sensitivity and 96% specificity.

Discussion: The GMA has predictive value for CP in this unique population. It is a valid complementary assessment tool that should be incorporated into the multidisciplinary follow-up for infants following surgery in order to facilitate referral into specialised early intervention services. Not surprisingly, normal fidgety movements do not guarantee normal motor development in this population, given the impact of surgery and hospitalisation.
A Child’s Concept of Pain: An International Survey of Pediatric Pain Experts

Joshua W. Pate 1,2, Julia M. Hush 2, Mark J. Hancock 2, G. Lorimer Moseley 3, David S. Butler 3, Laura E. Simons 4 and Verity Pacey 2,5

Affiliations:
1 Westmead Hospital Pain Management Centre, Sydney, NSW 2145, Australia
2 Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW 2109, Australia
3 Sansom Institute for Health Research, University of South Australia, Adelaide, SA 5001, Australia
4 Department of Anesthesiology, Perioperative, and Pain Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
5 The Children's Hospital at Westmead, Westmead, NSW 2145, Australia

Aims: A child’s ‘concept of pain’ refers to how they understand what pain actually is, what function pain serves, and what biological processes are thought to underpin it. We aimed to determine pediatric pain experts’ opinions of: (1) the importance and usefulness of assessing a child's concept of pain in clinical and/or research settings; (2) the usefulness of the content of items within currently published adult-targeted resources for assessing a child's concept of pain; and (3) important domains of a child's concept of pain to assess.

Methods: Forty-nine pediatric pain experts (response rate = 75.4%) from 10 countries completed an online survey. Descriptive statistics and frequency of responses were analyzed.

Results: Experts from all included disciplines reported that assessing a child’s concept of pain is important and useful both clinically and in a research setting (>80% reported very or extremely useful for each item). Experts considered that the content of 13 items from currently published adult-targeted resources was useful, but the wording was too complex for children aged 8–12 years. Experts considered that all seven of the proposed domains of a child’s concept of pain was important to assess.

Discussion: The findings can be used to inform the development of an assessment tool for a child’s concept of pain.
Explaining Pain to Children: What Terminology Should We Use? Qualitative Interviews Describing Pain-Related Neuroanatomy

Joshua W. Pate 1,2, Timothy Noblet 2, Julia M. Hush 2, Mark J. Hancock 2, Renee Sandells 3, Meg Pounder 3 and Verity Pacey 2,3

1 Westmead Hospital Pain Management Centre, Sydney, NSW 2145, Australia
2 Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW 2109, Australia
3 The Children's Hospital at Westmead, Westmead, NSW 2145, Australia

Aims: To (1) explore terminology and language that children diagnosed with persistent pain use to describe pain-related neuroanatomy, and (2) compare this terminology and language with children who are pain-free.

Methods: This qualitative study used in-depth semi-structured interviews. Interviews were 30-45 min and included questions and drawings about pain-related neuroanatomy. Stratified purposive sampling was used for children with persisting pain (>3 months) and children who are pain-free. All children (aged 8-12 years) lived in New South Wales, Australia and had not received multidisciplinary treatment or a program of pain education. Data collection continued until saturation was reached. Audio recordings from interviews were transcribed verbatim. Using an adapted grounded theory approach, transcripts were independently reviewed line-by-line by 3 authors who developed a preliminary coding scheme. Analytical themes were inductively developed and agreed upon through an iterative process. Themes were then scrutinised by a team of experts to ensure consensus.

Results: Sixteen children [mean (SD) age = 9.8 (1.4); 7 females] were interviewed. Three main themes emerged from the data: the important role of the brain, the forgotten spinal cord, and confusion about nerves. The words ‘controls’, ‘sending’ and ‘messages’ were commonly used by children with and without pain to explain the brain’s involvement in the experience of pain. Nerves were commonly described as being ‘everywhere’, but terminology was confused with feelings of nervousness and with veins or carrying blood.

Discussion: Australian children aged 8-12 years who have not received pain education had varying descriptions of pain-related neuroanatomy. The nervous system is typically conceptualised without inclusion of the spinal cord and is considered as having two parts; the brain and a peripheral component. When providing education of pain-related neuroanatomy to children, phrases such as ‘spinal cord’ and ‘nerves’ are likely to be poorly understood and potentially misinterpreted.
Feeding Practices in infants with Pierre Robin Syndrome

Bonnie Dorise1, Amit Trivedi1,2, Claire Galea1,2, Karen Walker1,2

1 Grace Centre for Newborn Intensive Care, The Children's Hospital at Westmead, NSW, Australia
2 University of Sydney, Children's Hospital at Westmead Clinical School, NSW, Australia
3 Cerebral Palsy Alliance, NSW, Australia

Aims: We aimed to identify the feeding practices of infants with Pierre Robin Syndrome (PRS) during their inpatient stay in a neonatal intensive care unit in a large tertiary paediatric hospital and assess the impact of these practices on growth.

Methods: A retrospective review of feeding practices in infants with PRS was conducted between January 2006 and September 2017. Baseline demographics, nutrition-related and general outcomes were collected. Feeding difficulties, length of stay (LOS) and malnutrition were primary outcome measures. Feeding difficulties included episodes of aspiration, gastro-oesophageal reflux disease (GORD) or vomiting. A weight-for-age Z score of <-1 was classified as malnutrition. Data were analysed using Fisher's exact tests.

Results: 49 infants, (22 boys and 27 girls) with a median admission age of 6.9 ± 10.0 days met eligibility criteria. The majority of infants were discharged on the same milk type as on admission (p<0.001*). Feeding difficulties correlated with a longer LOS (27 vs 16 days) (p=0.02*). Z-scores differed significantly between birth and discharge (0.18 vs -1.21) (p<0.001*) with malnutrition being evident in 26 infants (55%) of which only 17 infants (35%) were seen by a dietitian. Presence of intrauterine growth restriction (IUGR) increased the likelihood of malnutrition (OR 1.40(CI-1.11-1.77)).

Conclusions: Infants with PRS are at high risk of feeding difficulties and malnutrition, warranting additional intensive support from a dietitian. Infants with a longer LOS and IUGR should have their growth and feeding routinely monitored due to their increased susceptibility.
Identifying research priorities for childhood chronic conditions

Pamela Lopez-Vargas1,2, Allison Tong2,3, Sally Crowe4, Stephen I Alexander2,6, Patrina H.Y. Caldwell2,5,6, Dianne E Campbell5,7, Jennifer Couper8,9, Andrew Davidson10,11,12, Sukanya De6, Dominic A Fitzgerald15, Suzy Haddad13, Sophie Hill14, Martin Howell2,3, Adam Jaffe16,17, Laura J James2,3, Angela Ju2,3, Karine E Manera2,3, Anne McKenzie18, Angie Morrow5,19, Harrison L Odgers2,3, Ross Pinkerton20, Angelique F Ralph2,21, Peter Richmond22,23, Peter J Shaw24, Davinder Singh-Grewal25, Anita van Zwieten2,3, Melissa Wake10,12,26, Jonathan C Craig2,27 on behalf of The Kaleidoscope Project workshop investigators

Background: Chronic conditions are the leading cause of mortality, morbidity and disability in children. However, children and caregivers are rarely involved in identifying research priorities, which may limit the value of research in supporting patient-centred practice and policy.

Objective: To identify priorities of patients, caregivers and health professionals for research in childhood chronic conditions and describe the reason for their choices.

Methods: Participants included patients aged 8 to 14 years with a chronic condition (n=3), parents/caregivers of children aged 0 to 18 years with a chronic condition (n=19), representatives from consumer organisations (n=13) and health professionals (n=38). The workshop included small group discussions which enabled participants to identify and discuss their priorities for research. Discussions were audio recorded and transcripts were thematically analysed.

Results: Seventy-eight research questions were identified by the participants. Five themes underpinned participants’ priorities: maintaining a sense of normality (enabling participation in school, supporting social functioning, promoting understanding and acceptance); empowering self-management and partnership in care (overcoming communication barriers, gaining knowledge and skills, motivation for treatment adherence, making informed decisions, access and understanding of complementary and alternative therapies); strengthening ability to cope (learning to have a positive outlook, preparing for home care management, transitioning to adult services); broadening focus to family (supporting sibling well-being, parental resilience and financial loss, alleviating caregiver burden); and improving quality and scope of health and social care (readdressing variability and inequities, preventing disease complications and treatment side effects, identifying risk factors, improving long-term outcomes, harnessing technology, integrating multidisciplinary services).

Conclusion: Research priorities identified by children, caregivers and health professionals emphasize a focus on life participation, psychosocial well-being, impact on family and quality of care. Our study indicates the need for greater investment towards research that addresses the broader priorities explicitly identified by patients and those involved in their care.
A strife of interests: oral health workforce policy and planning

Balasubramanian M 1,2,3, Brennan D S3, Short S D4 & Gallagher J E2

Affiliation:
1 The University of Sydney Faculty of Health Sciences, Sydney, Australia; The University of Sydney School of Dentistry, Sydney, Australia; Western Sydney Local Health District, New South Wales Health, Australia.
2 Kings College London Dental Institute, Population and Patient Health Division, London, United Kingdom
3 Australian Research Centre for Population Oral Health, Adelaide Dental School, The University of Adelaide, Australia.
4 Faculty of Health Sciences, the University of Sydney, Sydney Australia; Sydney Asia Pacific Migration Centre, Faculty of Arts and Social Sciences, the University of Sydney, Sydney, Australia.

Correspondence: madhan.balasubramanian@sydney.edu.au

Introduction: Oral health workforce policy has often lacked systematic connections with broader health policy, and system-based reforms that would enable more effective responses to future needs of the population. The aim of the study was to learn from ‘elite’ senior leaders across the globe regarding challenges facing oral health workforce policy and planning, and to identify possible solutions.

Methods: In-depth interviews with 23 senior oral health leaders and health workforce or health policy experts at national, regional and global levels were conducted in 2016-17. Grounded theory principles using the Straussian school of thought guided the qualitative analysis.

Results: First, a ‘narrow approach towards dental education’ seems to have generally siloed the oral health system. Second, arguments have been raised pertaining to ‘imbalances in skills, jobs and competencies,’ due to increasing credentialism, and a failure to recognise and expand the skill-mix. Third, whilst ‘geographic maldistribution’ is evident across countries, several ‘health system deficiencies’ seem to have placed dentistry at the periphery of the general health care system. These three subthemes supported the development of an overarching theme – a strife of interests – which sheds light on the tension between the profession’s interest, and the needs of the population. A key aspect exemplifying the strife is the clash for power, dominance and authority within the oral health workforce and across health professions, most especially dental vis a vis medical. The evolving theory supports the thesis that the health workforce (as a whole) is mainly organised around professional interests and professional groups, rather than the needs of the population.

Conclusion: Solutions to address oral health workforce challenges in education, regulation and practice will strengthen the effectiveness of policy and planning by recognising the strife of interests as central. Innovative approaches and new models of care are required: to focus on greater oral health workforce integration; and to bring oral health more in line with general health, in order to better address unmet oral health needs nationally and globally.
Clinician-led improvement using video-reflexive methods: A workshop trial and evaluation

Su-yin Hor1,2; Mary Wyer1; Lyn Gilbert1; Zhao, Ya (Cynthia)3; Parmar, Rajesh3

1 CIDM-PH, Westmead Institute for Medical Research
2 Faculty of Health, University of Technology, Sydney
3 Westmead Hospital

Video-reflexive methods (VRM) have been used for more than a decade to successfully foster practice-improvement in healthcare settings. VRM are designed to grapple with the complexity of healthcare work, and to harness the expertise of frontline staff and stakeholders, through video feedback of everyday clinical practices and guided group reflection on this feedback. Staff are often enthusiastic and engaged in these innovative projects, and find them valuable. However, few healthcare units in Australia have adopted the method for use in clinician-led research or quality improvement projects.

This project provided a 1.5-day VRM workshop for nurses in Westmead hospital, to support them in designing, implementing and evaluating their own projects, to address issues in their own units. Support also included in-person advice during participants’ projects, and the loan of equipment. Of our six workshop participants, four were able to initiate projects, and three projects are currently underway, focusing on clinical handover, and hand hygiene.

Through semi-structured interviews and a focus group with participants, we have identified the factors that make it difficult for clinicians to undertake these projects, as well as the factors that enable them to do so. Our findings have immediate implications for developing future workshops and support for clinicians in undertaking projects using VRM. However, they also help us understand the challenges and opportunities involved when we ask healthcare professionals to undertake continuous learning and practice improvement, in order to provide safe care in increasingly complex circumstances.
A comparison of disease and treatment characteristics in people living with Human Immunodeficiency Virus (HIV) attending community-based (CB) versus hospital-based (HB) care settings in Western Sydney

Doshi J1, Ramlochun H2, Lewis DA2,3,4, Gilroy N1,3
1 Infectious Diseases Department, Westmead Hospital
2 Western Sydney Sexual Health Centre
3 Faculty of Medicine and Health, University of Sydney
4 Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney

Background/Purpose: More effective and early initiation of antiretroviral therapy after HIV diagnosis has shifted care from hospital based (HB) to community based (CB) settings. The current assessment of screening, prophylaxis and therapy aims to describe the status of service delivery.

Approach: This retrospective study compares patients presenting to Westmead Hospital (HB) and/or Western Sydney Sexual Health Centre (CB) from 2011 to 2016. Patient and disease characteristics, opportunistic infections, screening, prophylaxis and treatment variables were obtained from electronic and hard copy records. Summary data were analysed using descriptive statistics. Chi2 test was used for comparison of categorical data. Two sample comparisons of parametric and non-parametric data were determined using T-test or Wilcoxon rank-sum tests respectively. Two-tailed p value <0.05 defined statistical significance.

Outcomes/Impact: 237 new patients with HIV presented to hospital, community or combined services in Western Sydney over six years (2011-2016), with 172 (73%) seen in CB, 50 (21%) HB and 15 (6%) HB/CB. World Health Organization (WHO) Stage 1 disease at diagnosis was recorded in 177 (75%) of the combined cohort. Median CD4 count at treatment in those with HIV diagnosis before 2014 (prior to guideline changes) was 298/µL (IQR 150, 472), compared to 393/µL (IQR 194, 612) from 2014 (p=0.03). Antiretroviral treatment was accessed within 6 weeks, in accordance with year-appropriate guidelines, in 65% of the cohort. Opportunistic infections (OI) and cancers were reported in 15% and 2.5% of patients, respectively. Screening for tuberculosis was more frequent in HB compared to CB (88% vs 71%; p=0.01) and comprehensive hepatitis serostatus (Hepatitis A, B & C) more frequent in CB (70% compared to 56%(p=0.06). There were gaps in Measles, Mumps and Rubella (MMR) serostatus and prophylaxis across both centres.

Innovation and Significance: Data in this study provides a local profile of those seeking HIV care in Western Sydney and will inform future practice improvements. Disclosure of Interest Statement. There are no disclosures of interest.
Characteristics of patients attending community versus hospital-based HIV services in Western Sydney

Ramlochun H1, Doshi J2, Zabloska I1,3, Gilroy N2,3, Lewis D1,3,4.
1 Western Sydney Sexual Health Centre
2 Westmead Hospital Infectious Disease Department
3 Faculty Of Medicine and Health, University Of Sydney
4 Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney

Background: HIV has evolved from a life-threatening to a chronic illness and models of care have adapted accordingly. In Western Sydney Local Health District, most HIV out-patients are managed either at the community-based publically-funded Western Sydney Sexual Health Centre (WSSHC) or at the medicare-billed Infectious Diseases' outpatient clinic within Westmead Hospital (WH). We hypothesized that there would be a difference in demographic, sexual orientation and HIV prognostic factors among patients presenting to these two services.

Methods: We conducted a retrospective case-note review of patients who commenced or transferred their HIV care to WSSHC or WH over a 6 year period (2011-2016). We reviewed 237 patients' records (181 [76.3%] from WSSHC; 56 [23.6%] from WH). Categorical variables were analysed using contingency tables and Chi-square tests. Continuous variable were compared using t-test for 2 samples.

Results: There was a similar male:female patient ratio at both services (WSSHC, 3.6:1 vs. WH, 3.3:1; p=0.807). Those presenting to WSSHC were younger (mean age, 33.0 years [95% CI 31.5-34.5] vs. 38.9 years [95% CI 35.4-42.0]; p<0.001) and less likely to have been born in Australia (32.6% versus 55.4%; p<0.005). The heterosexual:homosexual patient ratio was higher at WH (1.47 vs. 0.96; p=0.085). Most WH patients (77.7%) were diagnosed due to clinical indications rather than through routine HIV screening. The mean viral load at presentation was lower at WSSHC (log10 4.42 [95% CI 4.22-4.62] vs. log10 4.93 [95% CI 4.69-5.18]; p<0.004). The mean CD4 count at presentation was higher in WSSHC patients (458 cells/mm3 [95% CI 419-498] vs. 325 cells/mm3 [95% CI 230-419]; p=0.003). More patients attending WH had advanced HIV disease (stage 4: 34.7%, WH vs. 6.0%, WSSHC; p<0.0001).

Conclusion: Patients presenting to WSSHC were more often younger, born overseas, diagnosed through screening and found to have less severe disease when compared to patients attending the local hospital for their outpatient care.
General Practitioner and Next of Kin details. A Ward Audit

Yeong C1, Swami V1, Smith T1,2
Affiliations: 1Westmead Hospital, WSLHD 2 Faculty of Medicine, University of Sydney.

Aim: Communicating with patients’ general practitioners(GP) and next of kin(NOK) is important for comprehensive care. We aimed to document the accuracy of GP/NOK details.

Methodology: Patients admitted to wards B5A/B5C on 09/03/18 under Respiratory Medicine were asked to nominate their GP and NOK. We cross-referenced the patient's nominated GP, GP practice, address, phone and fax numbers with records on Powerchart and Isoft patient manager (iPM). If practice name, address and contact number were available, this was recorded as complete. If details were incomplete, we undertook a simple internet search using available data. If missing information was found, this was recorded as partially complete. Otherwise details were recorded as incomplete. Post-hoc, we compared accuracy of GP details to admission method (via emergency or direct admission). NOK name and contact number were judged as correct if nominated details were concordant with iPM.

Results: 22 patients were recruited. Complete details were more often available on iPM (5 patients, 22.7%) compared to Powerchart (2 patients, 9.1%). Partially complete details reflected the same trend with iPM (13 patients, 59.1%) performing better than Powerchart (5 patients, 22.7%). Fax numbers were unavailable on Powerchart and recorded for 1 patient (4.5%) on iPM. The remainder (40.1%) had incomplete details. At the time of audit, NOK contact number was only available in iPM and was generally accurate (19 patients, 86.4%). Direct admissions had 100% of GP details recorded compared to 50% for patients admitted via Emergency.

Discussion: Correct, complete GP details were frequently unavailable. Despite combining available details with simple internet searching, GP details were unavailable for 40.9% of patients. NOK details were usually accurate. Recent changes linking iPM to Powerchart should improve data availability. Systems changes are needed to ensure GP details are accurately recorded regardless of route of admission.
Validity of routinely collected data in identifying hip fractures at a major tertiary hospital in Australia

Lieu Thi Thuy Trinh1, Helen Achat1, Sze Ming Loh2, Robert Pascoe2, Hassan Assareh1, Joanne Stubbs1, Veth Guevarra1
1Epidemiology and Health Analytics, Western Sydney Local Health District
2Orthogeriatric Service, Western Sydney Local Health District

Objectives: To examine the validity of routinely collected data in identifying hip fractures (HFs) and to identify factors associated with incorrect coding.

Method: In a prospective cohort study between January 2014 and June 2016, HFs were identified using physician diagnosis and diagnostic imaging and were recorded in a Registry. Records of HFs in the Health Information Exchange (HIE) were identified using ICD-10-AM/ACHI/ACS codes. New HFs were estimated by episode of care, hospital admission and with an algorithm. Data from the HIE and the Registry were compared.

Results: The number of HFs as the principal diagnosis (PD) recorded by episode (864) was higher than by admission (743), by algorithm (711) and in the Registry (638). The sensitivity was high for all methods (90%-93%) but the positive predictive value (PPV) was lower for episode (68%) than for admission (80%) or algorithm (81%). The number of HFs with surgery recorded in the PD by episode (639), algorithm (630) and in the Registry (623) was similar but higher than by admission (589). The episode and algorithm methods also had higher sensitivity (91%-92%) than the admission method (84%) for HFs with surgery. Factors associated with coding errors included subsequent HF, long hospital stay, intracapsular fracture, lower age, male, HF without surgery and death in hospital.

Conclusions: When it is not practical to use the algorithm for regular monitoring of HFs, using PD by admission to estimate total HFs, and PD by episode in combination with a procedure code, to estimate HFs with surgery can produce robust estimations.
Talking with patients: Improving clinician-patient communication around healthcare-associated infections using video-reflexive methods

Mary Wyer1, Suyin Hor1,2, Lyn Gilbert1,5, Ruth Barratt1, Tegan Dawson3, Elizabeth Hobby3, Kathy Dempsey4, Patricia Ferguson4,5, Mitchell Brown6, Rosemary Sadsad1,7,

1 CIDM-PH, Westmead Institute for Medical Research
2 Faculty of Health, University of Technology, Sydney
3 A6a Renal/Urology Unit, Westmead Hospital
4 Infection Prevention and Control, Westmead Hospital
5 Marie Bashir Institute, University of Sydney
6 Centre for Infectious Diseases and Microbiology, Westmead Hospital
7 Sydney Informatics Hub, University of Sydney

AIMS: Each year, around 165,000 healthcare-associated infections (HAIs) are acquired by patients in Australian acute healthcare facilities. Patient participation is increasingly recognised as a crucial component of successful infection prevention and control (IPC). However, limited clinician-patient communication about IPC and HAI means that patients may have inadequate understandings of transmission, and varying understandings of IPC strategies, and clinicians can find it challenging to have these conversations with patients.

This study is a researcher-led collaboration between Westmead Hospital Renal Unit staff, patients and families, IPC practitioners, laboratory staff and infectious disease physicians. It aims to improve clinician-patient communication around the screening and identification of multi-drug resistant organisms (MROs), with a broader aim of reducing HAIs by increasing patient involvement in IPC.

METHODS: This study used video-reflexive methods. Video recordings were made of the patient screening process on the renal unit, in the laboratory, and in the IPC offices. Clinician-patient communication was also recorded, and this was shown to patients, with their comments then added to the pool of video clips about the screening process. These clips were then shown to nurses, IPC practitioners and laboratory staff to generate discussion and strategies for improving current communication practices.

RESULTS: We report findings from the first phase of this study, where patients, nurses and IPC practitioners were able to identify communication gaps, opportunities and strategies when viewing the clips of the MRO screening process. In particular, nurses were able to better appreciate the informational needs of their patients, and to design resources to support their communication with patients during this process.

CONCLUSION: Video-reflexive methods enable healthcare professionals to view their communication practices from their patients' and colleagues' perspectives, and to better understand how they can shape patients' understandings and precautions around infection risks and behaviours.
Understanding access and equity: associations between barriers to health care and social marginalisation

Melissa Kang1,2, Fiona Robards1, Tim Usherwood1,3, Lena Sanci4, Catherine Hawke5, Marlene Kong6, Stephen Jan3, Kate Steinbeck7

Department of General Practice, The University of Sydney, Westmead, Australia.
University of Technology Sydney, Ultimo, Australia
The George Institute for Global Health, Sydney, Australia
Department of General Practice, University of Melbourne, Carlton, Australia.
School of Rural Health, University of Sydney, Orange, Australia.
The Kirby Institute, University of New South Wales, Randwick, Australia.
Discipline of Paediatrics and Adolescent Health, University of Sydney, Australia.

Aim: There is a need to address inequities in health access for different groups of young people. The purpose of this study was to identify and measure the prevalence of a range of barriers to health care, their sociodemographic correlates and the associations between barriers and social marginalisation.

Methods: Part of a mixed-method study series; this cross-sectional survey of 12 – 24 year olds across NSW with oversampling of five subpopulations of marginalised young people: Indigenous, homeless, sexuality and/or gender diverse, refugee background, geographically rural or remote. The questionnaire collected demographic information, technology use, barriers to health care, service utilisation in past six months, chronic health problems and psychological distress. A youth consultant committee assisted questionnaire development and study promotion. The questionnaire was available online and hardcopy.

Results: 1,416 young people completed the survey from 2016 – 2017, median age 18 years (IQR 4); 68.4% female, 28.7% male, 3.0% other. 426 were sexuality and/or gender diverse, 478 rural/remote, 169 Indigenous, 118 homeless and 75 of refugee background. 63.3% belonged to one or more marginalised groups. The number of chronic health conditions increased with increasing marginalisation (p<0.01), as did rates of very high levels of psychological distress (p<0.001). Young people were significantly more likely to have high or very high Kessler-10 scores if they belonged to at least one of the five marginalized groups (57.1% cf 43.4%; p <0.001).

Cost was the most prevalent barrier for all marginalised groups (26.7% to 56.1%), as well as for those belonging to no marginalised groups (45.0%). Confidentiality was only cited as a barrier by 15.2% of the whole sample. Increasing marginalisation was associated with identifying fewer barriers.

Discussion: The cost of health care for young people is a significant barrier regardless of social marginalisation.
In vivo costimulation blockade induced foxp3 Tregs from dereg mice with neonatal islet cell cluster tolerant xenografts exist memory tregs phenotype

Yuanfei Zhao

Introduction: In this study, we are verifying the Tregs from long-term tolerance mice contain the memory-Tregs, distinguished memory-Tregs from activated-Tregs phenotypically in the NICC transplant model, and assessing the suppressive function of memory-Tregs from spleens into islet transplanted Reg KO mice.

Methods: C57BL/6-DEREG mice who have eGFP attached to their Foxp3 gene were transplanted with 4000 IEQ NICC under the renal capsule, and treated IP with CTLA-4Fc (500ug) and MR-1 (500ug) at day 0, 2, 4 and 6. The phenotype of Foxp3 Tregs in the lymph nodes, draining lymph node and spleen were analyzed by flow-cytometry. CD4+GFP+ Tregs and highly selected (CD4+GFP-CD44+CD127+CD62L-) Tregs (>2×10⁵) were isolated from spleens of naïve (n=5), rejected (n=3) and tolerant (n=8) DEREG mice, all the tolerant mice over 100-day post-transplant. On day 25, those Tregs were injected into the immune-deficient transplanted Rag KO mice into the tail vein separately. On day 45, injected transplanted Rag KO mice were challenged by CD4+GFP- T cells at the ratio of 1:3 from the Naïve C57BL/6-C57BL/6-CD45.1GFP mice by tail vein. Transplanted Rag KO mice with no cells injected (n=2) and with only CD4+GFP- T cells injected (n=2) as positive and negative control groups. All mice would be sacrificed at day 100 after adoptive transfer.

Results: NICC xenograft tolerance was confirmed in recipient Foxp3-GFP mice, and rejected in control group. In phenotype, upregulations of CD44, MHC-II, CD39, especially CD127 (85.8% VS 4.3%) (p=0.002) in splenic CD3+CD4+Foxp3+Tregs occurred on the day 70 or more than 100 days after transplantation, and downregulations of CD27 (p < 0.0001) and CD62L (p = 0.0025) were found in the mouse with tolerant NICC grafts (5.6%, 3.7%), compared to Tregs of rejected group (73.8%, 36.6%) and naive-Tregs of non-transplanted mice (78.8%, 46.1%). Additionally, in pooled draining lymph nodes from the transplanted mice more than 100 days, tolerant-Tregs also showed the increased CD127 (71.3% vs 12.5%) and decreased of CD27 (15.2% vs 74.7%) and CD62L (14.6% vs 49.3%), compared to rejected-Tregs. However, the up and down in long-term pooled lymph nodes were only found in CD127 and L-selection of the comparison between rejected-Tregs and tolerant-Tregs, which from 19.4% to 43.9%, and 51.2% to 38.4% respectively. For suppressive function, the sorted adoptive-transferred Tregs and T cells can be detected separately on 71-day by flow after adoptive transfer in Rag KO mice.

Conclusions: Tolerant-Treg induced by costimulation-blockade by CTLA-4Fc and anti-CD154 antibodies in mice with NICC grafts may contain the memory-Tregs at the long-term time point, but the suppressive function need to be examined by histology further when they up to 100-day.
Correlation of Dynamic Immune Cell Profile with Individual Alloresponse and Outcome in Type 1 Diabetes Islet Transplantation Recipients

Min HU1, Elvira Jimenez VERA1, Yi Vee CHEW1, Heather BURNS3, Patricia ANDERSON1, Lindy WILLIAMS1, Karen KEUNG1, Suat DERYISH1, Xin Maggie WANG2, Shounan YI1, Wayne HAWTHORNE1, Natasha ROGERS1, Stephen ALEXANDER3, Philip O’CONNELL1

1Centre for Transplant and Renal Research, 2Flow Cytometry Core Facility, Westmead Institute for Medical Research, 3Centre for Kidney Research, Children’s Hospital at Westmead, University of Sydney, NSW, Australia

Overall objective: To identify immunosuppressive strategies that lead to enhanced proportion of Treg cells in islet-transplant (Tx)-recipients for better outcome.

Aim: To monitor the dynamic whole-blood-immune-profile (WBIP) and T-cell subsets of T1D islet-Tx-recipient pre&post-Tx, and evaluate for correlation with Tx outcomes.

Method & Material: WBIP of 4 islet-Tx-recipients (R1-4) was performed pre-&post-Tx [(at 2weeks (w), and 1, 3, 6, & 2 months (m)]. R1 received 3-islet-Tx, R2 received 2-Tx, and R3 & R4 had 1-Tx. The fold-change in cell numbers following Tx was compared to levels prior to 1st-Tx. The induction immunosuppression-regimen was ATG/Etanercept at 1st and 2nd Tx, Etanercept/Basiliximab at 3rd Tx, and additional Tacrolimus/MMF at Post-Tx.

Results: R1 had a significant reduction in insulin requirement after the 2nd-Tx, and was insulin free after the 3rd-Tx. R2 remains on insulin after 2-Txs (halved dosage), but is c-peptide positive. R3 and R4 has a partial reduction in insulin dosage. Prior to their 1st-Tx all recipients had immune-subset numbers (granulocyte, monocyte, NK, T, B cells) within normal range; except R2 who had an high granulocyte count (9383 cells/ul). We observed that the changes of dynamic WBIP for absolute cell numbers after immunosuppression differed between individual Tx-recipients (Table-1). There was substantial heterogeneity in the degree and proportions of T cell reconstitution after T cell depletion with ATG. The degree of reduction in T cell number did not correlate with successful outcomes. Reversal of CD4/CD8 T-cell ratio was observed in all recipients. Increased of CXCR3 expression on CD45RO+CD4+ memory T cells post-Tx for all recipients was observed. The proportion of FOXP3 Tregs increased in the first 2 weeks after ATG and the majority of these increased CD4+FOXP3+ Tregs were CD45RO+CD39+ memory Tregs.

Conclusion: Dynamic WBIP after transplantation can be performed and used to evaluate an individual’s response to transplantation and immunosuppression. Monitoring WBIP may be important after transplantation to guide the usage of immunosuppression to improve transplant outcome.

Table 1: The change of absolute immune cell numbers after islet transplantation in 4 recipients

<table>
<thead>
<tr>
<th>Time Point</th>
<th>CD45+ fold*</th>
<th>Granulocyte fold</th>
<th>Monocyte fold</th>
<th>B Cell fold</th>
<th>T Cell fold</th>
<th>NK fold</th>
<th>NKT fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE/R1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1w</td>
<td>1.15</td>
<td>0.99</td>
<td>1.49</td>
<td>1.17</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3M</td>
<td>1.17</td>
<td>1.18</td>
<td>1.08</td>
<td>0.87</td>
<td>1.21</td>
<td>1.07</td>
<td>1.07</td>
</tr>
<tr>
<td>5M (1W 2nd Tx)</td>
<td>2.37</td>
<td>2.07</td>
<td>1.48</td>
<td>1.61</td>
<td>41.52</td>
<td>2.85</td>
<td>14.82</td>
</tr>
<tr>
<td>6M (1M 2nd Tx)</td>
<td>2.47</td>
<td>2.03</td>
<td>2.18</td>
<td>21.15</td>
<td>2.33</td>
<td>6.70</td>
<td>6.70</td>
</tr>
<tr>
<td>PRE/R2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1w</td>
<td>2.24</td>
<td>2.28</td>
<td>2.21</td>
<td>1.19</td>
<td>2.5</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>3M</td>
<td>2.17</td>
<td>2.03</td>
<td>1.67</td>
<td>2.35</td>
<td>3.61</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6M (1M 2nd Tx)</td>
<td>2.65</td>
<td>2.42</td>
<td>2.7</td>
<td>2.993</td>
<td>6.475</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>PRE/R3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1w</td>
<td>1.52</td>
<td>1.22</td>
<td>0.87</td>
<td>2.4</td>
<td>8.83</td>
<td>2.87</td>
<td>5.93</td>
</tr>
<tr>
<td>3M</td>
<td>1.33</td>
<td>1.05</td>
<td>1.67</td>
<td>1.4</td>
<td>4.57</td>
<td>1.49</td>
<td>3.48</td>
</tr>
<tr>
<td>PRE/R4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1w</td>
<td>1.46</td>
<td>0.8</td>
<td>2.9</td>
<td>2.153</td>
<td>77.82</td>
<td>11</td>
<td>15.17</td>
</tr>
<tr>
<td>3M</td>
<td>0.88</td>
<td>0.36</td>
<td>1.45</td>
<td>3.27</td>
<td>38.21</td>
<td>6.03</td>
<td>9.38</td>
</tr>
</tbody>
</table>

*Fold: 1 indicate no change, >1 indicate decrease and <1 (in red) indicate increase compared to Pre-TX.
Measurement of mitochondrial metabolism: a rapid evaluation of regulatory T cell (Treg) function

Lei Sun, Shounan Yi, Philip O Connel
Westmead Institute for Medical Research

Aims: Treg quality assurance is a key issue to enable their therapeutic efficacy. Currently Treg function test is performed by MLR assay which needs 5-7 days to detect cell proliferating status, which is unable to predict Treg function in real time, thereby affecting Treg therapy outcomes. Therefore developing a sensitive and rapid evaluation of Treg suppressive function in vitro prior to their adoptive transfer is required to achieve controllable and efficient immunotherapy. In this study we tested whether determination of energy utilization of human PBMC cells could be developed as a rapid and reliable measurement of Treg suppressive function.

Methods: Human PBMC as responder cells were cultured alone or with polyclonal or xenogeneic stimulators in the absence and presence of autologous Treg. After 24 hr in culture, mitochondrial energy utilization was measured by a Seahorse Extracellular Flux Analyzer and presented as oxygen consumption (OCR) and extracellular acidification rate (ECAR).

Results: Responder cells were activated with high levels of OCR and ECAR after 24 hr in culture with either polyclonal or xenogeneic stimulation. However, the increased levels of OCR and ECAR in responder cells were markedly downregulated when cocultured with Treg, suggesting the impaired activation or proliferation of responder cells in the presence of Treg. These results are consistent with conventional CFSE dilution measurement to evaluate activated and/or proliferating status in effector cells.

Discussions: Mitochondrion function indicates cell activation, which may reflect early proliferating status of effector cells upon immunological stimulations, and could be used as an indicator to reveal Treg suppression of immunoresponses by a rapid and real-time detection of effector cell activation.
THE USE OF DRUG REPURPOSING TO IDENTIFY NEW TREATMENT OPPORTUNITIES FOR ACUTE KIDNEY INJURY

Mary El-Rashid1,2, Barkha Sanganeria 1,2, Sohel Julovi1,2 and Natasha M. Rogers1,2

1 Centre for Transplant and Renal Research, Westmead Institute for Medical Research
2 Westmead Clinical School, University of Sydney

Aim: To determine the role of drug repurposing to reduce acute kidney injury.

Background: Acute kidney injury (AKI) is a major contributor to mortality and morbidity in hospitalized patients. Despite decades of research there are no therapeutics available to treat or prevent AKI. Drug development is a lengthy, complex and costly process. Drug repurposing, using already approved drugs to treat diseases that differ from the primary indication, can circumvent these issues.

Methods: Human kidney biopsy samples with and without AKI were subject to RNA sequencing and gene profiling to identify dysregulated gene pathways using Integrated Pathway Analysis. Drugs that target these pathways and are already approved for clinical use were identified. Age- and gender-matched C57BL/6 mice were challenged with renal ischemia-reperfusion injury (IRI) (a model of acute kidney injury) and treated with relevant drugs. All animals underwent analysis of renal function and biomolecular phenotyping.

Results: Human kidney biopsies with AKI showed dysregulation of PTEN, STAT3 and pro-inflammatory cytokine signalling pathways compared to non-AKI controls. Metformin, doxycycline and colchicine were readily identified as affecting these pathways, respectively. C57BL/6 mice subjected to AKI were treated with intraperitoneal injections of a vehicle control, metformin (0.25 mg/kg/day, 3 days prior to IRI), doxycycline (10 mg/kg/day, 3 days prior to IRI) or colchicine (0.4 mg/kg, 1 hour prior to IRI). All drug treatments led to a statistically significant decrease in serum creatinine (untreated: 140±29 mmol/L versus metformin: 42±25 mmol/L, p<0.0001; doxycycline: 53±26 mmol/L, p<0.001; colchicine: 66±31 mmol/L, p<0.01). These findings correlated with improvements in histology by light microscopy and a decrease in pro-inflammatory cytokine infiltrates in whole kidney homogenate by qPCR.

Discussion: The use of bioinformatics is a powerful tool for identifying relevant molecular pathways in diseases and can lead to new treatment opportunities.
CD47 BLOCKADE MODULATES FIBROSIS IN CHRONIC KIDNEY INJURY

Sanganeria Barkha 1, Rogers Natasha 1,2,3

1 Westmead Institute For Medical Research, Westmead NSW, Australia
2 Westmead Hospital, Westmead NSW, Australia
3 The University of Sydney, Camperdown NSW, Australia

Aim: Acute kidney injury triggers a complex cascade of molecular responses that can culminate in maladaptive repair and fibrosis. We have previously reported that the matrix protein thrombospondin-1 (TSP1) and its receptor CD47 are induced following kidney injury. However, the role of this axis has not been characterized in chronic kidney disease (CKD).

Methods: Age and gender-matched wild type (WT), CD47 knockout (CD47-/-) and WT mice treated with CD47 blocking antibody (WT+CD47Ab) were compared in two chronic kidney injury models: unilateral ischemia-reperfusion and contralateral nephrectomy (RN), and unilateral ureteric obstruction (UUO). All animals underwent analysis of renal function and bimolecular phenotyping. Human and murine WT and CD47-/- renal tubular epithelial cells (rTEC) were studied in vitro. TSP1 plasma levels from patients at different stages of CKD were measured.

Results: WT, WT + CD47Ab, and CD47-/- RN mice showed no difference in serum creatinine regardless of injury model, however there was clear amelioration of renal histological changes and fibrosis with the blockade or knockout of CD47. WT RN mice showed upregulated mRNA and protein levels of TSP-1 and pro-fibrotic markers TGF-ß, SMAD2, smooth muscle actin, fibronectin and collagen I. These markers were significantly abrogated in both CD47-/- and WT + CD47Ab counterparts. Interestingly, both WT and CD47-/- UUO mice showed equivalent increases in pro-fibrotic tendency, regardless of overall TSP1 expression. Renal tubular epithelial cells isolated from WT mice showed robust upregulation of TSP1 and pro-fibrotic markers under hypoxic stress, which was mitigated in CD47-/- cells. ELISA results from patient samples showed proportionate correlation in TSP1 levels with the increased CKD stage and the patients on dialysis showed maximum increase in TSP1 protein.

Discussion: These data suggest that renal tubular epithelial cells contribute to fibrosis by activating TSP1-CD47 signaling, and point to CD47 as a target to limit fibrosis following injury.
IN VITRO SCREENING OF GENES ASSOCIATED WITH KIDNEY FIBROSIS

Xiaoqian Ma, Lei Sun, Lu Cao, Padma Srikaranth, Dandan Huang, Yuanfei Zhao, Shounan Yi, Philip O’Connell
Centre for islet transplantation and renal disease, Westmead Institute for Medical Research.

Aims: Chronic injury in kidney transplants remains a major cause of allograft loss. Our GoCAR multicenter study has identified a set of 13 genes were independently predictive for the development of fibrosis at 1 year after kidney transplantation. The high predictive capacity of the gene set was superior to clinical indicators. The aim of this study was to identify one or few of these genes which are associated with pathogenesis of kidney fibrosis.

Methods: The murine C1.1 tubular epithelial cell line, FOXO−/− C1.1 and TCF−/− C1.1 cell line were treated with or without TGF-β for 48h. Then the cells were harvest for real-time PCR to detect the expression of the 13 genes.

Results: We found there were big changes for four genes’ expression. In C1.1 cell lines, FJX1, CHCHD10, KLHL13 and ASB15 were low expressed while TGF-β induced their expression. It seems they may be regulated by TGF-β. In FOXO−/− cell line, when cells lost the fibrosis suppressor FOXO, the expression of FJX1, CHCHD10, KLHL13 and ASB15 were significantly upregulated by TGF-β, compared to the untreated control group, up to more than 5 times’ fold. In TCF−/− cell line, without the fibrosis promotor TCF, the expression of FJX1, KLHL13 and ASB15 were not upregulated even with the TGF-β treatment. While the CHCH10 was highly expressed by TGF-β treatment in TCF−/− cell line.

Discussion: The results suggested the four genes may be involved with the signaling pathway of fibrosis and we will further confirm their function by CRISP/CAS9 technique.
DEVELOPING PHOSPHOLIPASE A2 RECEPTOR ScFv FOR CAR TREGS FOR THE TREATMENT OF AUTOIMMUNE RENAL DISEASE

Jevin Karunia1, Yuan Min Wang1, Geoff Y. Zhang1,2, Andrew Wilaras1, Mahnoor Bakhtiar1, Hugh McCarthy2, Stephen I Alexander1,2,3
1Centre for Kidney Research, Kids’ Research, The Children’s Hospital at Westmead, Sydney; 2University of Sydney; 3Department of Nephrology, The Children’s Hospital at Westmead, Sydney

Background: Idiopathic Membranous nephropathy (IMN) is a leading cause of autoimmune renal disease driven in many cases by the recently described cognate antigen M-type phospholipase A2 receptor (PLA2R). Chimeric antigen receptors (CAR) T cells use antibody fragments to direct T cells to specific antigens, and have achieved clinical success in cancer. The strategy can be translated to treat IMN, an autoimmune condition that involves PLA2R, a target antigen that is exclusively expressed on the podocyte epithelial lining of the kidneys.

Aims: We aim to use PLA2R as a target antigen for treating IMN and design a single chain fragment of variable region (ScFv) to use in PLA2R-CAR-Tregs directed towards this antigen. PLA2R-specific monoclonal antibodies are generated against this antigen on human, mouse and rat podocytes.

Method: By using genetic sequence search tools, the PLA2R sequence across three species of human, mouse and rat, were aligned and compared to generate common peptide immunogens. Immunofluorescence (IF) staining was performed on a conditionally-immortalized podocyte (ciPod) cell line and frozen kidney sections of human, mice and rat for M-Type PLA2R expression in vitro as an assay for antibody testing. Mice were immunized with the PLA2R peptides to produce monoclonal antibodies against PLA2R. Hybridomas were established and screened and the hybridoma antibody sequenced for use in making the ScFv for the CAR construct.

Results: We have confirmed expression of the M-type PLA2R in human, mouse and rat podocytes on their cell membrane by IF. The anti-PLA2R monoclonal antibody (mAb) has been detected in the mouse sera of immunized mice by Western Blot and ELISA. The mAb hybridoma is being sequenced. The anti-PLA2R mAb from these hybridomas is reactive for M-Type PLA2R.

Conclusion: We have developed hybridomas against a podocyte target antigen that is also a disease antigen in membranous nephritis and are developing this as a kidney targeting strategy.
A20 regulation of inflammation in a mouse model of ischemic acute kidney injury

Danny Nguyen-Ngo1,2, Mary El-Rashid1,2, Barkha Sanganeria1,2, Shane Grey3, and Natasha M. Rogers1,2

1 Centre for Transplant and Renal Research, Westmead Institute for Medical Research
2 University of Sydney
3 Garvan Institute of Medical Research

Aim: To determine the role of A20 in acute kidney injury.

Background: Acute kidney injury (AKI) is a major contributor to mortality and morbidity in hospitalized patients. A20 is a zinc finger protein that is upregulated in cells during inflammation, specifically in response to the production of tumor necrosis factor-alpha (TNF-α). The role of A20 is to mitigate apoptosis and inhibit NF-kB activation. Therefore, it is vital in regulation of the inflammatory response. Overexpression of A20 has been shown to be protective during inflammation. However, investigation on the role of A20 in acute kidney injury is unknown.

Methods: C57BL/6 mice underwent ENU mutagenesis to produce mutant A20 (∆) mice, which possess a single nucleotide polymorphism (SNP) within the A20 locus. Age- and gender-matched littermate control mice (wild-type, WT), heterozygous mice (A20∆/+ ) and homozygous (A20∆/∆) were challenged with renal ischemia reperfusion injury (IRI). All animals underwent analysis of renal function and biomolecular phenotyping.

Results: WT mice subjected to IRI demonstrated upregulation of the pro-inflammatory cytokine TNF-α but no significant change in A20 expression compared to sham-operated animals. A20∆/∆ and A20∆/+ mice both demonstrated lower serum creatinine (mean 56.3 ± 33.74, 91.69 ± 51.24 µmol/L respectively) compared to their littermate controls at 24 hrs reperfusion (mean 119.3 ± 57.62 µmol/L). There was a significant difference between A20∆/∆ and WT serum creatinine (p<0.01) and no difference in weight loss. This result was reflected in decreased histological damage by light microscopy. qPCR of whole kidney tissue revealed significant increased expression of inflammatory markers IL-1β, CXCL2, TNF-α, and A20 between mutant mice and littermate controls.

Discussion: This SNP variant of A20 seems to be paradoxically protective in ischemic AKI, despite increased inflammatory marker expression in A20∆/∆ mice. Identifying human A20 homologs to this SNP variant may provide an additional screening marker in the clinic.
ACTIVATED CD47 PROMOTES ACUTE KIDNEY INJURY BY LIMITING AUTOPHagy

Mary El-Rashid1,2, Barkha Sanganeria1 and Natasha M. Rogers1,2,3

1Westmead Institute for Medical Research
2The University of Sydney
3Westmead Hospital

Aim: To explore the role of CD47 in acute kidney injury (AKI) and its therapeutic potential. Background: AKI is a common clinical disorder that initiates a complex pathophysiological cascade leading to epithelial cell death. Recent studies identify autophagy, the mechanism of intracellular degradation of cytoplasmic constituents, as important in protection against injury. We have reported that the protein thrombospondin-1 (TSP1), and its receptor CD47, are induced in AKI, however their role in regulating renal injury is unknown.

Methods: Age and gender-matched wild-type (WT) and CD47-/- mice were challenged with renal ischemia reperfusion injury, a model of AKI. All animals underwent analysis of renal function and biomolecular phenotyping. Human and murine WT and CD47-/- renal tubular epithelial cells (rTEC) were studied in vitro.

Results: CD47-/- mice were resistant to AKI, with decreased serum creatinine (140±26 versus 59±15 mmol/L, p<0.001), and ameliorated histological changes compared to WT animals. CD47-/- mice demonstrated concurrent upregulation of key autophagy genes, including Atg5, Atg7, Beclin-1, and LC3 at baseline and post-AKI. Electron microscopic examination of kidneys revealed increased autophagosome and autolysosome numbers in CD47-/- animals (2.8±1.6 versus 6.9±3.3, p<0.01). WT mice demonstrated negligible autophagy expression at all time points. rTEC from CD47-/- mice displayed basal upregulation of autophagy that was preserved under hypoxic stress and correlated with enhanced viability when compared to WT cells. Treatment of WT rTEC with a CD47 antagonist antibody or oligonucleotide to block TSP1-CD47 signalling increased autophagy. Finally, in a syngeneic mouse kidney transplantation model, treatment with a CD47 blocking antibody improved renal function and decreased histologic damage compared to control mice, and this was associated with increased autophagy.

Conclusions: These data suggest activated CD47 is a proximate promoter of AKI through inhibition of autophagy and point to CD47 as a target to restore renal function following injury.
Generating Chimeric Antigen Receptor Tregs to suppress the allogeneic response against HLA-A2

Samuel J. Robinson1,2, Yuan Min Wang1,2, Geoff Y. Zhang1,2, Jevin Karunia1, Mahnoor Bakhtiar1, Victor F. Shen1,2, Min Hu2,3 & Stephen I. Alexander1,2,4
1Centre for Kidney Research, The Children's Hospital at Westmead; 2The University of Sydney; 3Centre for Transplant and Renal Research, The Westmead Institute for Medical Research; 4Department of Nephrology, The Children's Hospital at Westmead

Background: Chronic allograft nephropathy (CAN) driven by the immunological rejection of mismatched antigens on allograft tissues remains a key driver of long-term graft loss. Such rejection is characterised by sustained adaptive immune responses directed against the graft. Regulatory T cells (Tregs) are CD4+Foxp3+ lymphocytes that mediate self-tolerance and suppress autoimmunity in vivo. Tregs have been shown to exert protective effects in cases of renal inflammatory diseases, and it has been suggested that alloantigen-specific Tregs generated through chimeric antigen receptor (CAR) technology may effectively suppress alloimmune responses in renal transplant. Human Leukocyte Antigen A2 (HLA-A2) is the most commonly mismatched alloantigen in renal transplantation. We have obtained A2Kb mice that express HLA-A2 fused to H-2Kb. We suggest that Tregs directed against HLA-A2 through insertion of a CAR will mediate suppression of the alloimmune response against HLA-A2 in vitro.

Aim: To generate CAR Tregs against HLA-A2 and assess their immunosuppressive capacity against the allogeneic immune response in vitro.

Methods: CAR Treg specific to HLA-A2 (CAR-A2) have been produced via retroviral transduction of murine Foxp3+ Tregs isolated from Ly5.1+Foxp3-GFP reporter mice. HLA-A2 transgene presence on A2Kb mouse kidneys was assessed through immunofluorescent staining of kidney sections for HLA-A2 and glomerular nephrin expression. CAR-A2 activation will be assessed through co-culture with K562-HLA-A2+ and HLA-A2- cells. CAR-A2 cytokine expression will be assessed by cytokine bead array for IL-2, IL-4, IL-6, IL-10, IL-17a, TNF-β, and IFN-γ; RT-qPCR for transcription factors FOXP3, T-bet, RORyt, Blimp-1, and GATA3; and proliferative capacity by CellTrace Violet staining. CAR-A2 activation will be compared to that of polyclonal Tregs and control CAR Tregs directed against HER2.

Results and Conclusion: HLA-A2 expression on A2Kb kidney cells has been confirmed by immunofluorescence. In addition, data detailing the cytokine profile, transcription factor activation, and proliferation of CAR Tregs stimulated with HLA-A2 will be presented.
Lipoplysaccharide Exacerbate Complement-associated Kidney Disease and the Development of AAV therapy in a Mouse Model of Known Human CFH Mutation

Victor F. Shen1,3, Geoff Zhang1,3, Samuel J. Robinson1,3, Mahnoor Bakhtiar1,3, Jevin Kurunia1,3, Min Hu2,3, Jimmy Zhou2,3, Hugh McCarthy1,3, Yiping Wang2,3, Qi Cao2,3, Guoping Zheng2,3, Natasha Rogers2,3, Tom D. Barbour4, Ian E. Alexander3,5, David Harris2,3, Stephen I. Alexander1,3 & Yuan Min Wang1,3
1Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia
2Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney, Australia
3The University of Sydney
4Department of Nephrology, Royal Melbourne Hospital, Melbourne, Australia
5Gene Therapy Research Unit, Children's Medical Research Institute, The Children's Hospital at Westmead, Sydney, Australia

Background: Atypical haemolytic uremic syndrome (aHUS) is commonly associated with complement factor H (CFH) mutation. CFH is a liver-secreted glycoprotein and a negative regulator of the alternative complement pathway. aHUS patients rapidly progress to acute kidney injury (AKI) due to complement-mediated renal insult. CFH mutation is predisposing, and additional infections are required to exacerbate disease. However, the role of infection in triggering disease remains elusive. Recombinant Adeno-Associated Virus (rAAV) have shown immense therapeutic potential and AAV8, in particular, transduce murine hepatocyte potently.

Aims: To evaluate whether lipopolysaccharide (LPS) induces acute kidney injury in CFH mutant mice and assess the therapeutic potential of a liver-mediated AAV therapy.

Methods: C57BL/6 mice carrying a single nucleotide polymorphism in CFH resembling human aHUS was generated by N-Ethyl-N-Nitrosourea mutagenesis. Homozygous (HOM), heterozygous (HET) and wildtype (WT) mice were treated with LPS via intraperitoneal injection. Renal function, histology and C3 immunofluorescence were assessed post-treatment. Serum and liver CFH levels were also measured. A heparin sulfate assay will be performed to assess the function of mutated CFH. A rAAV8 containing a liver-specific promoter and functional CFH will be administered intravenously into susceptible mice. Hepatic vector transduction will be assessed by vector copy number qPCR and therapeutic outcome evaluated by renal function and C3 immunofluorescence.

Results: HOM mice showed spontaneous C3 deposition in glomeruli and had minimal serum concentrations of CFH compared to HET and WT. Following LPS treatment, HOM mice showed significantly higher proteinuria (3.1±0.2mg/16h) and serum creatinine (28.3±3µmol/L) compared to HET (1.3±0.4mg/16h, 20.3±2µmol/L, p<0.01 and p<0.05 respectively) and WT mice (1.2±0.2mg/16h, 17.6±1.6µmol/L, p<0.01 and p<0.05 respectively). Functional assay and AAV data will be presented.

Discussion: Homozygous mice accumulated glomerular C3 and are sensitive to LPS, developing AKI, suggesting a significant role for infection in triggering aHUS in susceptible individuals. Therapeutic potential of AAV will be discussed.
DIETARY SODIUM INTAKE IS CORRELATED WITH SERUM COPEPTIN IN EARLY-STAGE AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

JQJ ZHANG1,2,3, C MANNIX1,2, A RANGAN3, ATY WONG1,2, GK RANGAN1,2
1Centre for Transplant and Renal Research, Westmead Institute for Medical Research, University of Sydney, NSW
2Department of Renal Medicine, Westmead Hospital, NSW
3University of Sydney, Camperdown, NSW

Aim: To determine the association between dietary sodium and protein intakes with serum copeptin (a marker of arginine vasopressin (AVP)) in patients with autosomal dominant polycystic kidney disease (ADPKD).

Background: Elevated levels of AVP are hypothesised to worsen renal cyst growth in ADPKD. Apart from low fluid intake, the dietary intake of sodium and protein also influence AVP levels, but this has not been specifically investigated in ADPKD.

Methods: Patients with ADPKD (18-65 yrs, eGFR≥30 mL/min/1.73m2) underwent a structured diet history interview to assess usual intake over the past 3 months, as part of the PREVENT-ADPKD study. Twenty-four-hour urinary sodium and urea excretion were measured to validate the diet history-reported sodium and protein intakes, respectively. Serum copeptin was measured by a sandwich immunoassay (B.R.A.H.M.S).

Results: Twenty-nine participants (aged 42±12 yrs; BMI 26±5 kg/m2; mean±SD) were analysed. The diet history was a valid method for estimating dietary protein, as reported intakes demonstrated a strong correlation with 24-h urine-derived estimates (r=0.658; P<0.001), and there was no evidence of systematic bias by the Bland-Altman method. Median±IQR serum copeptin concentration was 4.09±8.55 pmol/L. Multivariate analyses (adjusted for age, gender, 24-h urine volume and serum creatinine) revealed that higher serum copeptin was strongly associated with higher 24-h urine sodium (B=0.695; P=0.017) (the gold-standard for dietary sodium intake), but not with diet history-reported sodium or protein intake, 24-h urine urea, urine osmolality or dietary solute intake.

Conclusions: These cross-sectional, observational data support the hypothesis that dietary sodium intake stimulates AVP release in ADPKD. An ongoing randomised clinical trial will determine the combined long-term effects of adequate hydration and lowering dietary sodium intake on serum copeptin and the renal progression of ADPKD.
Preclinical assessment of anti-Midkine antibodies in reducing kidney injury and dysfunction induced by Adriamycin nephropathy

WANG JEFFREY1, CAO QI1, WANG YIPING1, HARRIS DAVID1, ROBERTSON GRAHAM 2, BURG DOMINIC 2, HALASZ MARIA2, LEE VINCENT1
1Centre for Transplantation and Renal Research, University of Sydney at Westmead Institute of Medical Research, Westmead, Australia.
2Cellmid Limited, Sydney, Australia.

Aim: To determine the efficacy of anti-Midkine (MK) antibodies in ameliorating kidney damage and dysfunction in the Adriamycin (ADR) nephropathy model.

Background: MK is known for its role in development and cancer where it promotes cell survival, proliferation and migration. Additionally, MK is implicated in inflammation and autoimmunity where it modulates chemotaxis, chemokine expression and immunological tolerance. Clinical studies have found progressive increases in systemic and urinary MK levels related to disease severity in chronic kidney disease (CKD)1-CKD5 patients. MK levels are also elevated in diverse experimental kidney disease models post injury or pathogenesis, and are associated with renal inflammation, fibrosis and progressive injury. More importantly, MK-deficient mice exhibit markedly reduced glomerulosclerosis, tubulointerstitial inflammation and damage than MK +/- mice in ischaemic, diabetic and nephrectomy models, suggesting that MK's pathological role in exacerbating renal injury and dysfunction operates primarily via its immunomodulatory functions.

Methods: ADR was administered once to male Balb/c mice via tail vein injections. Anti-MK antibody treatment was performed in prophylaxis mode, and antibodies were administered by intraperitoneal injections to ADR-treated mice periodically every 5 days starting from one day prior to ADR treatment. All mice were sacrificed 27 days after the initial antibody treatment for subsequent tissue processing and biochemical analysis for assessment of renal histology and function.

Results: ADR-treated mice administered with anti-MK antibodies exhibited significantly reduced overall kidney tissue damage in contrast with those administered with vehicle. Reduced proteinuria, serum creatinine and improved serum albumin levels were also observed in anti-MK antibody administered mice.

Conclusions: Our results indicate that MK plays a crucial role in modulating kidney injury and dysfunction in a murine model of FSGS, and therapeutic antibodies against MK may contribute to the treatment of chronic proteinuric renal disease.
Targeting CD103+ Dendritic cells using Flt3 inhibitors for treatment of kidney disease; relevance to human kidney disease

Aims: 1. To examine CD141+ dendritic cells (DCs; human homologue of CD103+ DCs) in human kidney diseases.
2. To explore the role of CD103+ DCs and therapeutic potential of targeting CD103+ DCs by repurposing Flt3 inhibitors in experimental kidney diseases.

Background: Whereas CD103+ DCs were previously considered to be a minor DC subset in kidney disease, we and others have proven that they have a major role. Flt3 is a receptor specifically expressed on tissue CD103+ DCs. Flt3 inhibitors are currently used for cancer treatment.

Methods: For a human study, we included 294 patients who underwent kidney biopsies from 01/07/2016 to 01/04/2017. For animal experiments, we are using Adriamycin Nephropathy (AN), anti-GBM disease and ischaemia reperfusion injury (IRI).

Results: In humans, the number and proportion of CD141+ DCs were significantly increased in proliferative glomerulonephritis and acute tubular necrosis (ATN). CD141+ DCs were found mainly in tubulointerstitium, except in lupus nephritis where they were also present in glomeruli. CD141+ DC numbers correlated with increasing severity of ATN (P<0.001) as well as increasing severity of fibrosis in IgA nephropathy (P=0.025), but not in diabetic nephropathy.

In murine AN, anti-GBM disease and IRI, the number and proportion of kidney CD103+ DCs were significantly increased. In AN, CD103+ DCs played a pathogenic role through activation of CD8+ T cells. Treatment with a Flt3 inhibitor specifically depleted CD103+ DCs and significantly reduced renal injury. The effect of Flt3 inhibition is currently being studied in anti-GBM disease and IRI.

Conclusions: Kidney CD103+ DC numbers correlate with severity of human kidney disease. In experimental kidney disease, CD103+ DCs play a pathogenic role through activation of CD8+ T cells. Targeting CD103+DCs with Flt3 inhibitors effectively reduces renal injury, suggesting a novel therapeutic strategy with accelerated translational potential through drug repurposing.
VALIDATION OF A SCORED SALT QUESTIONNAIRE TO SCREEN FOR HIGH DIETARY SALT INTAKE IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

A TY WONG1,2, C MANNIX1,2, J ZHANG1,2, A RANGAN3, G RANGAN1,2
1Centre for Transplant and Renal Research, Westmead Institute for Medical Research, University of Sydney, Westmead, NSW
2Department of Renal Medicine, Westmead Hospital, Western Sydney Local Health District, Sydney, NSW
3Nutrition and Dietetics, School of Life and Environmental Science, The University of Sydney, Sydney, NSW.

Aim: To test the hypothesis that the SSQ is reliable for detecting HSI in adults with ADPKD.

Background: The restriction of dietary salt (≤100mmol/L) may reduce the growth of kidney cysts in autosomal dominant polycystic kidney disease (ADPKD). The Scored Sodium Questionnaire (SSQ) is a validated tool to screen for high dietary salt intake (HSI) in elderly patients with Stage 3-5 Chronic Kidney Disease but its use in ADPKD is not known.

Methods: Patients with ADPKD (18 to 67 years old; eGFR³30 mL/min/1.73m2) participating in the PREVENT-ADPKD study self-completed the SSQ at the Screening Visit. HSI was defined as 24-hour urine sodium excretion >100mmol/L. Receiver operating characteristic (ROC) analysis was used to determine the ideal cut-off for SSQ score in ADPKD population.

Results: The majority (78%) of the cohort (n=126; 43% male; 44±12 years old; 24-hour urine sodium 144±56mmol/d mean±SD) were HSI consumers. HSI positively correlated with younger age and male gender but not height-corrected total kidney volume. The SSQ score was higher in HSI (76.4±22.1 vs. 65.3±24.9; P=0.02) but correlation with 24-hour urine sodium was weak (Spearman rho r=0.184, P=0.040). By multivariate analysis, the combination of age, gender and the SSQ score predicted HSI (P<0.001). ROC analysis identified the ideal SSQ cut-off for was 74 to identify HSI (area under the curve, sensitivity and specificity was 0.644, 61% and 75%, respectively).

Conclusion: Consideration of demographic factors (age<40 years old; male gender) together with the SSQ score (>74) may assist in identifying ADPKD patients at risk for HSI who might benefit from formal dietetic consultation. Further refinement of the SSQ scoring system might help improve the performance of this approach in the ADPKD population.
SEASONAL CHANGES IN FLUID INTAKE IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

CJ MANNIX1,2, A RANGAN3, JQI ZHANG1,2, GK RANGAN1,2, ATY WONG1,2
1Centre for Transplant and Renal Research, Westmead Institute for Medical Research, University of Sydney, Westmead, NSW
2Department of Renal Medicine, Westmead Hospital, Westmead, Sydney, NSW.
3Nutrition and Dietetics, School of Life and Environmental Science, University of Sydney, Sydney, NSW.

Aim: To determine the influence of season on fluid intake in patients with autosomal dominant polycystic kidney disease (ADPKD).

Background: Adequate hydration has been hypothesised to reduce the progression of ADPKD. Multiple factors influence fluid intake behaviour and specifically the role of seasonal variations in patients with ADPKD are not known.

Methods: The mean baseline daily fluid intake was assessed in patients with ADPKD (18-65 years old; eGFR≥30ml/min/1.73m²; n=100 and living primarily in Sydney and Perth) who were screened for the PREVENT-ADPKD study (a randomised controlled trial to determine if adequate hydration will slow kidney growth in ADPKD). Fluid intake was assessed using a beverage frequency questionnaire (BFQ) (previously validated for this purpose in this population), 24-hour urine volume, osmolality and other serum markers of hydration. One-way ANOVA was used to analyse inter-group differences.

Results: Fifty-three percent of the cohort were male with a mean age of 43±11 years old and BMI 27±5kg/m². The mean daily fluid intake in summer (n=20, 3040±930ml), autumn (n=28, 2843±1076ml), winter (n=18, 2822±1138ml) and spring (n=34, 2715±1027ml) were similar and the inter-seasonal differences were not significant (P=0.748). Similarly, 24-hour urine volume did not vary by season (P=0.221), nor did 24-hour urinary osmolality and sodium, or serum sodium and osmolality.

Conclusion: Fluid intake was stable through the year with no increase during the warmer months. These baseline data suggest that additional coaching during summer may be warranted in patients who are randomised to the prescribed fluid intake group (the intervention) in the PREVENT-ADPKD study. The limitations of this data are the sample size and that further studies are needed to determine if seasonal variations in intra-individual fluid intake exist.
Targeting CD47 and aryl hydrocarbon receptor axis to prevent the cardiovascular events in uremia

Sohel M Julovi1, Natasha M Rogers1

1, Kidney Injury Group, Centre for Transplant and Renal Research, Westmead Institute for Medical Research

Aims of the study:
Patients with chronic kidney disease (CKD) are exposed to uremic toxins and have an increased risk of cardiovascular disease. Some uremic toxins, like indoxyl sulfate (IS), are agonists of the transcription factor aryl hydrocarbon receptor (AhR). Thrombospondin-1 (TSP1)-CD47 signaling pathway is a major mechanism for driving endothelial cell senescence, leading to suppression of cell proliferation. Little is known about the link between TSP1-CD47-AhR axis and human vascular smooth muscle cell (hVSMC) senescence and cell proliferation. This study investigated the effects of TSP1 and IS on hVSMC proliferation and underlying mechanisms.

Methods:
Primary human aortic vascular smooth muscle cells at passage 3 were used in this study. Cells proliferation assay was measured by the XTT-assay at 24h and 48h. Immunoblotting and immunofluorescence (IF) microscopy were used to see the expression of AhR. Activated ERK2/1, CD47 and proliferation marker of Ki67 were assessed by immunoblotting.

Results:
TSP1 significantly down regulates hVSMC proliferation at low concentrations, while high concentrations of IS is required. Preincubation with anti-CD47 antibody, reversed the downregulation effects of TSP1 and IS on cell proliferation. IF microscopy suggest that hVSMCs constitutively express AhR mostly in cytosol, while TSP1 increased expression of AhR in both cytosol and nucleus. Western blotting confirmed the increased expression of AhR by low doses of TSP1, while high doses of IS down regulates cytosolic AhR possibly due to the increased translocation into the nucleus. Interestingly, IS activates ERK2/1 in a dose depended manner. Suggesting that IS induced ERK2/1 activation independent to AhR, remain to be elucidated.

Discussion:
Combined with their inability to proliferate, VSMC senescence promotes fibrous cap thinning by compromising its repair after rupture. Our findings demonstrate that TSP1-CD47 signaling is an important mechanism driving hVSMC proliferation. Thus, TSP1 and CD47 provide attractive molecular targets for treatment of CKD associated cardiovascular dysfunction and diseases involving hVSMC dysregulation.
Partial versus Complete Thrombosis Moderated by Intra-operative Vasopressor use in SPK Patients

Sara Shahrestani1, Erin Spike2, Amy Hort2, Thomas Gibbons1, Kerry Hitos1,3, Rebecca Lendzion2, Paul Robertson4, Henry C Pleass1,2, Kathy Kable4, Vincent Lam2, Ronald de Roo2, Lawrence Yuen2, Wayne J Hawthorne1,2,5.

1Sydney Medical School, University of Sydney, Australia; 2Department of Surgery, Westmead Hospital, Westmead, Australia; 3Westmead Research Centre for Evaluation of Surgical Outcomes, Department of Surgery, Westmead Hospital, Westmead, NSW, 2145, Australia 4Department of Transplantation, Westmead Hospital, Westmead, Australia; 5Centre for Transplant and Renal Research, The Westmead Institute, Westmead, Australia

Aims: Simultaneous pancreas-kidney (SPK) transplantation is the gold standard treatment for patients with type 1 diabetes and end stage renal failure. Thrombosis is a devastating complication of SPK that can result in graft loss and return to theatre for pancreatectomy.

Methods: We reviewed 235 SPKs performed at Westmead hospital over the past decade (2008-2017). We examined risk factors (donor and recipient) and characteristics of thrombosis in order to ascertain the clinical course for patients.

Results: 41 (17.4%) of patients experienced a thrombosis. In 85% (35/41) of cases, this thrombosis occurred early, in the first 6 weeks following transplantation. The majority of thromboses (68%, n=28/41) were venous. Importantly thrombosis associated with graft loss and pancreatectomy only occurred in less than half of the patients with a thrombosis (n=17, 7.2%). Graft loss was strongly associated with the use of intraoperative vasopressors with 71% (n=12/17) of the patients that lost their graft requiring intraoperative vasopressors, while only 46% (n=11/24) of those with partial thrombosis required this intervention.

Conclusion: While graft thrombosis is a devastating complication of SPK transplantation that leads to graft loss, it is reassuring to know that less than half of the grafts that thrombose are lost and require return to theatre for pancreatectomy. A strong risk factor for thrombosis leading to graft loss is the use of intra-operative vasopressors, leading us to believe careful management of blood pressure may be key to reducing the devastating outcomes of this not uncommon complication.
Background: The use of ante-mortem interventions in transplantation remains contentious due to ethical concerns and potential for harm to donors. There is variability in the acceptance and use of ante-mortem interventions across individual centers.

Methods: We conducted semi-structured interviews with 42 clinicians (transplant physicians, surgeons, ICU physicians, and donation specialist nurses), purposively sampled from eight countries including Australia, Italy, Japan, Korea, United Kingdom, United States, New Zealand, and Vietnam. We used thematic analysis to analyse the data.

Results: Four themes were identified: respecting the donor family’s experience of grief; optimising ‘the gift’ as a duty to the donor; ambiguity in operationalising ‘informed’ consent, and fears of harming the donor. Participants feared burdening the grieving family with organ donation, in often-traumatic circumstances and the donation specialist role was necessary for sensitive discussion of wishes. Clinicians felt a tension between their duty to enact donor wishes, protect donors as ‘patients in their own rights,’ and prevent ‘unsuccessful’ transplantation. The complete dissemination of information for consent in a time-pressured and emotionally-charged context was described as unrealistic. Instead, the legal concept of ‘authorisation,’ with less onus of information, was raised. The principle of ‘first do no harm’ applied to the potential harms of interventions and adhering to the donor’s wishes.

Conclusions: Respect for the rights and wishes of donors, minimisation of harm and optimisation of ‘the gift’ were paramount to clinicians. Clarity around what constitutes ‘benefit’ and ‘harm,’ along with informed discussion with families, will help clinicians resolve tensions regarding the acceptability of interventions in the donation process.
Comparison of Islet Transplantation Outcomes in Donation After Circulatory Death compared with Donation after Brain Death: The Australian Setting

Yi Vee Chew2, Christian Haron1,2, Lindy Williams2, Kerry Hitos1,2, Lina Mariana3, Tom Kay3,4, Philip O'Connell2, Tom Loudovaris3,4, Wayne Hawthorne1,2.
1Discipline of Surgery, Sydney Medical School, University of Sydney, Sydney, Australia; 2Centre for Transplant and Renal Research, Westmead Institute of Medical Research, Westmead Hospital, Westmead, Australia; 3St. Vincent's Institute of Medical Research, Melbourne, Australia; 4Department of Medicine, St. Vincent's Hospital, University of Melbourne, Melbourne, Australia

Aims: Islet cell transplantation provides long-term insulin independence restoring normoglycaemia, preventing the effects of hyperglycaemia and hypoglycaemic episodes in patients suffering Type 1 Diabetes (T1D). Unfortunately, significant organ donor shortages result in patients remaining on waitlists for years. Other groups have reported Donation after Circulatory Death (DCD) donors are a source of islets, with up to 60% of isolations transplanted, yielding outcomes similar to Donation after Brain Death (DBD) donors. Here, isolation and transplantation outcomes are compared from DCD and DBD donors within the same time period in the Australian program.

Methods: DCD (n=27) and DBD (n=73) islet donor pancreata from the Australian National Islet Transplant program were compared for donor variables (sex, age, weight, BMI, cause of death, CIT, WIT, pancreas weight), isolation outcomes (islet yield, purity, viability), and transplantation outcomes (islet number transplanted, abrogation of hypoglycaemic unawareness).

Results: Donor characteristics were comparable between DCD and DBD groups. However, DCD pancreata showed significantly lower post-purification yields in terms of total IEQ and IEQ per gram pancreas compared to DBD pancreata (146,518±28,971 vs 256,986±17,652 IEQ; p=0.001 and 2,154±504 vs. 2,681±372 IEQ/g; p<0.0001).

Post-culture yields from DCD were also significantly lower compared to DBD yields (37,634±27,786 vs 234,860±18,132 IEQ, and 455±305 vs 2,280±199 IEQ/g; p<0.0001). Viability (p=0.017) and purity (p=0.001) were also lower in DCD islets. However, stimulation index and beta cell viability index outcomes were comparable. Only 4% (1/27) of DCD islet preparations proceeded to transplant compared to 40% (29/73) of DBD preparations (p=0.001).

Discussion: In the Australian setting, vast distances to ship pancreata for islet isolation result in poorer outcomes from DCD compared to DBD pancreata. Earlier intervention, ante-mortem heparin use and faster transport may improve outcomes from DCD organs, providing a viable source of islets, helping alleviate donor shortages and improving access to cell transplantation therapy for T1D patients.
Enteric Leaks Following Simultaneous Pancreas and Kidney Transplantation: Associated Risk Factors and Management in the Westmead Hospital Transplantation Program

Amy Hort1, Sara Shahrestani1, Kerry Hitos1,2, Ronald De Roo1, Lawrence Yuen1, Brendan Ryan1, Richard Allen1, Paul Robertson1, Kathy Kable1, Wayne J Hawthorne1,2,3, Henry Pleass1,2.

1Department of Transplant Surgery, Westmead Hospital, Sydney, Australia; 2Westmead Clinical School, The University of Sydney, Sydney, Australia; 3The Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Westmead, Australia

Introduction: Simultaneous pancreas-kidney transplantation (SPK) is the gold standard treatment for patients suffering Type I Diabetes Mellitus and End-Stage Renal Failure. Enteric drainage is utilised to handle the exocrine drainage, however, enteric leaks are one of its more specific and challenging complications. There remains a lack of published research regarding risk factors for enteric leaks, particularly associated with vascular disease.

Aims: To identify the prevalence of enteric leaks in our major transplantation unit over the last decade. To identify additional risk factors that increase the risk of enteric leaks.

Methodology: SPK transplants performed at Westmead Hospital over ten years (between 2008-2017, n = 234) were analysed to identify enteric leaks. Donor, patient and transplantation procedure risk factors for enteric leaks were collected and analysed. Adjusting for possible confounders, a multivariate logistic regression model was used to assess the risk and predictors of enteric leaks.

Results and Discussion: Of the 234 patients, 12 (5%) experienced an enteric leak. Of these recipients, 9 (75%) had vascular disease, 6 (50%) were ex-smokers, 1 (8%) a current smoker and 3 (25%) were obese with a BMI >30kg/m2. All 12 patients were returned to theatre and converted to bladder drainage. At time of publication, no patients had experienced graft failure. The risk of an enteric leak increased by as much as 4.4 fold in recipients with vascular disease (OR: 4.4; 95% CI: 0.80-24.21; P=0.088). Other factors such as recipient BMI >24.2kg/m2 increased the risk of EL by as much as 1.8 fold (OR: 1.8; 95% CI: 0.4-9.3; P=0.46).

Conclusions: We have identified a possible trend between vascular disease and enteric leaks. These findings also identify other possible risk factors for enteric leaks and the need for further research in this area including careful screening of recipients for vascular disease.
Effects Of Live And Product-Based Helminth Therapy On Allograft Survival Within Animal Models Of Allogeneic Transplantation, A Systematic Review

1,2Michelle Kiss, 1Heather Burns, 3Sheila Donnelly, 1,2,4Wayne J. Hawthorne

1Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, Westmead Hospital, Sydney
2University of Sydney
3School of Life Sciences, University of Technology, Sydney
4Western Clinical School, University of Sydney

Aims: The unique immunomodulatory capacity of helminth parasites has long been exploited in the treatment for a number of autoimmune disorders. Research has previously shown such immunomodulation to be effective in the prevention of allograft rejection for allogeneic transplantation. This review aims to examine the literature to characterise the specific effects of both live and product-based helminth therapy on allograft survival within animal models of allogeneic transplantation.

Methods: Following PRISMA protocol guidelines, a literature search was conducted using PubMed, MEDLINE via OvidSP, along with additional manual searches of selected reference lists. Publications were screened for relevance to eligibility criteria, with all studies including helminth intervention across various allograft models. Primary and secondary outcomes were extracted by the use of standardised data collection tables. The SYRCLE risk of bias assessment tool was used for quality assessment. Due to heterogeneity of study designs, Meta-analysis was not able to be performed; rather outcomes were presented as a narrative synthesis with additional concept mapping.

Results: The literature search yielded 1,443 publications that were screened for relevance to the eligibility criteria, giving 14 relevant publications. These publications were included in this review for qualitative analysis. All 14 publications reported significant improvement to allograft survival when utilising helminth therapy. Differences between live and product-based helminth therapy were minimal in their effect on prolonging allograft survival. Additionally, the extent of impact of helminth therapy on allograft survival was noted to be dependant on study design factors such as parasite burden, allograft type and helminth species/genus.

Discussion: This review has shown that both live and product-based helminth therapy have potential applications as novel immunosuppressants or adjuncts for the prevention of allograft rejection. Aspects of parasite burden, type of allograft and helminth species/genus ultimately impact on the extent of effectiveness of this potential application for clinical use.
NORMOTHERMIC MACHINE PERFUSION OF NON-UTILIZED HUMAN KIDNEYS

A. Hameed1,2,3, N. Rogers1,3,5, B. Lu1,5, C. Zhang5, R. Gaspi5, P. Robertson5, G. Wong1,3,5, R. Miraziz4, R. Allen1,2,3, L. Yuen2,3, R. De Roo2, Wayne Hawthorne1,2,3 & H. Pleass2,3

1. Centre for Transplant and Renal Research, Westmead Institute for Medical Research; 2. Department of Surgery, Westmead Hospital; 3. Sydney Medical School, University of Sydney; 4. Department of Anaesthetics, Westmead Hospital; 5. Department of Renal/Transplant Medicine, Westmead Hospital

Aims: Normothermic machine perfusion (NMP) preservation prior to transplantation may provide superior kidney transplantation outcomes compared to traditional static cold storage (CS) methods, especially in less optimal, higher kidney donor profile index (KDPI) organs. Current human evidence is very limited. We aimed to test a clinically translatable NMP system using discarded or non-utilized human kidneys, and compare NMP and CS with respect to perfusion parameters and likely transplantation outcomes.

Methods: Kidneys (n = 9) were obtained from deceased kidney donors in the event that they were deemed unusable for any reason during/after planned procurement, or in the event of planned liver-only procurement. After a period of CS, kidneys underwent NMP using a customized perfusion circuit, using a packed red blood cell-based perfusion solution at 37°C. In the event that paired kidneys were available, one kidney underwent CS and the other NMP, followed by simulated ‘transplantation’ using ex vivo whole blood reperfusion. Perfusion parameters were compared, and sequential blood, urine, and kidney samples were also taken.

Results: Kidneys were obtained from both donation after circulatory (n = 3) and brain death (n = 3) donors. All kidneys were effectively perfused, and showed favourable perfusion characteristics (increasing flow and reduced intra-renal resistance) during NMP. Furthermore, all kidneys produced urine on the circuit. NMP was able to normalize the appearance of one kidney discarded due to poor perfusion in the donor, thereby potentially reducing kidney discard rates. In paired kidneys where CS and NMP were directly compared, the NMP kidneys had better flow/resistance parameters during simulated transplantation, in addition to a greater urine output, creatinine clearance, renal tubular function, and oxygen consumption.

Discussion: This is the first Australian study outlining the utility of NMP for the preservation of human donor kidneys. NMP is a feasible and effective preservation modality for higher risk kidneys, potentially reducing graft discard rates and enhancing graft function in comparison to CS alone.
**Randomised clinical trial of term induction of labour with Foley catheter silicone versus latex**

**Dr Marjan Khajehei**, Dr Therese McGee, Ms Beata Gidaszewski

*Department of Women’s and Newborn Health, Westmead hospital, Westmead NSW 2145*

**Aim:** Foley catheters are a popular tool for cervical ripening prior to induction of labour. Both silicone and latex single-balloon catheters are widely available but no literature exists to compare them. The purpose of the study was to compare the performance of silicone versus latex Foley catheters with respect to immediate and subsequent outcomes in an outpatient cervical ripening service.

**Methods:** Women undergoing Foley catheter cervical ripening were randomized to a silicone or latex catheter. The primary outcome was insertion-related accidental rupture of membranes. Secondary outcomes included catheter insertion failure, need for unplanned hospital admission, insertion-related bleeding and insertion-related discomfort together with general obstetric and neonatal outcomes.

**Results:** In all, 534 women were recruited, 371 nulliparous and 163 parous. Accidental membrane rupture was significantly more common with a silicone compared to a latex catheter at 7.2% (19/265) versus 1.5% (4/269) (RR 4.8; 95% CI 1.7 – 14.0).

Insertion failure was significantly less common in the silicone compared to latex cohort at 2.6% (7/265) versus 9.3% (25/269) (RR 0.3; 95% CI 0.1 – 0.6). However, when the alternative catheter was subsequently tried, the final failure rates were much closer at 1.9% (5/265) versus 2.6% (7/269).

Insertion-related hospital admission was higher with silicone at 9.4% (25/265) than latex 4.8% (13/269) (RR 2.1: 95% CI 1.1 – 4.1) with most of the difference due to accidental membrane rupture. All other outcomes were no different between the two groups.

**Conclusion:** When used for cervical ripening, a silicone Foley catheter is associated with a higher rate of accidental membrane rupture than a latex catheter but a lower rate of insertion failure. It may therefore be reasonable to attempt insertion with a latex catheter initially and manage insertion failures with a silicone catheter.
Women’s experience of pain and discomfort during Foley catheter insertion.

Beata Gidaszewski, Dr Marjan Khajehei, Dr Terry McGee

Aim: This study aimed to examine discomfort/pain associated with speculum and Foley catheter insertion in term pregnant women undergoing cervical ripening in an outpatient setting and to explore factors affecting their discomfort/pain.

Methods: This was a prospective cohort study conducted in the context of larger RCT comparing silicone and latex Foley and conducted between May 2015 and July 2017.

Results: We asked 330 out of 534 outpatient pregnant women who participated in the main study about the discomfort/pain experienced during the digital vaginal examination and during insertion of the speculum, insertion of the Foley catheter and while the catheter was in-situ.

Women rated digital vaginal examination and speculum insertion (mean pain score=4.6-4.7/10) to be significantly more uncomfortable than Foley catheter insertion (mean pain score=3/10), while having the catheter in-situ for a median of 14 hours was mid-way in discomfort (mean pain score=3.7/10). Only 12-13% of women experienced no discomfort during digital vaginal examination and speculum insertion, while about 40% experienced no discomfort during Foley catheter insertion. We identified no factors that influenced the experience of discomfort during speculum insertion. However, being overseas-born (OR=1.91, 95%=1.10, 3.33) and experiencing discomfort during the speculum insertion (OR=8.15, 95%=3.19, 20.79) increased the chance of discomfort on catheter insertion.

Discussion: In our study in heavily pregnant women, we found no significant association between speculum insertion discomfort and women's physical factors such as BMI, the expectation of pain or knowledge of the procedure, or with the inserter’s recorded difficulty with the procedure.

Being overseas-born was associated with more discomfort during Foley catheter insertion but not during speculum insertion. The most significant predictor of pain on catheter insertion was pain on speculum insertion. Staff designation and level of experience appear to play little role in women’s discomfort. This is reassuring for those who wish to teach and learn this common procedure.
2018 NURSING & MIDWIFERY ABSTRACTS
<table>
<thead>
<tr>
<th>Time</th>
<th>Presenter</th>
<th>Abstract No.</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.45 – 09.55</td>
<td>Stephen Kivunja</td>
<td>170</td>
<td>Experiences of giving and receiving care in traumatic brain injury: an integrative review</td>
</tr>
<tr>
<td>09.55 – 10.05</td>
<td>Dr. Marjan Khajehei</td>
<td>171</td>
<td>Increasing prevalence of diabetes in pregnant women attending Westmead hospital over a period of 7 years.</td>
</tr>
<tr>
<td>10.05 – 10.15</td>
<td>Dr. Christine Atsalos</td>
<td>172</td>
<td>Meeting the challenges posed by an escalating diabetes healthcare burden: a mixed methods study to identify new strategies to enhance diabetes care.</td>
</tr>
<tr>
<td>10.15 – 10.25</td>
<td>Suzanne Pagett</td>
<td>173</td>
<td>Audit of the use of Methoxyflurane via Penthrox inhaler for the management of procedural pain by the Acute Pain Service at Westmead Hospital.</td>
</tr>
<tr>
<td>10.55 – 11.05</td>
<td>Kathy Kable</td>
<td>174</td>
<td>Clearance of BK Virus Nephropathy by Combination Antiviral Therapy with IVIG.</td>
</tr>
<tr>
<td>11.05 – 11.15</td>
<td>Donna Tilley</td>
<td>175</td>
<td>Developing a care pathway for patients with kidney function decline when taking Pre-exposure prophylaxis for HIV prevention.</td>
</tr>
<tr>
<td>11.15 – 11.25</td>
<td>Bincy Kottukappallil</td>
<td>176</td>
<td>Staff knowledge and adherence to supplemental oxygen therapy guidelines within Acute Aged Care (AAC) inpatient settings.</td>
</tr>
<tr>
<td></td>
<td>Abraham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.25 – 11.35</td>
<td>Margaret Murphy</td>
<td>177</td>
<td>Enhancing the training of trauma flash teams: Results from a mixed methods study.</td>
</tr>
</tbody>
</table>
A New Diabetes Education Program for Nursing Staff at Westmead Hospital.

Lauren Stonnill, Diabetes Clinical Nurse Consultant, Westmead Hospital.

**Background:** Diabetes is a complex condition with a growing incidence. Research conducted by the Diabetes Centre has identified a greater need for nursing education in Diabetes management.

**Aim:** To identify gaps in knowledge and begin to provide a Diabetes Education program facilitated by a Diabetes Clinical Nurse Consultant (CNC) to nursing staff at Westmead Hospital.

**Methods:** After consultation with Nurse Educators, Kahoot online interactive quizzes were created to address important aspects of nursing care for patients with Diabetes. In early 2018, all departments were offered in-service sessions for nursing staff facilitated by a Diabetes CNC. Staff answered quiz questions on their phones with correct answers immediately available after completing each question, allowing for identification of knowledge gaps and further discussion. A survey was completed by all staff post quiz.

**Results:** 47 in-services were provided by a Diabetes CNC which were attended by 360 nursing staff. Overall, 82% of questions were answered correctly. Questions which had the most incorrect answers related to target blood glucose levels, insulin administration and appropriate Diabetes Educator referral. Staff feedback showed that most preferred this style of learning and felt their knowledge improved. The sessions also allowed for further Diabetes-related issues specific to each department to be raised during discussion.

**Conclusion:** This program was the first part of a new approach to education on Diabetes for nursing staff. While a strong knowledge base has been established, knowledge gaps have been identified and new issues in Diabetes management have been raised. Future education will address these issues.
Late Referrals for Inpatient Insulin Education

Natasha Diwakar Diabetes CNS

Patients who are started on insulin injections are often referred to the inpatient diabetes nurse educator (DNE). However, often nursing and medical staff refer patients for an insulin start on the day of discharge. An audit was conducted to check which wards are referring patients for an insulin start on the day of discharge and to ensure nursing and medical staff understand that the DNE requires a minimum of 24 hours notification for an insulin start.

Data was collected from January 2016 until December 2016 for a quality audit. An audit tool was developed which included the source of referral, the notifier, the total number of ward visits and the day of referral.

260 referrals were received for insulin starts. 124 patients (47%) had not had previous education and 51 (19%) of the insulin start referrals were late referrals. 143 (55%) of the insulin start referrals were urgent referrals and were seen on the day of discharge and three patients were discharged before they were seen by a DNE. If patients are referred on the day of discharge, it is difficult to fit them in if there are other patients to be seen, who may also be scheduled for discharge.

Inservices should be conducted around the hospital to educate ward staff about what an appropriate and specific referral involves. The endocrine team should also be notifying the DNE about all patients they see who require diabetes education, particularly if they are starting insulin.
The relationship between Pulmonary Rehabilitation and Sleep Quality in COPD

M Roberts, J-G Cho and JR Wheatley

Poor sleep quality is a common complaint reported by patients with COPD. Exercise has been shown to improve sleep quality. However, the effect of pulmonary rehabilitation (PR) on sleep is unknown.

Aim: To examine sleep quality in patients with COPD before and after PR, to identify factors associated with improvement in sleep quality.

Methods: Retrospective chart review of patients with COPD who completed PR. Primary outcome was sleep quality measured by Pittsburgh Sleep Quality Index (PSQI). We identified patients with poor sleep quality at baseline (PSQI>5 units), and compared baseline and post-PR characteristics of patients whose PSQI improved following PR by ≥3 units (i.e. responders) with those who did not (i.e. non-responders).

Results: Data were available for 331 patients (52% male, 69.6±8.8 years, FEV₁ % predicted 47±16%). 219 (67%) had poor sleep quality (PSQI>5). Following PR, group mean PSQI decreased by 0.95±3.14 units (p<0.0001) however this did not meet the minimally clinically importance difference. Only 28% of patients were classified as PSQI responders. Patients whose PSQI decreased by ≥ 3 units also had greater improvements in quality of life and mood following PR than non-responders. The absence of supplemental oxygen and higher baseline level of sleepiness were both significantly associated with improved PSQI following PR.

Conclusion: Poor sleep quality is common in patients with COPD referred to PR. Not all patients' sleep quality improves with PR. Further studies are needed to determine if other interventions to improve sleep quality can be implemented in PR programs to further improve sleep quality.
CoCo Study: Service redesign opportunities with families experiencing continuity of care for congenital anomalies requiring newborn surgery

Susan Heath¹ and Kim Psaila²

¹Women’s and Newborn Health, Westmead Hospital, NSW, Australia
²School of Nursing and Midwifery, Western Sydney University, NSW, Australia

Background: For over a decade, professionals providing perinatal services have emphasised the importance of “continuity of care” for pregnant women and their families. Research addressing impact on patient outcomes, especially within high risk maternity populations, is lacking, but health care organisations working in silos are reported as suboptimal.

Study Aims:

1. Map the current service provision for pregnant women and their newborn infants who require surgical neonatal intensive care unit care, from diagnosis of a congenital anomaly.

2. Examine the facilitators and barriers to the delivery of effective family centred care

3. Identify opportunities and strategies for service redesign

Methods: In phase one of this mixed methods study, data from women’s records, and also data from 18 parental interviews, and members of the high risk pregnancy care team. Data was analysed thematically with basic codes initially organised using a chronological coding framework, based on the phases of the continuum of care mapped, then themes extracted.

Results: Relational, managerial and informational continuity were reported by families as contributing to a successful transition across services. Aspects of service provision contributing to parental perception of discontinuity of care were primarily identified prior to PEARLS care, followed by ‘a contrast in support’. A single area for improvement was identified relating to informational and relational discontinuity.

Conclusions: Exploring continuity from the perspective of families and professionals identified a variety of existing strategies to support continuity of care. Translation of findings related to discontinuity, can improve the family’s journey across multiple health care organisations.
Integrated care review of chronic obstructive pulmonary disease inpatient management

Vinita Swami1, Tracy Smith1,2, Jin-Gun Cho1,2,3, Archit Chawla1, Mary Roberts1,2,3, & John Wheatley1,2,3

1Department of Respiratory and Sleep Medicine, Westmead Hospital, Westmead NSW, Australia
2University of Sydney at Westmead Hospital, Westmead NSW, Australia
3Ludwig Engel Centre for Respiratory Research, Westmead Institute for Medical Research, Westmead NSW, Australia

In 2014–15, 66,540 patients were hospitalised with an acute exacerbation of COPD (AECOPD) in Australia. National and international guidelines to optimise inpatient management of COPD exist, however compliance to these remains unclear.

Aim: To review management of AECOPD in inpatients admitted to a tertiary teaching hospital.

Methods: The Integrated Care Team (ICT) developed an evidence-based checklist to monitor the care of inpatients with AECOPD and adherence to COPD guidelines. ICT performed a chart review of checklist adherence for AECOPD admissions at Westmead Hospital from 5/2016 - 9/2017.

Results: ICT reviewed 276 inpatients with AECOPD (55% male; mean age 69.9±10.4 years). ICT identified good adherence to oxygen prescription on medication charts, early antibiotic rationalisation, and spirometry measurement (all>75% compliance). However, ICT found inhaler prescription errors in 37% of reviews including incorrect or missing prescriptions, and duplication of inhaler classes. 36% of patients were still on nebulised therapy at time of ICT review, 30% of patients were current smokers and only half of current smokers had been offered smoking cessation measures. Respiratory vaccinations were not up-to-date in 62% of patients, and only 5% had been offered pulmonary rehabilitation.

Conclusion: ICT has identified areas for improvement in inpatient management of AECOPD including inhaler therapy prescriptions, smoking cessation measures, referral to pulmonary rehabilitation and assessment of immunisation. There is a role for ICT in education and optimisation of inpatient AECOPD management. Further research is needed to understand the impact of improved compliance to AECOPD guidelines on long-term ambulatory outcomes in patients with COPD.
Eat well, Move well with your cancer treatment.

¹N Taylor, ²C Ceprnia, ³M Quinlivan, ⁴R Hammond, ⁵P Talbot.

¹Melanoma CNC, Crown Princess Mary Cancer Centre, ²Physiotherapy, ³Nutrition and Dietetics.

Diet and exercise have long been associated with improved health outcomes but what has become evident that they are just as important in the Oncology setting. The most studied cohorts include Breast and Prostate cancer.

Studies have helped establish select exercise units for these patients;

UNSW—Lifestyle clinic.

Western Australia Cancer Council—Life Now Exercise—12 week programme for cancer patients on treatment.

With no formal programme available for Cancer pts undergoing treatment in the LHD a working group of Nurse/Physio/Dietician submitted a research design to support this initiative in a new emerging cancer group; Melanoma pts undergoing Targeted and Immunotherapy treatments.

Aim: The aim of this study was to determine changes in physical activity, nutrition status with structured education and activity sessions. Quality of Life EORTC QLQ-C30 questionnaires were used to map health improvements.

Method: This pilot study comprised of 4 education sessions spaced over 6 months; incorporating physio assessment and exercises, dietary assessment and nursing assessment and QOL data.

Results: Ongoing. Initial review of QOL indicate better QOL and health outcomes.

Discussion: The post session surveys indicate good satisfaction with content/ frequency and presentation style.

Further assessment of facilitators and barriers is needed to support and monitor sustained life-style changes for health improvements.
“If the glove fits, wear it!”

Rachel Paton- Clinical Nurse Specialist & Nicole Tolhurst- Clinical Nurse Specialist

Healthcare workers (HCW) wear gloves to prevent the transmission of microorganisms. Gloves should be changed and discarded between specific tasks, between patients, as soon as integrity has been altered and must not been cleaned or reused. Hand hygiene must immediately be performed before and after glove use. (NSW Infection Prevention & Control Policy PD2017_013)

World Health Organisation 2009 states “It is important that health care workers are able to differentiate between specific clinical situations when gloves should be worn and changed and those where their use is not required.”

The aims of this quality improvement study are to determine:

- Are HCW wearing gloves for the correct reasons and/or are there inappropriate uses of gloves?
- What are patient’s perspectives of appropriate glove use for HCW?

The results indicated that health care workers (100 participants) wore gloves appropriately 57% during a 20 min observation. Inappropriate glove use was 47%. Infection Prevention and Control (IPAC) now highlights appropriate glove usage in all educational sessions.

IPAC have evaluated data regarding patient’s (100 participants) perspective when HCW should be wearing gloves during specific tasks. Results indicate that the majority of patients have an understanding that high risk fluid exposure tasks require gloves however 50 % indicated low risk tasks (skin contact) requires staff to wear gloves.

Future plans will focus on educating HCW and patients and then determine if implementing education initiatives has improved glove use knowledge.
The journey of a novice research team – the highs and the lows.

Catherine Hardman, Neurosurgical Data Manager; Kim Foxall, Neurosurgical Case Manager; Katherine Schaffarczyk, Nurse Educator; Diane Lear, Clinical Nurse Consultant Neurosurgery; Summer Byrne, Clinical Nurse Educator Neuroscience/Trauma Close Observation Unit and Annie Black, Clinical Nurse Specialist, Neuroscience/Trauma Close Observation Unit. Westmead Hospital, Sydney.

Background: Current pain management strategies for post-operative craniotomy patients are limited due to concerns about the effect of stronger analgesic agents on level of consciousness and respiratory status. There was an opportunity for practice change to optimise the care and experience of patients undergoing elective craniotomy. Patient controlled analgesia (PCA) fentanyl was introduced for this patient cohort.

Aim: To provide an overview of the journey taken by the research team from inception to commencement of the study.

Method: Extensive consultation was undertaken with key stakeholders during the planning phase. Challenges were experienced which required a tenacious, determined and cohesive approach from the research team. These challenges included gaining support from Neurosurgeons, allocating time to do the research whilst balancing clinical and administrative roles; and having little funding.

Patients in the treatment group were interviewed three times during their patient journey and administered questionnaires; patients in the non-PCA group were interviewed once. Review of patient medical records was conducted. Recruitment and patient tracking required an organised and systematic approach.

Thematic analysis of the qualitative data was chosen with team members contributing to the analysis of each transcript. The potential impact of reflexivity during this analysis phase was acknowledged given the neurosurgical nursing background of researchers.

Preliminary results indicate the use of PCA fentanyl in this patient cohort is safe with patient satisfaction noted.

Conclusion: The undertaking of a research study requires a coordinated team approach. The benefits to patient care and to researcher professional development are rewarding.
The Tortuous Journey of Introducing the Nurse Practitioner as a New Member of the Healthcare Team

David Collins RN BSc MN (NursPrac) MAppMgt (Nursing) Nurse Practitioner Acute BMT

The Nurse Practitioner has been a member of the health care team in Australia since 2000 when the initial authorisation of the role began. In the first 18 years the numbers of nurses authorised to practice and the areas of practice have grown.

Many positions were primarily based in the community or the emergency department, however, this is now not so, with many Nurse Practitioners practicing in specialist areas.

This paper will outline the first 18 months of a NP role in Blood and Marrow Transplantation (BMT). It will discuss how the model of care used has developed to allow for a reduction in readmissions and faster access to specialised care after discharge for this patient group.

It will debate how the role interacts with medical and nursing staff and compare how these interactions match with the experiences of Nurse Practitioners in the published literature. It will further demonstrate how such a specialised role can save inpatient bed days and support patients to achieve intended health outcomes.
Learning to Lead - Exploring Team Leader Roles and Responsibilities

Patricia Walsh NUM A4B/C Westmead Hospital. Laila Pethani CNE A4B/C Westmead Hospital

In the busy and chaotic health care environments, clinical staff rely on the coordination and leadership that the team leader provides. Their role is vital to ensure the day-to-day provision of evidence based person centred care.

Aim: To develop team leaders in A4C and provide mentoring for staff transitioning to the role.

Method: Collaborative, facilitated discussions with current team members to identify knowledge regarding the role. Using the Claims, Concerns and Issues evaluation tool, team leaders identified areas that they required development in and worked with the Nurse Unit Manager and Clinical Nurse Educator to achieve their learning goals. All staff were included in discussions to ensure awareness of the Team Leader role and responsibilities and their role in supporting the team leaders to achieve person centered care. A program was developed that all aspiring team leaders were mentored through to increase their skills. We established numerous resources. We then surveyed the staff following the implementation of the project.

Results: Staff in the team leader role rated their confidence and ability to lead, as improved following this program.

Other positive outcomes for this leadership mentoring program included improvement of staffs’ understanding of their impact on the patient’s journey and awareness of roles and responsibilities around communication, person centred care, conflict resolution, care coordination, role modelling, clinical expertise, clinical supervision and problem solving.

The vision for the future is the expansion of the program to other clinical areas to continue the support and upskilling of clinical staff.
Co-creating unit values with multidisciplinary staff and highlighting the importance of incorporating them in our workplace.

Bincy Kottukappallil Abraham, Russell Roxburgh and Susan Rebolledo

Workplace culture in a multidisciplinary team is vital and contributes to job satisfaction, staff retention, and improved patient outcomes. Workplace culture is often not a topic that comes naturally as part of any general conversation, although it does have a huge impact on staff. The organizational culture seeks to provide guidance to staff around unified behaviours, reducing conflicts and creating a consistent healthy environment to conduct work effectively and efficiently.

**Aim:** To co-create unit values with multidisciplinary staff and highlight the importance of incorporating those unit values into workplace culture in B4B/C.

**Results:** In B4B/C, with assistance from Essentials of Care (EOC), we conducted several focus groups to discover the unit values, based on our organizational values, and used Kahoot™ play system to group 10 core values within the unit. The B4B/C team used the CORE values as a basis for the unit values and built the unit values on that stem. Staff found several value display formats from different websites and collaborated to decide on a format to create the team values chart. Staff were provided regular feedback at various stages throughout this process and the team values will now be part of our induction for new staff and will be used to facilitate further development of existing staff.

**Conclusion:** Unit values are identified with an intention towards improving and maintaining job satisfaction and advancement of staff performance. The values model is in the process of being printed for official display in the unit.
Audit of the lifestyle effects of SCIg on patients with PID at Westmead Hospital

Itoya A. 1, Wells K. 1, Stone P. 1 Stewart G. 1,2,3 Brown D. 1,2,3 Swaminathan S. 1,2,3 Berglund L. 1,2,3 Suan D. 1,2,3 and Lin MW. 1,2,3 on behalf of the Department of Clinical Immunology, Westmead Hospital

1 Department of Clinical Immunology, Westmead Hospital. 2 Department of Immunopathology, ICPMR, NSW Health Pathology. 3 Faculty of Medicine, University of Sydney, Sydney NSW

Patients with Primary Immunodeficiency (PID) rely on lifelong Immunoglobulin (Ig) treatment to improve quality of life (QOL), slow and/or stop the progression of associated organ damage, prevent recurrent infections and associated comorbidities. Ig treatment could be administered either Intravenously (IV) as Intravenous Immunoglobulin (IVIg) or subcutaneously as subcutaneous Ig (SCIg). IV treatment requires hospital admission once or twice per month depending on the patient’s regime and SC treatments is in the home setting, hence making this a more attractive alternative to the busy patient. SCIg has been successful and patients’ preference in many European countries.

Australia is slowly converting long established IVIg patients to SCIg. Since 2014, Westmead Hospital have successfully trained and converted 50 patients from IVIg to SCIg. 80% of these patients have PID and the others have secondary immunodeficiency from immunosuppressive agents for underlying autoimmune disease.

The aims of this poster are firstly, to evaluate the QOL of patients on SCIg, focusing on the frequency of infections as reported by the patients in terms of looking at number of days off work/school, number of days on antibiotics and general wellbeing. Secondly, monitoring of random Ig levels and evidence of end organ damage. Lastly, healthcare cost savings to the department of immunology with SCIg will also be addressed.

Our audit shows that SCIg is beneficial for patients with PID in terms of reduction in the number of infections, improvement of QOL, with minimal/tolerable side effects. There was also significant reduction in healthcare costs to the Day Stay unit.
Success & Challenges with SCiG training and treatment; a nursing perspective

Stone P.¹, Itoya A.¹, Wells K.¹ and Wu Y.¹ on behalf of the Department of Clinical Immunology, Westmead Hospital

¹ Department of Clinical Immunology, Westmead Hospital.

Subcutaneous Immunoglobulin (SCIg) has been treatment of choice for patient with Primary Immunodeficiency Disease (PID). Although, Australia is slowly converting suitable IVIg patients to SCIg, this has been the preference in many European Countries for many years and many success stories has been recorded, targeting patients' better quality of life since the switch from Intravenous Immunoglobulin (IVIg) (Chapel & Gardulf, 2013). The department of Clinical Immunology at Westmead Hospital initiated a SCIg program in 2014 with over 50 PID patients successfully attending their treatment at home.

The majority of the patients were transitioned from IVIg to SCIg and a few naive to Ig treatment. SCIg training is performed in a nurse led clinic with the trained registered nurses working very closely with the specialist Immunologist. The clinic treats patients from other departments such as Neurology, Rheumatology as well as Immunology and allergy. All staff working in the clinic are capable to treat and train SCIg patients. This poster will cover the challenges from a nurse's perspective that the clinic has encountered since the start of the program.

Challenges such as recruiting, patient training, patient compliance, adverse reaction, equipment (usage and ordering process), and also issues encountered with product ordering and pickup processes. Discussion will also highlight trouble shooting of these issues and recommendations will be made. The poster will also cover the success so far of involving and training the patients' General practitioner in cases where patients require some assistance.
Experiences of giving and receiving care in traumatic brain injury: an integrative review

Stephen Kivunja RN, BN, MCN, PhD Candidate, Dr Jo River PhD, BN Hons, RN, Senior Research Fellow & Janice Gullick PhD, RN, Associate Professor

This presentation reports on the findings from an Integrative Review which synthesised the research literature on the experiences of giving and receiving care in traumatic brain injury (TBI) for people with TBI, their family members and nurses in hospital and rehabilitation settings. TBI is a major source of physical, social and economic burden. In the hospital and rehabilitation settings, people with TBI report being excluded from decision-making processes, and families report receiving inadequate support. Nurses also face challenges providing TBI care due to limitations in skills and organisational-resources.

This study used an integrative review framework. Five databases were searched for relevant articles including; CINAHL, PubMed, ProQuest EMBASE and Google Scholar. The inclusion and exclusion criteria mandated the inclusion of primary studies that were published in peer-reviewed journals, and the exclusion of papers that were not published in English.

Important themes identified in the review indicated that; patients struggled to live with physical changes, regretted the loss of independence, and experienced insensitivity from caregivers. Family members wanted to be involved in patient-care, and culturally appropriate services to support their understanding of TBI care. Nurses expressed serving as advocates for patients and families by ensuring patient-safety and offering reassurance. Overall, the findings suggested that there is a mismatch in the perceptions of what constitutes person-centred TBI care among patients and nurses.

This study provides important information on the importance of formal inclusion of people with TBI and families in care planning and case-management to guide access to services and funding.
Increasing prevalence of diabetes in pregnant women attending Westmead hospital over a period of 7 years

Marjan Khajehei

**Aim:** To assess changes in the prevalence and risk factors of diabetes in pregnant women from 2011 to 2017.

**Methods:** In this retrospective study, data from 38,851 pregnant women who attended Westmead Hospital (2011-2017) were considered for evaluation using SPSS for statistical analysis.

**Results:** Out of 38,851 pregnant women, 4,672 (12%) had gestational diabetes and 462 (1.2%) had pre-existing diabetes. There was steady increase in gestational diabetes (from 9% to 14%) and an increase in pre-existing diabetes (from 1% to 2%) (p<0.05) in the general population of pregnant women from 2011 to 2017.

The rate of endocrine diseases, multiparty, and Body Mass Index>35 significantly increased from 2011 to 2017 in pregnant women with diabetes during pregnancy (p<0.05).

After regression analysis adjusting for baseline characteristics, hypertension and endocrine diseases were shown to be significant risk factors for diabetes during pregnancy (p<0.05). The odds of diabetes during pregnancy in women with hypertension increased from 1.86 (95%CI=1.37-2.52) in 2011 to 1.90 (95%CI=1.43-2.53) in 2017. On the other hand, the odds of diabetes during pregnancy in women with endocrine diseases decreased from 1.67 (95%CI=1.32-2.12) in 2013 to 1.28 (95%CI=1.03-1.59) in 2017 (p<0.05).

**Conclusions:** The prevalence of diabetes among pregnant women has increased from 2011 to 2017.

**Implications for practice:** The steadily increasing rates of diabetes in pregnancy indicate the burden of high-risk pregnancies is increasing. Although we have come a long way in improving care for these women, further efforts are needed to reverse the trend toward increased diabetes in women of child-bearing age.
Meeting the challenges posed by an escalating diabetes healthcare burden: a mixed methods study to identify new strategies to enhance diabetes care

Dr Christine Atsalos, CNC Diabetes, Westmead Hospital; Associate of the University of Technology Sydney.
Ms Marlene Payk, NP Diabetes, Westmead Hospital.
Ms Ann O’Neill, Nurse Manager, DEACC, Westmead Hospital.
Ms Sally Inglis, CNC Diabetes, Royal North Shore Hospital, Sydney.
Prof. Wah Cheung, Director, Department of Diabetes and Endocrinology, Westmead Hospital; Clinical Professor, University of Sydney.
Prof. Debra Jackson, Professor of Nursing, University of Technology Sydney; Principal Fellow Oxford Biomedical Research Centre; Professor of Nursing Research, Oxford Health NHS Foundation Trust, UK; Editor-in-Chief, Journal of Clinical Nursing.

Background: The ongoing escalation in the incidence of diabetes is contributing to a growing burden associated with diabetes management.

Aim: The primary aim of this study was to identify innovative new strategies to maintain optimal care for patients with diabetes while in hospital.

Methods: Mixed methods underpinned by Appreciative Inquiry, using an online survey and focus group interviews with nurses and midwives, and individual interviews with recently discharged hospital patients.

Findings: Suggest a need for further education and knowledge on diabetes management for nursing, midwifery, medical and ancillary staff. This, together with improved communication and teamwork, is required to prevent delays in prescribing and reviewing insulin requirements, along with timely access to appropriate food for people with diabetes.

Discussion: The introduction and implementation of innovative educational and organisational strategies are needed to assist in meeting the challenges posed by an escalating diabetes healthcare burden. The safety of patients with diabetes can be optimised with the timely availability of appropriate meals and snacks, and enhanced coordination and communication between and within multidisciplinary teams.

Conclusions: In seeking solutions to the challenges in caring for hospitalised patients with diabetes there is a need to work across the entire hospital workforce and to develop effective and efficient methods for ensuring appropriate skills and knowledge of diabetes management for staff across complex and rapidly changing hospital systems.
Audit of the use of Methoxyflurane via Penthrox inhaler for the management of procedural pain by the Acute Pain Service at Westmead Hospital

Suzanne Pagett

In 2010 the Acute Pain Service (APS) introduced the administration of Methoxyflurane via Penthrox Inhaler (MVPI) to manage procedural pain in wards and units throughout Westmead Hospital.

This modality of analgesia has been utilised for many years by emergency services as a single dose delivery for the management of pain in the pre hospital setting. There is no literature however on multiple administrations of MVPI to the same patient. Since its introduction here the APS have delivered this modality of analgesia several times to the same patient with close monitoring of their renal and hepatic function.

**Aim:** Provide an overview of the use of MVPI by the APS for the management of procedural pain and prove that repeat administrations to patients with normal renal and hepatic function is safe.

**Method:** A retrospective audit reviewing the medical records and blood results of the 156 patients who have had MVPI administered by the APS

**Results:** 37% of the patients who had MVPI administered were admitted following trauma. The indication for use in 89% of the patients was for change of vacuum assisted closure dressings or wound packing changes. 14% of patients had received five or more administrations on MVPI. Only one patient in the 156 patients had a decline in renal function following several doses of MVPI, however this patient had other clinical issues likely to have contributed to this.

**Conclusion:** MVPI is an effective modality for the treatment of procedural pain. Multiple appropriate doses to the same patient appears to be safe.
Clearance of BK Virus Nephropathy by Combination Antiviral Therapy with IVIG

Kathy Kable, RN, MN (NP); Carmen D. Davies, RN; Philip J. O’Connell, FRACP; Jeremy R. Chapman, FRACP; Brian John Nankivell, MD, FRACP

**Background:** Reactivation of BK polyoma virus causes a destructive virus allograft nephropathy (BKVAN) with graft loss in 46%. Treatment options are limited to reduced immunosuppression and largely ineffective antiviral agents. Some studies suggest benefit from intravenous immunoglobulin (IVIG).

**Methods:** We evaluated effectiveness of adjuvant IVIG to eliminate virus from blood and tissue, in a retrospective, single-centre cohort study, against standard-of-care controls. Both groups underwent reduced immunosuppression, conversion of tacrolimus to cyclosporine, and mycophenolate to leflunomide, oral ciprofloxacin, and intravenous cidofovir.

**Results:** Biopsy-proven BKVAN occurred in 50 kidneys at 7 (median, IQR 3-12) months after transplantation, predominantly as histological stage B (92%), diagnosed following by dysfunction in 46%, screening viraemia in 20%, and protocol biopsy in 34%. Following treatment, mean viral loads fell from 1,581±4,220x10^3 copies at diagnosis to 1,434±7,0639 mid-treatment, and 0.138±0.331 after 3 months (P<0.001). IVIG at 1.01±0.18g/kg was given to 22 patients (44%). The IVIG group more effectively cleared viraemia (HR 3.68; 95%CI, 1.56-8.68, P=0.003) and BK immunohistochemistry from repeated tissue sampling (HR 2.24; 95%CI, 1.09-4.58, P=0.028), and resulted in faster (11.3±10.4 vs. 29.1±31.8 months, P=0.015) and more complete resolution of viraemia (33.3% vs. 77.3%, P=0.044). Numerically fewer graft losses occurred with IVIG (27.3% vs. 53.6% for control, P=0.06), although graft and patient survivals were not statistically different.

**Conclusions:** Combination treatment incorporating adjuvant IVIG was more effective eliminating virus from BKVAN, compared with conventional therapy. Validation by multi-centre randomised trial is needed.
Developing a care pathway for patients with kidney function decline when taking Pre-exposure prophylaxis for HIV prevention

Donna Tilley 1,4, Vincent W. Lee 2,3, Melissa Power 1, Deborah Couldwell 1, David Lewis 1,5

1Western Sydney Sexual Health Centre, 162 Marsden Street, Parramatta, NSW; 2Department of Renal Medicine, Westmead Hospital, NSW; 3Sydney Medical School, University of Sydney, NSW, 4Sydney Nursing School, University of Sydney, NSW, 5Marie Bashir Institute for Infectious Diseases and Biosecurity & Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, NSW.

Background: Pre-exposure prophylaxis (PrEP) for HIV infection is a safe and effective biomedical prevention approach. High levels of efficacy have been demonstrated in multiple trials using an oral fixed drug combination of tenofovir disoproxil and emtricitabine. The risk of adverse events needs to be assessed in the context of risk of HIV acquisition. Clinical trials found small but significant changes in renal function with up to 2.2% of participants experiencing a sustained fall in estimated glomerular filtration rate (eGFR) to below 60mL/min/1.73m². Currently there is no clear guidance on the clinical management of declining renal function prior to or following a decision to cease PrEP.

Analysis: A large NSW demonstration project to scale up PrEP access commenced at Western Sydney Sexual Health Centre (WSSHC) in May 2016. Of the patients enrolled, 1.2% (6/512) have had an eGFR that has fallen below 60mL/min/1.73m². This nurse-led project involved a collaboration between WSSHC and the Renal Medicine Department at Westmead Hospital. Together we developed a multidisciplinary care pathway to guide investigations, referral and clinical decision making.

Outcome: The care pathway is now used at WSSHC for assessing risk factors and clinical indicators of kidney disease. The development of the pathway has streamlined the referral process for patients, provides consistent assessment parameters and has strengthened collaboration between the renal and sexual health teams.

Applications: The care pathway will be disseminated among the NSW statewide sexual health services, HealthPathways and included in the clinical practice guidelines at WSSHC.
Staff knowledge and adherence to supplemental oxygen therapy guidelines within Acute Aged Care (AAC) inpatient settings.

Bincy Kottukappallil Abraham and Mary Roberts

Supplemental oxygen therapy is a commonly used therapeutic to treat hypoxaemia. In the healthcare setting, oxygen is considered to be like a drug. Like any drug, when used inappropriately, it can cause harm. National guidelines state supplemental oxygen should be prescribed on the medication chart with target oxygen saturation ranges based on patient risk. Anecdotal evidence and IMS reports suggest that this is not occurring.

Aim: To assess the knowledge of staff working in the Acute Aged Care inpatient setting regarding supplemental oxygen therapy guidelines and to assess current adherence to national guidelines.

Method: Ward audits to identify adherence to oxygen guidelines and administration of questionnaires to staff to assess level of knowledge.

Results: 69 subjects (58% registered nurses, 17% medical officers, 14% allied health staff & 10% enrolled nurses) were enrolled in the study following informed consent. Knowledge regarding oxygen therapy was variable (scores ranging from 17 – 89%) with 89% of respondents acknowledging oxygen was like a drug however 85% were unable to identify the correct flow rates for a simple face mask. There was no correlation between the total score of the knowledge questionnaire and years of experience, and minimal differences between the mean scores of the different disciplines.

Conclusion: Knowledge regarding supplemental oxygen is variable within the AAC inpatient settings with associated poor adherence to national guidelines. To improve adherence (and patient safety), the research team will develop a targeted educational program to improve knowledge regarding the administration of supplemental oxygen and reassess outcomes after the intervention.
Enhancing the training of trauma flash teams: Results from a mixed methods study.

Margaret Murphy, Clinical Nurse Consultant, Emergency Services

Aim: To understand what is required to produce a high performing trauma team in real resuscitation encounters following multidisciplinary simulated trauma team training (TTT).

Background: Management of critical trauma requires high level team performance. Trauma teams mobilise quickly and comprise of staff from numerous specialties and disciplines. Simulation is promoted as a platform for training such teams. There is need to understand what is required in the real world clinical environment to enable high performing teams.

Design: A mixed methods embedded experimental study with a primary quantitative drive.

Methods: This paper describes integration of a mixed methods study. The primary quantitative results (time to critical operation, ED length of stay, mortality, facilitators and barriers to teamwork) were compared, contrasted and amalgamated with the qualitative results (team members’ experience and perspective), to identify the impact of TTT on team performance, skills and patient outcomes. It also evaluates application of training to the clinical environment.

Results: Integration of data produced four new findings; Trauma teams are rapidly constructed ‘flash’ teams requiring collaborative decision making for safe performance; Communication techniques need to support the uncertain and complex context of trauma resuscitation; Standardisation promotes safe trauma care; Leaders need to enable teamwork.

Conclusion: Multidisciplinary TTT enhances team performance as it teaches a quickly constructed group of individuals how to work as an expert team. Factors that facilitate or impede team performance in real resuscitation encounters need to be considered in training program.
The benefits of screening in cardiac rehabilitation for obstructive sleep apnoea – a single site experience.

Robert Zecchin, Justine Thelander, Julie Hungerford, Gail Lindsay, Jan Baihn, Yeng Chai, Inga Saliba, A. Robert Denniss

**Background:** Obstructive sleep apnoea (OSA) is considered an independent risk factor for coronary heart disease. There is a paucity of knowledge of OSA screening in cardiac rehabilitation (CR) patients, especially in Australia.

**Methods:** Consecutive patients who attended the CR program at Westmead Hospital and who had OSA screening using the STOP-BANG questionnaire were included. This study compared low risk (LOSA) and high risk (HOSA) patients for OSA in relation to socio-demographics, anthropometrics, functional capacity, risk factors, medications and quality of life (QOL).

**Results:** In 18 months, 479 patients (mean age 61 +/- 13; 80% male) were screened. There were 26% patients in the LOSA groups and 29% in the HOSA group. One patient was previously diagnosed with OSA in the LOSA group, whereas 25 (24%) in the HOSA group were diagnosed prior to CR. Therefore 80 patients were undiagnosed as high risk for OSA at CR assessment. Analysis showed that HOSA patients were often older (p<0.01), male (p<0.01), have a history of atrial fibrillation (p=0.002), increased weight, waist circumference and BMI (p<0.001), have diabetes (p=0.002) and hypertension (p<0.001), greater digoxin (p=0.03), ACE-I/ARB (p=0.003) and calcium channel blockers (p=0.01) use, and lower physical functioning QOL score, compared to LOSA. No differences were found in diagnoses, functional capacity, smoking and hyperlipidaemia rates, beta-blocker usage and depression scores. Untreated high risk patients were referred for further assessment.

**Conclusion:** OSA is an underdiagnosed risk factor in patients attending CR. CR is an ideal setting for OSA screening and to make referrals for further assessment.
Fundus Photography in the ED: Saving lives, sight and time

Julia Costello, A/Clinical Nurse Consultant and Transitional Nurse Practitioner, Emergency Department, Westmead Hospital; Jason Montgomery Nurse Practitioner, Emergency Department, Westmead Hospital; Megan Greig, Nurse Practitioner, Emergency Department, Westmead Hospital; Dr Hamish Dunn, Research Fellow, Dept of Ophthalmology, Westmead Hospital; A/Prof Andrew White, Head of Dept of Ophthalmology, Westmead Hospital; A/Prof Clare Fraser, Neuro-ophthalmology and Ophthalmic Education Clinical Ophthalmology & Eye Health, University of Sydney School of Medicine; Dr Matthew Vukasovic, Head of Dept, Emergency Department, Westmead Hospital; Alison Pryke, Clinical Trials Coordinator, Dept of Ophthalmology, Westmead Hospital; Dr Kai Zong Teo, Research assistant, Summer Research Scholarship; Lakni Weerasinghe, Medical student research assistant, Summer Research Scholarship

Around the world, Emergency Department’s (ED) are missing up to 13% of patients with clinical signs of blinding and life-threatening pathologies. These missed neuro-ophthalmic emergencies are avoidable because they are detectable using fundoscopy.

Fundoscopy, looking at the back of the eye, gives a live picture of the brain and blood vessels, revealing critical information including detecting vascular changes, and raised pressure around the brain. However fundoscopy in ED with the direct ophthalmoscope is awkward to use and hard to interpret. A groundbreaking study in Atlanta found ED doctors were only examining 14% of patients who needed fundoscopy and were missing 100% of management changing pathology.

Westmead ED and Ophthalmology Departments collaborated on a research project which implemented the use of a portable non-mydriatic fundus camera (NMC) to take fundus photographs without dilating the pupil.

The project recruited a core group of Nurse Practitioners (NP) and doctors to highlight patients needing fundoscopy early in their ED journey, and conduct NMC photography. The photos taken were uploaded to the eMR and reviewed by the Ophthalmology team within 24 hours. The detection of neuro-ophthalmic emergencies by ED improved from 0.06% to 11.9% within two months. The fundoscopy rate improved from 6.4% during the same period the previous year to 89.5% during the trial.

This was the first portable NMC fundus photography program in Australia and demonstrates the value of collaborative fundus imaging for the safety of patients presenting to ED. It lead to rapid translation of evidence-based best practice at Westmead ED.
2018 ALLIED HEALTH RESEARCH ABSTRACTS
<table>
<thead>
<tr>
<th>Time</th>
<th>Presenter</th>
<th>Abstract No</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.30 - 10.40</td>
<td>Kim Hobbs</td>
<td>193</td>
<td>Progressing the Allied Health research agenda in Health Literacy.</td>
</tr>
<tr>
<td>10.40 – 10.50</td>
<td>Cassandra Wong</td>
<td>194</td>
<td>Rigidity but not tremor is associated with pain in people with Parkinson’s disease.</td>
</tr>
<tr>
<td>10.50 - 11.00</td>
<td>Dragana Ceprnja</td>
<td>195</td>
<td>Coping with pelvic girdle pain during pregnancy: a qualitative study protocol with Australian women.</td>
</tr>
<tr>
<td>11.00 - 11.10</td>
<td>Stephen Harvey</td>
<td>196</td>
<td>A novel approach to falls prevention: Associations between bicycling and falls related physical performance in older adults.</td>
</tr>
<tr>
<td>11.10 - 11.20</td>
<td>Matthew Sproats</td>
<td>197</td>
<td>Evaluating and enhancing upper limb prosthetic use during everyday activities.</td>
</tr>
<tr>
<td>11.20 - 11.30</td>
<td>Fadila Najem</td>
<td>198</td>
<td>Implementing follow-up phone calls to help identify post treatment side effects at The Crown Princess Mary Cancer Centre.</td>
</tr>
<tr>
<td>11.30 - 11.40</td>
<td>Rachael Hammond</td>
<td>199</td>
<td>Changes to nutrition status and activity levels in melanoma cancer: the Eat well, move well with your cancer treatment study.</td>
</tr>
<tr>
<td>11.40 - 11.50</td>
<td>Elizabeth Parker</td>
<td>200</td>
<td>Changes in resting energy expenditure in female adolescent patients hospitalised with anorexia nervosa during higher caloric nutritional rehabilitation: A pilot study.</td>
</tr>
<tr>
<td>11.50 - 12.00</td>
<td>Deirdre D’Souza</td>
<td>201</td>
<td>When all trials are not equal: introducing a complexity scoring system to assist pharmacy workload and resource planning.</td>
</tr>
<tr>
<td>12.00 - 12.10</td>
<td>Melissa Loos</td>
<td>202</td>
<td>Domestic violence presentations to WSLHD emergency departments.</td>
</tr>
</tbody>
</table>
Problematic ankle pain in an adult with spastic quadriplegic cerebral palsy: A case study

H Barden, I Baguley, A Alchin

Brain Injury Rehabilitation Service, Westmead Hospital, Australia; Sydney Medical School, The University of Sydney, Australia; Integratedliving Australia.

Background/Aims:

Botulinum Toxin-A (BTX-A) is a well recognised treatment for lower limb spasticity in people with cerebral palsy (CP). In Australia, BTX-A receives government funding for children under 18 and adults are only subsidised if they were injected as children. This creates discrepancies in access to clinically appropriate spasticity management for a subset of adults with spasticity. This case study presents the clinical problem solving of an adult patient with acute onset ankle pain related to spasticity and examines the costs associated for this individual.

Methods:

A 33 year old male with spastic quadriplegic CP presented with acute left ankle pain limiting mobility. Plain x-rays were unremarkable and an ultrasound found tibialis posterior tenosynovitis. An MRI identified a left distal tibial stress fracture and osseal oedema in talus and calcaneus. He was managed NWB in a CAMboot for 6 weeks. Follow up MRI showed tibial healing, however, during graduated return to FWB he developed increased pain and was found to have a new calcaneal stress fracture. While bone density scanning showed borderline osteopenia, his fractures were diagnosed as being secondary to spasticity.

Results:

A small literature base supports the occurrence of ankle/foot stress fractures from spasticity in both adults and children with CP. In this patient's case, mobility and capacity to work were restricted over an almost seven month period. Direct costs (health, plus time off work) were estimated at around $23,100. Due to funding restrictions, he had not received BTX-A injections for equinovarus for 20 months pre-fracture.

Discussion:

This study presents a patient with recurrent ankle/foot stress fractures on the basis of under treated spasticity. The inability to treat adults with CP who were not injected as children appears based on rationing practices that are not supported by the literature or the costs of not treating.
What outcomes do patients achieve with SMART acute-rehabilitation?

J Brugman¹, N Gupta²

¹ Occupational Therapy, Westmead Hospital; ² Rehabilitation Medicine, Westmead Hospital

Background/Aims:

The Specialist Management with Acute Rehabilitation Team (SMART) provides multi-disciplinary rehabilitation to acute care patients at Westmead Hospital. The service has expanded from the surgical wards to now include renal, haematology and neurology.

SMART works with a complex and diverse group of patients who experience a wide range of rehabilitation outcomes. As a team we are keen to understand the factors that may predict rehabilitation outcomes.

The aim of this project is to create a profile of patients admitted to the SMART program, examine their rehabilitation outcomes, identify possible predictors of these outcomes and use these findings to guide future quality improvement activities.

Methods:

A retrospective audit has been conducted using routinely-collected data gathered from SYNAPTIX, Westmead Hospital’s Australian Rehabilitation Outcomes Centre (AROC) reports and electronic medical records. All patients admitted to the SMART program in 2017 were included in this audit.

Group data will be analysed using descriptive statistics, and Spearman rank correlations will be used to evaluate the relationship between variables, with a P<.05 used for significance. Results will be used to identify sub-groups of patients who achieved functional and discharge outcomes above or below expectations. An audit of medical records for patients in these sub-groups will be used to identify characteristics that may influence rehabilitation outcomes.

Results:

Data for all 209 SMART admissions in 2017 has been collected and is in the process of being analysed. Preliminary data on the profile of SMART patients and the relationships between key variables will be presented.

Discussion and Conclusions:

It is expected that this project will result in a profile of patients who are typically referred to SMART as well as profiles of patient groups who benefitted most and least from admission to SMART. Results will also be used to guide SMART quality improvement activities.
Improving knowledge about eating well and moving well during cancer treatment

D Ceprnja, R Hammond, M Quinlivan, P Talbot, N Taylor

Physiotherapy, Nutrition & Dietetics, Crown Princess Mary Cancer Centre, Westmead Hospital

Background/Aims: There is increasing evidence that lifestyle modifications, such as physical activity and good nutrition, can contribute to improving survivorship by reducing cancer and chronic disease risk. Despite this evidence, there is limited opportunity for people with cancer to access information and education about nutrition and exercise within the public health system. The aims of this study are to: 1. determine the effect of an education session on participant knowledge about eating well and moving well during cancer treatment; 2. evaluate participant satisfaction with the education session; 3. identify potential barriers and facilitators to participant ability to implement healthy eating and exercise into their daily lives after the education session.

Methods: As part of this pilot study, four education programs about eating well and moving well during cancer treatment in an emerging cancer group, Melanoma patients undergoing targeted and immunotherapy treatments, were held over a 6 month period at Westmead Hospital and provided by a team including nursing, physiotherapy and dietetics and nutrition. Questionnaires were completed about satisfaction with the session and knowledge gained. A qualitative approach was undertaken to explore perceived facilitators and barriers to making lifestyle changes.

Results: Six participants completed the four education programs. The data reveals that participants report high levels of satisfaction with the education program and increased knowledge about eating well and moving well. Reported barriers and facilitators to making healthy changes will be presented.

Discussion: The service has been shown to be effective in improving knowledge amongst participants with Melanoma cancer about eating well and moving well during their cancer treatment. Further investigation into the facilitators and barriers is warranted in order to support people with melanoma cancer to make more sustainable changes. This model utilised the growing evidence base to redesign the delivery of care to people with cancer.
Aseptic & Cytotoxic Production: Implementation of an Action Plan for Positive Glove Print Results

M Clifford¹

¹Senior Pharmacy Technician, Sterile Production Services, Westmead Hospital Pharmacy Department

Background/Aims:
To implement an action plan to ensure consistency of response should any production staff (pharmacists/technicians) present with a positive glove print result due to poor hand hygiene.

Methods:
On reviewing current departmental procedures a gap was identified in terms of what action to take when production staff present an unusual or high count result on their routine glove prints. A new procedure, including a flow chart, was created to ensure all staff are aware of what action should be taken depending on how much and what type of growth has occurred. In consultation with the Microbiology Department it was also suggested that affected staff undergo a hand hygiene refresher session with the use of an ultraviolet (UV) lotion and torch. Staff with unusual or high count results would apply the UV lotion to their hands prior to usual hand hygiene procedures required to enter the clean room. The UV torch would then highlight any residual lotion left behind (if any) identifying potential issues with hand hygiene techniques which could then be appropriately addressed.

Results:
Routine glove prints cultured Escherichia coli for one staff member and Proteus for another. Both staff underwent refresher training with the UV lotion and subsequent glove print results were negative. The new procedure and action plan was demonstrated to all production staff. An 85% reduction in Staphylococcus growth was seen in the two months after the demonstration in comparison to the two months before with the same production staff involved (29 in total). Bacillus counts were slightly higher however these organisms are considered environmental contaminants not related to the operator.

Discussion/Conclusion:
Production staff are now aware of procedures to be followed should positive results occur. In addition they have a better awareness of the importance of hand hygiene and of potential microbial contamination in the cleanroom environment.
Workflow mapping within the Cancer Care Pharmacy: Reviewing practice 10 years on, is there scope for innovation and redesign?

D D’Souza, S Cuan, F Chiu, K Chung, L Sundmark, D Kerr, K Engelhardt, J Paton, D Ng

1 Cancer Care Pharmacy, Westmead Hospital.
2 Workflow Innovation and Redesign Team, WSLHD
3 Department of Pharmacy, Westmead Hospital.

Background: In July 2007, the Cancer Care Pharmacy (CCP) was integrated within the Westmead Cancer Centre. Changes in pharmacy workload and service provision 10 years on (new processes, equipment, treatment and clinical trial expansion, PBS drug management and staffing enhancements) had led to space limitations that were impeding workflow.

Aim: To define and map existing workflow processes and develop recommendations for practice innovation and redesign.

Method: A project group was established involving CCP staff and WSLHD workflow redesign consultants. A project plan encompassing workflow capture, staff consultation, workflow-development and validation was mapped. Issues and risk identification undertaken and service end-users surveyed.

Results: The project was undertaken in June-July 2017. Distinct processes were identified through high-level workflow-mapping of core business (treatment referral, treatment planning, treatment write-up, treatment verification, production and/or dispensing, education and information). Detailed workflow-mapping was undertaken for each process, sorted by inpatient-versus-outpatient provision or urgent-versus-non-urgent service delivery. Consultants observed on-site practices to validate workflow-processes. A key role map outlining staff reporting lines was drafted. Fourteen key issues/risks were raised by CCP staff, mainly relating to equipment, work, health & safety, workload-burden and space limitations. Positive feedback was returned from end-user stakeholders through a qualitative survey.

Discussion: The CCP team were positively engaged in the workflow-mapping process, staff reported feeling validated as the detailed documentation process allowed them to visually appreciate how busy and complex processes and practices had become. The workflow innovation and redesign team observation of procedures found CCP service delivery to be highly efficient, collaborative and patient focused. Recommendations to improve workflow included space-saving furniture and storage units, purchase of hands-free telephone head-sets to allow multi-tasking in the cytotoxic production suite and suggestions for planned ‘clean-up’ days for review and archiving of old documents. Findings will assist future business-case requests for enhanced space-allocation.
The Role of Clinical Pharmacists in the Emergency Department

C Hidayat¹,², D Kwan¹,²

¹Department of Pharmacy, Westmead Hospital, Westmead NSW, Australia.
²Emergency Department, Westmead Hospital, Westmead NSW, Australia.

Background/Aims: Prior to October 2017, there were no designated pharmacists for the Emergency Department (ED). Given the increased risk for medication errors in the ED, a 1.5 full time equivalent (FTE) clinical pharmacist service (7.30am-5.20pm, Monday-Friday) was implemented from October 2017.

To evaluate the impact of the service, key performance indicators (KPIs) were explored including the number of clinical interventions and medication reconciliations performed. The types of clinical enquiries received and clinical interventions categorised as severe ± life threatening were also analysed.

Method: From October-December 2017, clinical interventions were recorded using the in-house paper-based recording system, where potential medication prescribing errors are flagged with a 'red dot'. Errors are then classified according to the type of error and the severity. During the period, the types of clinical enquiries received and number of medication reconciliations performed were also recorded into a database.

Results: Table 1 Summary of KPIs

<table>
<thead>
<tr>
<th>Number of medication reconciliations</th>
<th>801</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of clinical interventions</td>
<td>475</td>
</tr>
</tbody>
</table>

With respect to severe non-life threatening clinical interventions (n=36), there was a common theme of sub-therapeutic dosing of anticoagulants, antibiotics and immunosuppressants. For all severe life-threatening clinical interventions (n=4), the offending drug involved was methotrexate. Most significantly, there were two incidents where methotrexate was prescribed as a daily regimen instead of weekly.

With respect to clinical enquiries from medical staff, the majority were regarding appropriate medication dosing and alternative pharmacotherapy in view of e.g. medication shortages. Regarding clinical enquiries from nursing staff, the majority revolved around medication administration advice e.g. appropriate rate and concentration of intravenous medications.

Discussion: Our findings demonstrate the initial, positive impact of a 1.5 FTE clinical pharmacist service in the ED. These findings reinforce the value of clinical pharmacists in the ED in providing timely medication advice, reducing medication errors and significantly improving medication safety.
Discharge summaries and medication lists – Are we getting any better?

C Hidayat¹, C Coorey¹

¹Department of Pharmacy, Westmead Hospital, Westmead NSW, Australia.

**Background/Aims:** Discharge summaries form the main communication channel between inpatient and outpatient healthcare providers. This channel continues to suffer, as information is often missing from the medication list or inaccurate despite training and educating doctors.

To assess the accuracy, currency and completeness of medication lists in discharge summaries.

**Method:** A prospective audit of adult patients admitted to a university teaching hospital in Sydney was conducted over one week in March 2017. Patients were included if they were admitted for greater than 24 hours and taking one or more medications at discharge. Data was collected using the National Quality Use of Medicines Indicator 5.8 for Australian Hospitals – Percentage of patients whose discharge summaries contain a current, accurate and comprehensive list of medicines.

**Results:** A total of 54 patients (median age 62 years, interquartile range 41-77) were included in the audit in which of these, 37 had a discharge summary and of these 37, 35 had a medication list included in the discharge summary. It was found that only 16 patients (46%) had a medication list that was current, accurate and comprehensive. Only 21 patients (60%) had all ongoing medications listed. Surprisingly, 11 patients (31%) had the dose, route, frequency and/or duration omitted on at least one medication in the list and 2 patients (6%) had at least one medication that should not have been continued on discharge. Lastly, for all 54 patients, only 21 patients (39%) had a best possible medication history.

**Discussion:** Our findings reinforce the notion that information is often missing from the medication list or inaccurate despite educational interventions. This informs the need for a stronger presence of hospital pharmacists at the time of discharge, assuming an integrative role in the discharge process where medication lists in discharge summaries are finalised or verified by pharmacists.
MAKING OVER THE DAY PROGRAM: PRELIMINARY EVALUATION OF OUTCOMES FOLLOWING REVISIONS TO A RELAPSE PREVENTION GROUP PROGRAM

C Huynh, J Pan, T Lam, P Bowden, K Hay

Western Sydney Drug Health, Sydney, NSW

Background/Aims: The Day Program is a relapse prevention group program that is offered to clients participating in two court diversion programs (MERIT and the Drug Court of NSW). In 2016, the WSLHD Forensic Drug Health Team implemented a number of revisions to the therapeutic content and structure of the Day Program following reports of high attrition rates and preliminary evidence indicating an increased prevalence of crystal methamphetamine users within the client population. The aim of this study is to examine the changes in treatment outcomes for clients who participated in the revised Day Program and how these outcomes differed from the previous Day Program.

Methods: Data was collected for clients who participated in the revised Day Program in 2016. Analyses explored client attendance and completion rates for the Day Program, and a number of psychometric measures related to clients’ wellbeing and self-efficacy. Relevant measures were also compared to available data collected for clients who participated in the Day Program in 2015.

Results: Preliminary evaluations suggest a number of improved treatment outcomes. Exploratory analysis indicates that clients in the revised Day Program on average participated in more sessions of treatment with a subsequent improvement in treatment outcomes compared to the previous Day Program.

Discussion: Results suggest that targeted changes to relapse-prevention group programs can effectively improve treatment engagement for forensic clients in the community. Findings from this study can contribute to our understanding of forensic populations within a group-based therapeutic framework. Implications and recommendations for treatment are discussed.
Improving Access for Patients with Low Back Pain Referred to the Neurosurgical Outpatient Clinics at Westmead Hospital

K Maka and C Segaram

Background/Aim:
The Neurosurgical clinics receive over 1000 referrals annually however only 1 out of every 6 patients referred will see the Neurosurgeon in a 12 month timeframe. Due to this demand patients wait over 463 days to be seen (expected wait time 365 days). Long waiting times have a direct impact on Did Not Attend (DNA) rates (29%) which limits timely access to care. Therefore the aims of this project were to:
1. Improve access for patients with back pain referred to the Neurosurgical outpatient clinics at Westmead Hospital
2. To decrease DNA rates through 2 strategies:
   a. Calling patients for appointments.
   b. Sending automated text-message reminders for appointments.
3. Improve the coordination of care through a multidisciplinary case conference meeting involving all spinal services at Westmead.

Methods:
This was a non-randomized observational study. All adult patients with a history of low back pain were recruited from Westmead Hospital’s Neurosurgical outpatient waiting list. Those excluded were patients with; spinal fractures, history of cancers, cauda equine/cord sings and patients requiring surgical input. Key outcome measures included; number of days waiting for an appointment, attendance rates and patient/staff satisfaction surveys.

Results:
1. Improved access as patients were seen within 199 days (improvement of 264 days). 98% of patients were satisfied with the new service.
2. DNA rates decreased from 29% to 16%.
3. 73% of patients had direct access to other spinal services they required through the establishment of the multidisciplinary case conference.

Discussion:
This pilot study has been successful in providing efficient, timely and cost effective multidisciplinary patient care. It has demonstrated through utilising existing resources, Physiotherapy led clinics can provide appropriate care improving access to services. Collaborative partnerships between spinal services has also provided priority access to patients who require input from another spinal service ensuring patients have access to ongoing care.
Association of nutrition on voice quality in MND patients – a pilot study

Niven, K. 1, Singer, Z. 1, Hammond, R. 2, Lau, T. 2, Bogaardt, H. 1, Menon, P. 2, Vucic, S. 1,2, Flood, V. 1,2

1 = University of Sydney, Fac. of Health Sciences, Sydney, Australia
2 = Western Sydney Local Health District, Westmead Hospital, Westmead, Australia

Background/Aims:
Patients with Motor Neurone Disease (MND) experience voice abnormalities. Voice difficulties are associated with muscle function of the larynx. Research highlights the importance of nutrition among people with MND, as good nutrition supports improved muscle function. This research aims to investigate the association of voice quality with nutrition parameters among people with MND.

Methods:
A voice assessment was conducted among people with MND participating in a pilot study of swallowing exercises and diet intervention. Participants were asked to produce a sustained /a/ three times, at a comfortable pitch and loudness. The following acoustic parameters were analysed: Jitter, Shimmer and Noise-to-Harmonics Ratio. Voice samples were recorded with an electronic recording device and analysed on a standard computer using voice analysis software (PRAAT). Participants also completed 3-day food records. These were analysed in Foodworks for energy intake and macronutrient consumption. Anthropometric measures were assessed using bioelectrical impedance analysis (BIA). The proportion of energy intake to estimated energy requirements (%EER) and estimated protein requirements (%EPR) were calculated. Hydration levels were estimated through total body water percentage (%TBW) from the BIA.

Results:
Data from seven participants with confirmed MND (mean age: 67.0 yrs (±11.6; 44.0-79.0); mean ALSFRS-r score: 36.5 (±7.8; 23-47)) were used in this preliminary analysis. Mean scores for acoustic analysis revealed that all voice samples indicated the presence of voice problems (mean scores for jitter, shimmer and harmonics-to-noise ratio: 1.14%, 10.98% and 11.00). Mean %EER was 115.6 (±15.11), mean %EPR 193.0 (±34.63) and %TBW 48.7 (±8.63). A regression model showed significant influences of %EER, %EPR and %TBW on harmonics-to-noise ratio (p=.023, p=.011 and p=.020 respectively), and a correlation between nutritional status and voice quality.

Discussion:
Preliminary analysis indicates that nutritional status is associated with voice disorders, suggesting that adequate management of nutritional status may be an important consideration for vocal quality.
Study protocol for a randomised controlled trial investigating two different refeeding formulations to improve safety and efficiency of hospital management of adolescent and young adults admitted with anorexia nervosa

E Parker, M Halaki, F Wilson, C Wearne, L Gomes, G Anderson, S Clarke, E Frig, J Russell, M Kohn & V Flood.

1 Westmead Hospital, Western Sydney Local Health District, NSW 2145
2 Faculty of Heath Sciences, The University of Sydney, NSW 2141
3 Royal Prince Alfred Hospital, Sydney Local Health District, NSW 2050

Introduction: Providing effective nutritional rehabilitation to patients hospitalised with anorexia nervosa is challenging, partly due to conservative recommendations advocating feeding patients at low energy intakes to prevent the possible development of refeeding complications.

Of particular concern, the reintroduction of carbohydrate in a starved patient can lead to electrolyte derangement and increased risk of developing organ dysfunction. Conversely, an ‘underfeeding syndrome’ can develop when patients are not provided with adequate nutrition during treatment, whereby malnourished patients fail to restore weight in a timely manner, and may even lose weight.

Aim: This proposed trial builds on earlier research that establishes the safety of more rapid refeeding. In that context, it aims to test the efficacy and safety of a lower carbohydrate enteral formula (28% carbohydrate) against a standard enteral formula (54% carbohydrate), in patients (aged 15-25 years), hospitalised with anorexia nervosa.

Methods and Analysis: A double blind randomised controlled trial will be used. Recruitment will occur in two hospitals in NSW. Participants will be randomly allocated to receive a standard enteral feeding formula (containing 1.5kcal/mL, 54% carbohydrate) or a lower carbohydrate enteral feeding formula (containing 1.5kcal/mL, 28% carbohydrate). Assessments will be conducted during the first 3 weeks of hospital admission. The primary outcome measure will be incidence of hypophosphatemia.
The X-men - Exercise classes for prostate cancer
G Regan, K Maka & C Segaram
Contact e-mail: Gerard.Regan@health.nsw.gov.au

Background/Aims: Prostate cancer is the most diagnosed cancer in Australia. Androgen deprivation therapy (ADT) is a standard method of oncology care. ADT can have physical and psychological effects, including decreased muscle and bone strength, increased risk of frailty, increased number of falls in the past 12 months and recurrent falling. The study aimed to increase lower limb strength (LL) via supervised exercise classes, as superior LL strength is a predictor of decreased falls. This is important as the greatest reason for hospital admission for individuals aged over 70 is falling.

Methods: All participants were referred by WSLHD oncologists and completed 8 weekly supervised exercise classes consisting of both cardiovascular and resistance circuit exercises, as supervised classes are more effective than individual programming for this population. A quality assurance study was completed to assess if the program significantly increased participant LL strength. LL strength was assessed prior to and after program completion via “A 30-s Chair Stand Test as a Measure of Lower Body strength in Community-Residing Older Adults”. A paired T-Test was used to measure significance.

Results:
- Total number of Participants referred on ADT by 26th April 2018: 38
- Total number of referred participants on ADT who did not wish to take part: 7
- Total number of participants lost to follow up at 26th April 2018: 3
  - Pre-Class data
    - Average A 30-s Chair Stand Test repetitions = 15.321
  - Post-Class data
    - Average A 30-s Chair Stand Test repetitions = 19.71
- LL strength significantly increased (p<0.001)

Discussion: Participants had a significant increase in LL strength after completing the program, which is important as it will decrease falls risk. This may decrease later risk of injury and hospital admission for this high risk population. Not applicable
ABSTRACT 192

THE ROLE OF CLINICAL PSYCHOLOGY IN DRUG HEALTH: A CASE STUDY

A BARBARO, B GARBER, L HARVEY, R HOPKINS, M MARCHANT, M TRUSCOTT

1 Centre for Addiction Medicine, Western Sydney Local Health District, NSW

Background/Aims: Psychological assessment and treatment has been shown to be invaluable with regards to achieving sustainable outcomes in substance using patients. At times, when general drug and alcohol treatment approaches are insufficient in formulating a patient's problems, specialised psychological input can help to specifically target mediating factors and barriers to treatment, which would otherwise not be taken into account and potentially increase the chance of relapse. This frequently applies in the case of comorbid substance use and mental health conditions, such as depression, anxiety, post-traumatic stress disorder (PTSD) and personality disorders. If substance use itself is conceptualised not as a temporary problem behaviour, but as a longstanding coping response to deal with difficult and painful underlying beliefs, memories and emotions, then evidence based psychological treatment may provide better outcomes than medical and general counselling interventions focused on substance only. The case study presented in this work, aims to demonstrate an example of a complex drug health patient, where specialised psychological interventions were used to address multiple mental health and physical comorbidities.

Methods: Client and Treatment Setting: The case study presents a 46-year-old male, with an extensive forensic background, and diagnoses of substance use disorder (ETOH, Methamphetamines, Cannabis), depression and complex PTSD. The patient initially completed a 12 week court diversion treatment program with WSLHD (MERIT Program), which provided Cognitive Behaviour Therapy (CBT) based psychological treatment and an introduction to schema therapy. He then commenced individual outpatient psychology treatment at WSLHD Drug Health and has attended 28 psychology sessions, mostly attending on a weekly to fortnightly basis. Psychological assessment and interventions utilised evidence-based modalities including Schema Therapy as well as Eye Movement Desensitisation (EMDR).

Results: Throughout the course of treatment, the patient’s overall functioning increased significantly. He successfully completed Hepatitis C treatment and started to actively seek employment; he reports maintaining abstinence from alcohol and methamphetamines and has reduced his reported use of Cannabis by 50%. The patient also paid off a large part of his state debt through a work and development order, which was linked to his regular attendance at therapy sessions.

Discussion: Despite limited availability of outcome data in this case, it highlights the role of clinical psychology in the treatment of complex presentations, as well as types of specialised interventions that can be used to address complex mental health presentations in substance using patients.
Progressing the Allied Health research agenda in health literacy

K Hobbs, M Balasubramanian, C Blumenthal, C Burns, D Cepnja,
J Gibson, T Lau, R Milad, V Flood

1 Allied Health, Division of Clinical Support, WSLHD
2 The University of Sydney, Faculty of Health Sciences
3 Western Sydney Local Health District, Allied Health Research Unit
4 Nutrition and Dietetics, Division of Women’s Health and Newborn Care, WSLHD

Background/Aims: People with lower health literacy (HL) often have multiple medical co-morbidities and poorer health outcomes. This research aimed to investigate the HL of patients attending Allied Health outpatient clinics of Westmead Hospital, and to further explore previously collected information by clinic group.

Methods: An observational study conducted in 2017 collected data from 230 patients attending clinics supported by Dietetics and Nutrition, Occupational Therapy, Physiotherapy, Social Work and Speech Pathology, using the Health Literacy Questionnaire (HLQ)™. This research further analysed the data by clinic groups: Women’s Health, Hand Therapy, Head and Neck, Musculoskeletal and Cancer Service clinics.

Patients who participated in the survey from these clinics were described for demographic details and using the nine subscale domains of the HLQ; a methodology known as “Ophelia”. Statistical analyses compared demographic details and the health domains between the clinic groups, using ANOVA, and post-hoc analyses as required.

Results: Generally, among the people who completed the survey, the HL scores in each domain were good. In preliminary analyses, patients who completed the survey at the Hand Therapy Clinic had significantly lower scores in several HL domains compared to other clinics (P<0.05). Patients who attended the Hand Therapy Clinic were significantly younger (45 years) and included a higher proportion of people working full-time (51.7%), compared to most other clinic groups (p<0.05). Case “vignettes” will be presented to describe the typical characteristics of the sub-groups, with reference to HL scores in each domain.

Discussion: Preliminary findings from this research suggests further support is required to improve HL in outpatients in Westmead Hospital. The provision of information and educational resources tailored to the known HL of our patients may improve understanding of the prevention and/or self-management of chronic health conditions and treatment compliance; thus facilitating better health outcomes.
RIGIDITY BUT NOT TREMOR IS ASSOCIATED WITH PAIN IN PEOPLE WITH PARKINSON’S DISEASE

Cassandra Wong, 1Natalie E Allen, 1Niamh Moloney, 1Colleen G Canning. Discipline of Physiotherapy, University of Sydney, PO Box 170, NSW 1825, Australia.

Faculty of Health Sciences, The University of Sydney, Sydney, Australia;

Department of Health Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia

Background/Aims: Up to 85% of people with Parkinson’s disease (PD) experience pain. Previous research has explored the presence and severity of pain in PD, but not the frequency of pain or the effect pain has on activities. The contributions of impairments to pain are poorly understood. This study aimed to:

1) examine the severity and frequency of pain and the extent to which pain interferes with work and

2) explore the contributions of motor impairments to pain in people with PD.

Method: Pain severity, frequency and the impact of pain on work were determined using subscores from the SF-36TM, Parkinson’s Disease Questionnaire and SF-12v2TM, respectively, in 231 people with PD. Motor impairments were measured using the Unified Parkinson’s Disease Rating Scale. Freezing of gait was determined as its presence or absence in the last month. Associations between impairments and pain were examined using logistic regression.

Results: Pain was reported by 187 (81%) participants, with 91 (39%) reporting pain of moderate severity or worse. Pain interfered with work to some extent in 158 (68%) participants. After adjusting for age and gender, increased rigidity was associated with higher pain frequency and more pain that interfered with work (for both models, Odds Ratio 1.14, 95% confidence interval 1.0–1.3). Tremor was not associated with any measures of pain and motor impairments were not associated with pain severity.

Discussion: Most people with PD experience pain at least monthly and pain interferes with daily activities. PD impairments are associated with more frequent pain and pain that interferes with work, with rigidity having the strongest association. Development of PD-specific pain assessments and further investigation into the association between PD impairments and pain is warranted.
COPING WITH PELVIC GIRDLE PAIN DURING PREGNANCY: A QUALITATIVE STUDY PROTOCOL WITH AUSTRALIAN WOMEN

D Ceprnja1,2, L Chipchase2,3, P Liamputtong2, A Gupta1,2

1Physiotherapy Department Westmead Hospital, 2School of Science and Health, Western Sydney University, 3Faculty of Health, University of Canberra

Background/Aims: Pelvic girdle pain is commonly experienced during pregnancy and results in significant physical, psychosocial and work-related challenges. Few studies have investigated the lived experiences of pregnant women with pelvic girdle pain and their coping strategies. There is a need to develop a greater understanding of this prevalent condition among Australian women. Thus, this study seeks to gain information about the impact of pelvic girdle pain on daily life and how women cope with this condition during pregnancy.

Methods: A qualitative research design, situated within a phenomenological framework, will be adopted. The participants will be invited to describe their lived experiences of pregnancy-related pelvic girdle pain, the impact on their daily life, and the strategies they use to cope with the condition. Different methods of data collection will be utilised, including face to face interviews, solicited diaries and focus groups. Ethical approval has been granted by the Human Research Ethics Committees of Westmead Hospital, Sydney, and Western Sydney University, Sydney.

Results: not applicable as this is a protocol. Data collection will commence in July 2018.

Discussion: This project will provide information about the lived experiences of pregnant women with pelvic girdle pain and the supports they see as beneficial in helping them to cope with this condition. An important strength of this study design is that it aims to include a diverse and broad sample of Australian women, such as those from varying socio-economic situations and different ethno-cultural backgrounds. This study will provide knowledge that will inform healthcare professionals and determine the best support(s) needed so that sensitive health care practice can be implemented in the management of pregnant women with pelvic girdle pain.
Associations between bicycling and falls related physical performance in older adults.

S.Harvey, C.Rissel and M.Pijnappels

Westmead Hospital, Sydney, Australia; School of Public Health, The University of Sydney; Faculty of Behavioural and Movement Sciences, Vrije Universiteit, Amsterdam.

Background/Aims: Falls remains a significant health issue in older adults. The WHO estimates that 28-35% of people over 65 years old fall each year, of which 20-30% result in an injury requiring medical attention. Bicycling positively influences falls risk factors including reduced balance, muscle weakness and low self-perceived confidence in maintaining balance. This association has not been systematically examined.

Methods: 107 community-dwelling participants aged 65 and over in the Netherlands were recruited to this cross-sectional study. Participants completed 3 questionnaires on cycling behaviour and balance confidence, and undertook 5 fall related physical performance tasks encompassing tests of balance, strength, gait and endurance. These were compared using two sample 't' tests.

Results: Current bicyclists showed significantly better scores in all physical tasks and confidence compared to non-riders ranging from a 10% difference in six metre walk time to a 141% difference in single leg balance time (all p=0.01). Type of bicycle used and duration of bicycling displayed varied associations (0.01<p<0.79).

Discussion: This study showed that at a higher age, people who still bicycle demonstrate better scores on falls related tasks and have a higher confidence in their balance. Causation cannot be established due to the cross sectional nature of the study, but people who continue to bicycle seem to represent active and healthy aging. Falls rates between the Netherlands and other western nations do not differ significantly, however, other research suggests those engaging in physical activity may have less injurious falls. Novel means of engaging and maintaining people in physical activity such as bicycle riding are worthy of further investigation.
Evaluating and enhancing upper limb prosthetic use during everyday activities.

1Mr Matthew Sproats, 2Dr Melissa Nott, 3Dr Judy Ranka.

1Western Sydney Local Health District, 2Charles Sturt University, 3University of Sydney.

Background: Individuals with an amputation of their upper-limb experience physical impairment that is often overcome by the use of a prosthesis. Despite advances in prosthetic technology, prosthetic non-use is high. Increased cognitive load when using a prosthesis may be a contributing factor in prosthetic non-use.

Methods: Two participants engaged in a four week intervention program that targeted specific participant goals. Each participant set three goals but only two goals were targeted. Goal three was retained and used to evaluate generalisation of skills learned. Cognitive strategy training was based on Perceive, Recall, Plan and Perform (PRPP) Intervention. This approach uses in-task prompting methods that promote the application of attending, sensing, remembering and thinking strategies during performance. Pre and post intervention assessment was conducted using Goal Attainment Scale methods and the Perceive, Recall, Plan and Perform (PRPP) Assessment: Performance Mastery and Cognitive Strategy Application.

Results: Both participants demonstrated improvements in task performance mastery and cognitive strategy use. Goal attainment scaling demonstrated that both participants met their occupational goals. Generalisation of skill to the non-trained goal was evident in both participants.

Discussion: The PRPP is a suitable assessment and intervention model for prosthetic training as it addresses both performance and cognitive strategy use. Its application in training novel or complicated tasks warrants further investigation.
Evaluating and enhancing upper limb prosthetic use during everyday activities.

1Mr Matthew Sproats, 2Dr Melissa Nott, 3Dr Judy Ranka.

1Western Sydney Local Health District, 2Charles Sturt University, 3University of Sydney.

Background: Individuals with an amputation of their upper-limb experience physical impairment that is often overcome by the use of a prosthesis. Despite advances in prosthetic technology, prosthetic non-use is high. Increased cognitive load when using a prosthesis may be a contributing factor in prosthetic non-use.

Methods: Two participants engaged in a four week intervention program that targeted specific participant goals. Each participant set three goals but only two goals were targeted. Goal three was retained and used to evaluate generalisation of skills learned. Cognitive strategy training was based on Perceive, Recall, Plan and Perform (PRPP) Intervention. This approach uses in-task prompting methods that promote the application of attending, sensing, remembering and thinking strategies during performance. Pre and post intervention assessment was conducted using Goal Attainment Scale methods and the Perceive, Recall, Plan and Perform (PRPP) Assessment: Performance Mastery and Cognitive Strategy Application.

Results: Both participants demonstrated improvements in task performance mastery and cognitive strategy use. Goal attainment scaling demonstrated that both participants met their occupational goals. Generalisation of skill to the non-trained goal was evident in both participants.

Discussion: The PRPP is a suitable assessment and intervention model for prosthetic training as it addresses both performance and cognitive strategy use. Its application in training novel or complicated tasks warrants further investigation.
Implementing follow-up phone calls to help identify post treatment side effects at The Crown Princess Mary Cancer Centre.

F Najem1, S Prosser1, J Harris1, W Sharp1

Crown Princess Mary Cancer Centre- Westmead Hospital

Aim: Several studies have investigated the benefits of follow-up phone calls for cancer patients. The findings showed proactive telephone calls to be effective for exchanging information between Radiation Therapists (RT’s) and patient’s in regards to post radiation therapy (RT) side effects and results in a high level of patient satisfaction. The purpose of this study is to evaluate the effectiveness of implementing follow up phone calls to help identify patients with post treatment side effects at Crown Princess Mary Cancer Centre Westmead (CPMCCW).

Methods: All curative RT patients, excluding paediatrics between January 2015 & February 2016 were selected for a follow-up phone call post radiation treatment. A questionnaire consisting of 8 questions was created in ARIA to capture data collected from the follow-up phone calls. Patients were assessed on current health status and on-going side effects post treatment as well as their satisfaction with the follow-up phone call.

Results: Data was collected from 850 patients, including 11 separate disease groups. Most patients (575/850) coped well and were getting better after their treatment. Lung cancer patients were not coping as well as other sites with more males than females reporting they were not coping well. Head and neck (H&N) and lower gastrointestinal (LGI) cancers had the lowest reports of side effects improving. Just 11% of patients contacted the hospital because they weren’t feeling well and most patients did not require any dressing supplies. The majority of patients had a follow up appointment and were aware they could contact the hospital regarding concerns. Almost all (835/850) patients were happy to have had the follow-up phone call.

Discussion: The follow up calls have proven to be a successful service that has enhanced the post treatment care of radiation oncology patients. Ongoing evaluation will occur to ensure patient’s needs will continue to be met.
Changes to nutrition status and activity levels in Melanoma cancer: the Eat well, Move well with your cancer treatment study

¹R Hammond, ²D Ceprnja, ³M Quinlivan, ⁴P Talbot, ⁵N Taylor

¹ Nutrition & Dietetics, ² Physiotherapy, ³ Crown Princess Mary Cancer Centre, Westmead Hospital

Background/Aims: Cancer is a leading cause of burden of disease globally. There is increasing evidence that lifestyle modifications, such as physical activity and good nutrition, can contribute to improving survivorship by reducing cancer and chronic disease risk. The aim of this study was to determine changes to nutrition status, activity levels, body composition, and malnutrition risk as a result of increased knowledge and facilitation of behaviour change.

Methods: As part of this pilot study, four education programs about eating well and moving well during cancer treatment in an emerging cancer group, Melanoma patients undergoing targeted and immunotherapy treatments, were held over a 6 month period at Westmead Hospital and provided by a multidisciplinary team including nursing, physiotherapy and dietetics and nutrition in direct response to identified patient needs.

Results: Improvements were seen in self-reported physical activity, and diet quality across the session times. At first session, participants reported moderate activity levels on average, compared to vigorous activity by the final session. Similarly, more participants reported undertaking strengthening exercises and flexibility practice at the final session. Participants achieved higher diet scores across the sessions, indicating improved diet quality, and intakes closer to the Australian Guide to Healthy Eating, with increased intake of fruit, vegetables, legumes and olive oil and reduce intake of discretionary choices and alcohol.

Discussion: Participants made small changes to their diet and moderate changes to their activity levels. The service has been shown to be cost-effective in managing these patients in an integrated group setting, rather than in individual appointments with each provider.
Changes in resting energy expenditure in female adolescent patients hospitalised with anorexia nervosa during higher caloric nutritional rehabilitation: A pilot study.


Aims: This study examined changes in resting energy expenditure (REE) during the first two weeks of higher caloric nutritional rehabilitation in 10 hospitalised female adolescent patients (aged 15-18) with anorexia nervosa (AN) (DSM-5).

Methods: REE was measured at baseline, week 1, and week 2, using indirect calorimetry (Cosmed FitMate™ v2.3), and compared against 10 age and sex matched healthy controls.

Results: The mean age of subjects was 16.2 years (±0.8). During the first 2 weeks of inpatient treatment, mean daily energy intake increased from 2590kcal (±202) to 4110kcal (±749), which equated to 54.5kcal/kg (±6.4) at baseline and 79.5kcal/kg (±17.9) at Week 2. Mean weight increased from 47.9kg (±4.8) to 52.3kg (±4.4), median %BMI increased from 82.7% (±5.9) to 90.5% (±5.3).

REE was significantly lower at baseline among patients with AN compared with healthy controls (1367kcal/day (±248) vs. 1653kcal/day (±209), p=0.012). There was a significant increase in REE from baseline to week 1 (1367kcal/day (±248) vs.1656kcal (±220), p=0.013) among the cases. The higher REE was maintained at week 2 among the cases, and there was no significant difference between the cases and controls at both week 1 and week 2.

Discussion: REE is depressed in patients with AN. REE increased after 1 week of inpatient higher caloric nutritional rehabilitation, and was similar to healthy controls 1 and 2 weeks after nutritional rehabilitation commenced.
When all trials are not equal: Introducing a complexity scoring system to assist pharmacy workload and resource planning.

D D’Souza, P Fa, D Ng

1 Cancer Care Pharmacy Service, Westmead Hospital, WSLHD
2 Investigational Drug Service, Westmead Hospital, WSLHD
3 Department of Pharmacy, Westmead Hospital, WSLHD

Background: The Westmead Cancer Care Pharmacy provides investigational product (IP) management services to 6 cancer clinical trial units in addition to providing clinical pharmacy, drug distribution and cytotoxic production services to non-clinical trial cancer patients. Expansion in the number of clinical trials (CTs), including early phase trials, greatly increased pharmacy workload. Predicting resource implications primarily on the number of open trials has not been an effective strategy as variations in complexity of IP management, production/dispensing processes, recruitment rates and treatment schedule frequency also impact workload.

Aim: To develop an objective rating system for CTs to predict pharmacy workload and assist with resource planning and allocation.

Methods: A pharmacy working group assessed existing CT workload scoring systems, none were found for pharmacy trial-related roles and activities; CT coordinator functions were the focus of published articles measuring workload complexity. Objective criteria for evaluating pharmacy CT workload complexity was developed and a consensus scoring system applied. Validation of the scoring system was confirmed by scoring consistency for the same CT by independent pharmacists. The scoring system was then applied to CTs supported by the cancer care pharmacy during August 2017.

Results: A total of 90 CTs were supported by the cancer care pharmacy in Aug 2017; medical oncology (47%), haematology (34%) and smaller numbers from other cancer trial units. The complexity scoring system rated 17% of CTs to have standard/basic workload implications, 48% moderate/intermediate workload and 34% having high workload impacts due to complexity.

Discussion: This scoring system provides an objective tool for quantifying pharmacy-specific CT activity related to workload complexity. Application of this tool will enable better planning and distribution of staffing resources and can be used as a key performance indicator of workload complexity when factored against recruited patients on the CT.
Domestic Violence Presentations to WSLHD Emergency Departments

M Loos11, T Lau21 33, A Vukovic41, A Shetty51 63, M Nittis72, F Pisani 82, V Flood91 103

111Westmead Hospital, WSLHD
122Blacktown/Mt Druitt Hospital WSLHD
133University of Sydney

Background/Aims: International research suggests that domestic violence (DV) may be a significant problem amongst females seeking healthcare, with scant local evidence to support same. The primary aims of this study were to document the specific injury types among females presenting to ED, and explore demographics of the females presenting to WSLHD ED with DV related injuries.

Methods: We conducted a retrospective medical file audit of females presenting to Westmead, Blacktown and Mt Druitt EDs from May 2016 to November 2016. Information was collected about presenting injury and confirmed / probable cases of domestic violence (DV) were identified.

Results: A total of 1747 files from female presenting with injuries were reviewed and preliminary descriptive analyses conducted. Eighty-eight (88) cases (5.04% 95% CI 4.11-6.17) were identified as being confirmed or probably DV-related occurrences (includes 52 cases of confirmed and 36 cases of probable DV). Fall related injuries were the most common injury type overall (814 out of 1747, 46.6% 95% CI); and among cases identified as DV, a combination of head, neck, face and eye injuries were the most prevalent injury types (49 of 88 cases, 55.7%, compared to 18.9% among those who were non DV cases, p < 0.0001). Females identified as DV cases were younger (33.1 years to 54.1 years, mean difference 20.9, p<0.0001) and more likely to be single (48.8% versus 23.7%. p<0.0001) compared to those who were non DV cases. Almost half (40 of 88 (45.5%) of the women identified as DV cases were seen by a social worker.

Discussion/Conclusion: This study provides local insight about females presenting to ED and likely to be DV cases. The most prevalent injury type of DV cases were head, neck, facial and eye injuries. In comparison to international literature the number of cases identified as DV cases in WSLHD are likely to be an underestimate highlighting the importance of information gathering and selective screening, with consideration to presenting injuries.
Acknowledgement of Assessors

Terry Amis
Jane Armes
Justin Beardsley
David Booth
Yuyan Chen
Helen Cheng
Tegan Cheng
James Chong
Zoe Clayton
Anna DeFazio
Odette Erskine
Peter Fahmy
Patricia Ferguson
Samantha Ginn
Wendy Gold
Kavitha Gowrishankar
Jin Gun Cho
Georgette Hanna
Andrew Harman
Wayne Hawthorne

Kerry Hitos
Vincent Lee
Sadia Mahboob
Richard McGee
Michael Nafisinia
Sarah Palmer
Zeb Rahman
Scott Read
Sam Rogers
Federica Saletta
Radek Szmyd
Joanne Tan
Varsha Tembe
Verlaine Timms
Naomi Truong
Karen Walker
Cameron Webb
Anthony Yeoh
Hans Zoellner