The aim of this symposium, the second of the biannual ECR symposium series for 2012, is to provide a forum for Early Career Researchers (ECRs) to present their work to fellow researchers and also to **foster collaborations, enhance networking** and **bring together researchers** located at the Alfred Medical Research and Education Precinct (AMREP).

The AMREP ECRs come from the Central Clinical School, BakerIDI, Burnet Institute and the School of Public Health and Preventive Medicine. The research undertaken is a ‘broad church’, embracing a variety of research strands from investigation of physiological mechanisms at their most detailed to population health statistical analyses and data registries.

Such a nexus provides great opportunities for development of cross disciplinary research initiatives, and for individuals to draw on a wide range of expertise for their research projects.

See more at [www.med.monash.edu.au/cecs/ecr-committee.html](http://www.med.monash.edu.au/cecs/ecr-committee.html)
## Programme

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<td>12.50-1.00pm</td>
<td>Closing Remarks and Awarding of Prizes by Dr Lachlan Gray, NHMRC Postdoctoral Fellow, Burnet, and CCS ECR Committee President and Chair</td>
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The prize selection panel consists of:

- Dr. Elizabeth Gardiner (CCS, ACBD)
- Dr. Alex Agrotis (CCS, Dept Immunology)
- A/Prof Melissa Churchill (Burnet, Centre for Virology)
- Dr Maree Powell (Burnet, Centre for Immunology)
Abstracts for Oral Presentations

Session 1: 9.20-9.32am

Kate Hoy (CCS, Alfred Psychiatry Research Centre)

Can transcranial Direct Current Stimulation restore learning effects in Schizophrenia?

Cognitive deficits in schizophrenia underlie more functional disability than any other symptom of the illness and existing treatments are largely inadequate. The development of effective pathophysiology based techniques are urgently required. Currently, the standard treatment approach is cognitive remediation, however the degree of improvement is modest at best (with low to medium effect sizes [on average d = 0.41]) and remediation requires many hours of intensive training. An approach which is able to produce cognitive improvements of large effect sizes within short durations would be transformative. Non-invasive brain stimulation, namely Transcranial Magnetic Stimulation, has very recently been shown to hold considerable promise in this respect (Barr et al, In Press). Here we present highly complementary preliminary data of the effects of another non-invasive form of stimulation, transcranial Direct Current Stimulation. These results suggest that brain stimulation techniques may represent a transformational approach to the treatment of cognitive impairment in schizophrenia.

Session 1: 9.32-9.44am

Renee Duncan (Burnet Institute, Centre for Virology)

Increased HIV-1 dependence on the CCR5 N-terminus and altered recognition of the CCR5 extracellular loops defines a common mechanism of clinical resistance to the CCR5 antagonist maraviroc in vivo, and exposes a new drug target

Background: The CCR5 antagonist maraviroc (MVC) inhibits HIV-1 entry by altering the CCR5 extracellular loops (ECL), such that the gp120 envelope glycoproteins (Env) no longer recognize CCR5. To elucidate the mechanism of resistance to MVC in vivo, we characterized Envs isolated from 2 subjects who experienced virologic failure on MVC.

Methods: Envs were cloned from patients 17 and 24 before commencement of MVC (17B and 24B) and after virologic failure (17F and 24F). Resistance was determined phenotypically by measuring the maximal percent inhibition (MPI). Resistance mutations were identified by Env mutagenesis. CCR5 affinity was measured by 293-Affinofile assays and mathematical modeling. The mechanism of CCR5 engagement was determined by entry assays in cells expressing CCR5 mutants, neutralization/inhibition assays and structural studies.

Results: The patients exhibited broad divergence in resistance levels, with 17F Env having a high MPI of ~95% and 24F Env having a very low MPI of ~0%, indicating “weak” and “strong” resistance, respectively. Affinity-profiling revealed that 17F and 24F Envs engaged MVC-bound CCR5 inefficiently or very efficiently, respectively. Despite highly divergent phenotypes, both resistant Envs exhibited an almost superimposable pattern of dramatically increased reliance on sulfated tyrosines in the CCR5 N-terminus, and on charged histidine residues in the CCR5 ECLs. This altered mechanism of CCR5 engagement rendered both the resistant Envs susceptible to neutralization by a sulfated peptide mimic of the CCR5 N-terminus.

Conclusions: Clinical resistance to MVC can involve vastly divergent Env phenotypes, but the molecular pathway to resistance appears to be common. The discovery of this common pathway, in particular the increased reliance on sulfated CCR5 N-terminus residues, exposes a new avenue to block HIV-1 entry by CCR5 N-terminus sulfopeptidomimetic drugs. These inhibitors could be useful for treating patients with resistance to CCR5 antagonists, or could prevent resistance from occurring if co-administered with CCR5 antagonists.

Session 1: 9.44-9.56am

Elizabeth Moore (CCS, Epidemiology and Preventive Medicine)

Acute Kidney Injury in Traumatic Brain Injury: Frequency and Implications

There is limited information on the incidence of acute kidney injury in patients with traumatic brain injury although acute kidney injury may contribute to morbidity and mortality. We investigated the incidence of acute kidney injury in patients with moderate and severe traumatic brain injury and the association of acute kidney injury with risk factors and outcomes in these patients.

We studied all patients with traumatic brain injury over 16 years of age admitted to the two designated major trauma hospitals in the state of Victoria, Australia from January 1 to December 31 2008. Patients were included if they had head trauma and presented with a GCS < 13. Prospectively collected data from the hospital trauma registries, Intensive Care Units and pathology databases were analysed retrospectively. RIFLE criteria were used to categorise renal function.

The incidence of acute kidney injury was 9.2% (19/207). Patients who developed acute kidney injury were older, had higher severity of illness scores, and a lower GCS. Overall 42.1% of these patients died in hospital compared with 18.1% in patients without acute kidney
injury. In univariable linear regression analysis, age, severity of illness and admitting hospital were associated with acute kidney injury. After multivariable logistic regression, the occurrence of acute kidney injury was associated with age (p<0.001) and higher APACHE III scores, a measure of severity of injury (p=0.016).

Acute kidney injury is relatively common even in patients with traumatic brain injury. Its association with age and APACHE III scores helps identify patients at higher risk of acute kidney injury.

Session 1: 9.56-10.08am

Megan Lim (Burnet Institute, Centre for Population Health)
Trends in chlamydia positivity from a sentinel surveillance network, 2007-2011

Introduction: The number of chlamydia infections diagnosed annually in Victoria has increased steadily, from 11149 in 2007 to 19184 in 2011. This may be due to increased frequency of chlamydia testing or an increase in population prevalence. This analysis describes trends in chlamydia positivity, 2007 to 2011, among heterosexuals tested for chlamydia at selected high risk clinics in Victoria, Australia.

Methods: The Victorian Primary Care Network for Sentinel Surveillance of BBV/STI collects data on all clients testing for chlamydia at eight sexual health and young peoples’ clinics. Pathology results were obtained from laboratories and demographic and behavioural data from a questionnaire administered at the time of testing. Trends were assessed by including ‘year of survey’ as a continuous variable in logistic regression models. Those aged less than 16 years, sex workers, and men who reported sex with men in the past 12 months were excluded from analysis.

Results: 36174 chlamydia tests were conducted among females and 23883 among males. Between 2007 and 2011, positivity among females increased from 4.7% to 5.8% (OR 1.06; 95%CI 1.03-1.10) and among males positivity increased from 7.4% to 8.2% (OR 1.02; 95%CI 0.99-1.06). Adjusting for age, country of birth, number of sex partners, condom use, and presence of symptoms, chlamydia positivity increased on average 7% per year (OR 1.07; 95%CI: 1.03–1.11) among females and 2% per year (OR 1.02; 95%CI: 0.99–1.06) among males. There were also changes over time in the prevalence of certain risk behaviours reported.

Conclusion: We identified a concerning increase in chlamydia positivity over time, particularly among females. Explanations for this trend may include changes in risk behaviours leading to increased transmission, an increase in the number of high risk women presenting for testing, increasing reinfections due to decreased chlamydia immunity, or declining rates of general antibiotic use.

Session 1: 10.08-10.20am

Roisin Worsley (CCS, Epidemiology and Preventive Medicine)
Sex steroids and lipids in postmenopausal women

Objective: The relationships between endogenous sex hormone levels and cardiovascular disease (CVD) risk in women is contentious. Our aim was to systematically investigate the relationships between sex steroids and lipid levels in postmenopausal women taking into account other potential risk factors.

Methods: A cross-sectional study of 624 naturally and surgically menopausal women not using any systemic hormones, lipid-lowering therapy, mean age 53.9 ± 5.8 years, recruited in the US, Canada, Australia, UK, and Sweden between July 2004 and February 2005. The relationships between total testosterone, dihydrotestosterone (DHT), estrone, estradiol and sex hormone binding globulin (SHBG), the homeostasis model assessment of insulin resistance (HOMA-IR) and each lipid variable were explored using multivariable linear regression.

Results: None of the sex steroids measured made an independent contribution to the multivariable models for total cholesterol, HDL-cholesterol, LDL-cholesterol or triglycerides. The best model for total cholesterol included race and age, and that for LDL-cholesterol included race and blood pressure with each model only explaining 4.8% and 3.3% of the variation in each lipid respectively. HOMA-IR, SHBG, age and surgical menopause explained 22.8% of the variation in HDL-cholesterol whereas HOMA-IR, SHBG, race and surgical menopause explained 25.4% of the variation in triglycerides.

Conclusions: Endogenous estrogen and androgen levels are not independent predictors of lipid levels in postmenopausal women. HOMA-IR and SHBG each make independent contributions to HDL-cholesterol and TG. These factors made little contribution to total and LDL-cholesterol.
TACI regulates T-independent marginal zone B cell responses through innate activation-induced cell death

Activation-Induced Cell Death (AICD) is essential for immune homeostasis and tolerance. In T cell-dependent humoral responses, AICD of B cells is facilitated by Fas ligand (FasL) on T cells triggering Fas signalling on B cells. In contrast, T cell-independent B cell responses involve innate-type B cells, such as marginal zone (MZ) B cells. Little is known about the mechanisms that control AICD of B cells during innate B cell responses to TLR activation. Here, we show that MZ B cells undergo AICD in response to TLR4 activation in vivo. This process requires engagement of the BAFF/APRIL receptor TACI. TACI and TLR4 cooperate to up-regulate expression of both FasL and Fas on MZ B cells and also repress Fas apoptosis signaling inhibitors. Furthermore, up-regulation of FasL and Fas, and AICD of TLR4-activated MZ B was not impaired in the absence of T cells. These findings demonstrate an unappreciated role for TACI and its ligands in the regulation of AICD during T-independent B cell responses.

Histone Deacetylase Inhibitors (HDACi) and HIV Latency Reactivation

**Background:** Histone deacetylases inhibitors (HDACi) can induce viral production in latently infected T-cells through activation of transcription from the HIV LTR and are currently being evaluated in clinical trials as a strategy to perturb latency. Ex vivo and in vivo responses to HDACi are variable and activity of HDACi in cells other than T-cells has not been well characterised. We aimed to develop a high throughput assay to determine the activity of HDACi in different cell types using integrated LTR sequences derived from patients on suppressive antiretroviral therapy (ART).

**Methods:** Integrated HIV LTRs were amplified using triple-nested Alu-PCR from total memory CD4+ T-cells (CD45RO+). NL4-3 or patient-derived LTRs were cloned into the plasmid pCEP4, which forms an episomal chromatin structure. The transcriptional activity of the luciferase gene is under the control of the LTR. Constructs were transfected into Jurkat (T-cell), SVG (astrocyte) and Hela (epithelial) cell lines. The activity of a panel of HDACi on LTR transcription was measured by quantification of luciferase activity.

**Results:** Using a wild-type NL4-3-pCEP construct, we transfected Hela, SVG and Jurkat cell lines and analysed the ability of HDACi to stimulate viral transcription. In Hela, the most potent HDACi that induced >100-fold increase in luciferase activity were panobinostat (0.05µM), Givinostat (1µM), vorinostat (2µM) and entinostat (10µM). Belinostat (0.5µM) and trichostatin-A (0.2µM) both induced 10-100 fold increase in luciferase activity. An increase in luciferase activity in Jurkat and SVG cell lines were also observed, but at significantly lower levels than in Hela for all HDACi. Integrated LTR sequences derived from memory CD4+ T cells collected from pre-ART and/or post-ART (n=4) were successfully amplified, sequenced, cloned and transfected into Hela. All HDACi significantly increased the luciferase activity of patient-derived LTRs similar to that seen with NL4-3 LTR. One unique clone that had no response to any HDACi was identified (1 of 6 clones) from one patient on ART.

**Conclusions:** HDACi activate transcription of patient-derived HIV LTRs in Hela with minimal cytotoxicity. This novel system allows rapid screening of drugs that potentially activate HIV transcription from patient-derived LTRs in different target cells, which could then be used to guide the appropriate design of clinical trials that examine latency activators used either alone or in combination.

Category fluency in schizophrenia research: Is it an executive or semantic measure? A new approach

**BACKGROUND AND AIMS:** Categorical/semantic fluency is a popular measure of semantic function in schizophrenia (SZ). Two meta-analyses have supported the tasks’ efficacy in measuring semantic function (Bokat and Goldberg, 2003; Henry and Crawford, 2005), while a more recent meta-analysis, which contrasted category/semantic and phonemic/executive fluency, suggested that executive function is the predominant determinant of semantic fluency performance (Doughty and Done, 2009). The current study employed a novel approach to examine this latter position by using a fluency task that incorporates both semantic and executive elements (animals by size). This task was matched to the traditional executive fluency task on difficulty, which allowed the two tasks to be contrasted and any differences attributable to semantic deficit.

**METHODS:** Forty two SZ participants and forty healthy controls performed three fluency tasks: executive, semantic and executive/semantic.

**RESULTS:** SZ executive fluency performance was not significantly different from controls, semantic fluency was impaired in SZ, and executive/semantic performance in SZ was impaired to a similar degree to semantic performance.

**CONCLUSIONS:** Given the intact executive fluency performance, and the similar level of deficit in the semantic and executive/semantic task, the SZ deficits likely arise from semantic deficiency. These data support the finding that the semantic fluency task is primarily a semantic measure. This result is discussed in relation to the conflicting literature.
The transcription factor GRHL3 functions as a tumor suppressor in head and neck squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. The 5-year survival for HNSCC patients has remained stationary at 50% for the past 20 years, despite the recent advances in our understanding of cancer biology, and improvements in supportive care post-chemotherapy. For this reason, delineation of the molecular basis of HNSCC is urgently required to inform new treatment paradigms and improve prognosis. Our laboratory has recently demonstrated that the developmental transcription factor, GRHL3 functions as a potent tumor suppressor in the context of skin SCC. Here we examine the role of this factor in HNSCC. Grhl3-K14-Cre conditional knockout mice, which display deletion of the Grhl3 gene in the oral epithelium by 12 weeks, and wild type control mice were exposed to the oral chemical carcinogen 4-NQO. The mutant mice displayed markedly enhanced susceptibility to 4-NQO induced HNSCC, with 39% of these animals developing malignant tumors compared to 3% of controls. Unlike its role in skin SCC, which centres on the PI3K/AKT signalling pathway, Grhl3 exerts its tumour suppressor function in HNSCC by transcriptionally regulating glycogen synthase kinase 3β (GSK-3β). Loss of GSK-3β leads to stabilisation of c-Myc and enhanced tumorigenesis in Grhl3 mutant mice. Grhl3 deletion during embryogenesis also causes epithelial keratinocyte hyperproliferation in the tongue, as result of loss of GSK-3β and enhanced stability of c-myc. Expression of both Grhl3 and GSK-3β in HNSCC patient samples is also markedly reduced indicating that Grhl3 functions as a tumour suppressor gene in HNSCC through enhanced stabilisation of c-Myc oncogene.
Abstracts for Poster Presentations

**Jo-Anne Chan (Burnet Institute, Centre for Immunology)**

**Evaluating the importance of PfEMP1 as a target of protective immunity to human malaria (Poster B1)**

Effective clinical immunity that protects against symptomatic malaria in humans develops gradually after repeated exposure to Plasmodium falciparum. However, the primary targets of immunity are poorly understood. During intra-erythrocytic development, P. falciparum expresses novel surface antigens on the membrane of infected erythrocytes (IE) and includes a protein known as PfEMP1. Antibodies to these surface antigens are variant-specific and associated with protection from symptomatic malaria. However, the significance of different surface antigens as targets of acquired immunity remains unclear. In this study, the importance of IE surface antigens as antibody targets was evaluated using transgenic P. falciparum with inhibited surface antigen expression. We used flow cytometry-based assays to measure the antibody reactivity to IEs and comparisons between the mutant and parental parasites allowed us to quantify the importance of different surface antigens as targets of naturally acquired human antibodies. This approach was applied to a cohort of adults and children in Kenya and Papua New Guinea. Our results showed that a protein known as PfEMP1 was a dominant target of naturally acquired human antibodies to the IE surface and of antibodies that mediate opsonic phagocytosis of IEs by monocytes. Furthermore, individuals with antibodies to PfEMP1 were associated with protection from symptomatic malaria. These findings enhance our understanding of acquired immunity to human malaria and have major significance for vaccine development.

**Emma Weaver (Burnet Institute, Centre for Population Health)**

**Increase in chlamydia positivity among young female attendees of family planning clinics, 2008-2010 (Poster B2)**

**Background:** Young people are disproportionately represented in national chlamydia notifications. We assess chlamydia testing and positivity trends among young attendees of family planning clinics (FPCs) participating in the Australian Collaboration for Chlamydia Enhanced Sentinel Surveillance (ACCESS) in 2008-2010.

**Methods:** Using routine clinical data among 16-29 year olds, we calculated the proportion tested at least once for chlamydia in a 12 month period and chlamydia positivity (proportion of individuals tested returning a positive result at any test) among four FPCs, by year, sex, and age group using non-parametric test for trends.

**Results:** Between 2008-2010, 12,612 individuals attended four FPCs (93% female). Among females, proportion tested was consistently higher in 16-19 and 20-24 year olds compared to 25-29 year olds (e.g. 44%, 44% and 24% in 2010, respectively). Overall testing rates in females increased between 2008 and 2010 (from 38% to 41%; p<0.01); similar trends were seen in 16-19 and 20-24 year olds (p<0.01). Chlamydia positivity was consistently higher in 16-19 year old females compared to 20-24 and 25-29 year olds (e.g. 15%, 9% and 4% in 2010, respectively). Among 16-19 year olds, but not other age groups, positivity increased significantly between 2008 and 2010 (from 9% to 15%; p<0.01). Of interest, this increase was largely attributed to an increase in positivity at one clinic.

The proportion of males tested was consistent between 2008-2010 (47%), while positivity rates showed an overall increase from 20% to 28% (p=0.11).

**Conclusion:** We identified a considerable increase in chlamydia positivity over time among young females, which may be due to higher risk females presenting for testing, more targeted testing or increased background prevalence in the population attending. A similar rise has been observed in young females attending sexual health clinics. These results highlight the importance of ongoing surveillance to monitor chlamydia testing and positivity trends.

**Emma Weaver (Burnet Institute, Centre for Population Health)**

**Comparing Australian born and non-Australian born men who have sex with men (MSM) testing for HIV and other STIs at high case load clinics in metropolitan Melbourne (Poster B3)**

**Background:** Victoria has seen recent increases in HIV notifications among non-Australian born men who have sex with men (MSM). We analysed HIV and other STI testing outcomes among MSM at high case load clinics in metropolitan Melbourne by region of birth.

**Methods:** Rates of HIV, chlamydia and syphilis testing and positivity among MSM attending the Victorian Primary Care Network for Sentinel Surveillance on BBV/STIs (VPCNSS) between 2007-2011 was analysed by region of birth.

**Results:** There were 79,745 HIV, chlamydia and syphilis tests conducted between 2007-2011, of which approximately 30% (n=24,005) were among non-Australian born MSM; most commonly from South East Asia (SEA) (25%) and Europe (25%).

Among 25,915 HIV tests, 7,679 (30%) were among non-Australian born MSM. Among 446 HIV diagnoses, 128 (29%) were among non-Australian born MSM. HIV positivity of both Australian and non-Australian born MSM was 1.7%. HIV positivity was highest among MSM born in SEA (2.3%).
Among 26,492 chlamydia tests, 8,130 (31%) were among non-Australian born MSM. Among 1,736 chlamydia diagnoses, 545 (32%) were among non-Australian born MSM. Relative to Australian-born MSM, chlamydia positivity was comparable among non-Australian born MSM (6.7%), but significantly higher (p=.03) among MSM born in SEA (7.7%).

Among 27,338 syphilis tests, 8,196 (30%) were among non-Australian born MSM. Among 448 syphilis diagnoses, 185 (41%) were among non-Australian born MSM. Relative to Australian-born MSM (1.4%), syphilis positivity was significantly higher (p<.01) among non-Australian born MSM (2.3%), and highest (p<.001) among MSM born in Europe (4.4%).

**Conclusion:** Disaggregating non-Australian born MSM by region of birth has indicated that SEA born MSM have higher positivity for HIV and chlamydia than Australian-born MSM. MSM born in Europe also show significantly higher syphilis positivity compared to other MSM. Further investigation of STI testing and predictors of positivity by non-Australian born MSM is warranted to inform prevention services.

**Hilary Veale (Burnet Institute, Centre for Population Health)**

**The use of social networking sites for sexual health promotion: key strategies for successfully engaging users (Poster B4)**

**Background:** Online social networking sites (SNSs) such as Facebook and Twitter are hugely popular and highly interactive, making them ideal platforms for health promotion. SNSs are increasingly used for sexual health promotion; however, their effectiveness is unknown. The aim of this study was to identify key strategies used to successfully reach and engage users of SNSs involved in sexual health promotion.

**Methods:** We identified active Facebook (n=60) and Twitter (n=40) profiles involved in sexual health promotion and reviewed their activity over a one month period. Quantitative measures of reach and interaction (engagement) were assessed and profiles ranked according to a composite score. Content of the top ten ranked ‘successful’ Twitter and Facebook profiles was analysed using a thematic framework, and compared with three poorly performing profiles to identify strategies for successfully reaching and engaging users.

**Results:** Strategies that were associated with having a large and interactive user base included: regular posts by the host (median of 46 posts per month for top ten Facebook profiles versus six for poorly-performing profiles, and a median of 78 tweets per month versus six for Twitter profiles); direct engagement with users 85% of top ten Facebook and Twitter hosts reply directly to users, versus 0% of poorly performing profiles); and encouraging interaction and conversation by posing questions (100% of the top ten, versus 47% of poorly performing profiles). Making content broadly relevant and engaging by uploading pictures, video and audio material (80% of top ten, versus 27% of poorly performing profiles), and posting time-relevant content (95% compared with 64%) also appeared to be important for success.

**Conclusion:** Compared to poorly performing profiles, successful SNS profiles posted regularly, directly engaged with users, encouraged conversation, and utilised uploads and relevant links. These findings can be used to develop a successful health promotion campaign using SNSs. Success can be measured using a combination of quantitative measures for reach and interaction and basic qualitative content analysis.

**Muriel Aldunate (Burnet Institute, Centre for Virology)**

**LACTIC ACID, A NATURAL MICROBICIDE IN THE FEMALE GENITAL TRACT (Poster B5)**

The vagina contains a ~1% racemic mixture of D- and L-isomers of lactic acid (LA) due to anaerobic glycolysis of epithelial cells and LA-producing bacteria. LA is likely responsible for maintenance of low pH which consequently influences microflora composition. Bacterial vaginosis (BV), an imbalance in the vaginal microflora, is a common condition associated with a decrease in lactobacilli sp. and an increase in vaginal pH. Notably, BV is a major risk factor for sexually transmitted infections, including HIV in women. Our studies show that L-LA has potent broad-spectrum HIV virucidal activity, which is more potent than HCl alone. However, the relative HIV-1 virucidal activities of LA isomers and acetic acid as well as the mechanism of viral inactivation are unknown.

**AIM:** Determine the virucidal properties of L-LA and understand its ability to directly and indirectly protect against HIV and other STIs.

**METHODS:** CCR5-utilizing HIVBa-L and transmitted/founder strains were treated with 0.3% D- and L-LA, and pH4.0 alone for 30 min at 37°C. Samples were subsequently neutralized and viral infectivity determined in TZMbl cells. Anti-HIV activity of 1% L-LA compared to 1% acetic acid and HCl alone was also determined in a time course experiment. The impact of LA on HIV-1 particles was determined by detection of viral proteins by Western blot.

**RESULTS:** At pH 4.0, 0.3% L-LA was 17-fold more potent at inactivating HIVBa-L than 0.3% D-LA. 1%L-LA (pH 3.8) rapidly inactivated HIVBa-L mediating a 10,000-fold reduction compared to 1000-fold and <10-fold for acetic acid and HCl (pH3.8), respectively. No loss of gp120 envelope or disruption of viral particles was observed.

**CONCLUSION:** L-LA has more potent HIV-1 virucidal activity compared to D-LA at threshold concentrations suggesting a stereospecific mechanism against viral protein and is more potent than acetic acid and HCl alone. These data suggest that using L-LA to acidify microbicide formulations could potentially provide greater protection against HIV-1.
Melissa Brown (CCS)

**Osteopontin and AML progression: A causative or collateral relationship? (Poster C1)**

Acute Myeloid Leukaemia (AML) is the most commonly diagnosed acute leukaemia in adults, and is characterised by the abnormal expansion of immature myeloid cells (myeloblasts). AML has a higher incidence in adults over the age of 50, and has a poor overall survival rate, with 80% of patients succumbing to the disease within 5 years of diagnosis. While clinical treatments have improved the overall survival of patients suffering from various leukaemia types over the past couple of decades, the development of AML therapeutics have lagged, and a significant improvement in the clinical treatment of AML hinges on the identification of novel therapeutic targets across AML subtypes.

Our group has recently identified a functional prognostic factor, Osteopontin, which is significantly overexpressed in the myeloblasts of AML patients compared to normal control cells, and increases in patients following relapse. Osteopontin is a secreted factor that has established roles in cell adhesion and cell survival, which are two classic hallmarks of cancer progression. While the relationship between DPN and AML progression is yet to be clarified as causative or collateral, the targeting of Osteopontin has been shown to decrease metastatic potential in mouse xenograft models of solid cancer types such as human lung cancers. With the use of mouse xenograft models of human AML, we are investigating the effects of Osteopontin expression on leukaemogenesis and cancer cell survival in response to conventional clinical therapeutics. Given the attractive nature of the bioavailability of Osteopontin as a secreted protein, our studies aim to establish whether Osteopontin could be a viable target for the development of clinical inhibitors for the treatment of AML.

Zeyad Nasa (CCS, Department of Immunology)

**In vivo generation of antigen specific T regulatory cells through the transduction of bone marrow cells (Poster C2)**

T regulatory cells (Tregs) have the capacity to suppress a wide range of immune responses and play a role in controlling autoreactive reactions in the periphery, making them an ideal candidate for cellular treatment of autoimmune diseases. They are mainly represented as CD4+ CD25high FoxP3+ T-cells. The transcription factor Foxp3 is crucial for Tregs function and it has been demonstrated that antigen specific T cells transduced with Foxp3 gene are converted to functional Tregs. Bone marrow gene modification can be used as a method to drive the expression of genes in bone marrow derived cells, including the expression of T cell receptors. We have developed a retroviral vector encoding the 2D2-TCR specific for the autoantigen MOG35-55, linked to Foxp3 gene. We hypothesized that this would generate an in vivo population of MOG35-55 specific Tregs and thus prevent the induction of MOG35-55 induced EAE. Retroviral constructs were tested in vitro in cell lines and in isolated splenic naïve CD4 and found that these transduced CD4 cells expressing 2D2-TCR and Foxp3, furthermore they displayed Tregs markers such as CD25, GITR and CTLA-4. Transduced BM with 2D2-TCR-Foxp3 retroviruses were used to create chimeric mice with a subpopulation of T cells with MOG35-55 TCR specificity and Foxp3 expression. Chimeric mice were immunized with MOG35-55 peptide to induce EAE, however these cells failed to demonstrate any suppressive activity and mice had a typical EAE symptoms. Thymic analysis of chimeric mice revealed that 2D2-TCR FoxP3+ cells were detected in the thymus but were largely absent in the peripheral. This may be due to the high affinity TCR used resulting in negative selection.

Amit Joglekar (CCS, Department of Immunology)

**The transplantation of bone marrow stem cells encoding the autoantigen MOG promotes antigen-specific T cell tolerance in the thymus and increased FoxP3 CD4+ T cells in the periphery (Poster C3)**

Multiple sclerosis (MS) is an autoimmune disease that targets the myelin sheath surrounding axons of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is a mouse model of MS and can be induced by immunisation with myelin oligodendrocyte glycoprotein (MOG35-55). We have previously we have shown that transplantation of bone marrow (BM) stem cells retrovirally transduced to express MOG, rendered mice resistant to MOG induced EAE. In this model we showed that thymic deletion was a key mechanism of tolerance as deduced by using the MOG35-55 specific 2D2-TCR. Since the normal T cell repertoire exists as a polyclonal population that will harbour varying TCR affinities to MOG, it is not known if in our model, deletion will be the key mechanism or whether it may encourage the generation of regulatory T cells. Using retrogenic technology and availability of MOG specific TCRs with varying affinities, mixed BM chimeras were generated expressing different MOG35-55 specific TCRs and MOG. Following engraftment, we found that the development of MOG35-55 specific thymocytes from all TCR affinities tested was reduced. In the periphery, MOG specific CD4+ T cell population were also significantly reduced compared to other cell populations. Further, examination of MOG specific T cells in the periphery revealed an increase in the proportion of MOG specific Tregs in mice receiving MOG encoded BM. These data suggest that following the transfer of BM encoding the autoantigen MOG, tolerance is associated with deletion of antigen specific T cells and the generation or survival of antigen specific Tregs.
Novel strategy for cell type specific gene expression through retroviral transduction of bone marrow stem cells (Poster C4)

Jie Yu Chung (CCS, Department of Immunology)

Gene therapy combined with bone marrow transplantation has enormous potential for treating immune related disorders. This is due to the unique capacity of transduced bone marrow stem cells to reconstitute patients with immune cells encoding corrective or therapeutic genes. Our lab however is pursuing genetically modified bone marrow transplantation as a means of inducing long-term immune tolerance as a preventative therapy or cure for autoimmune diseases. Exposure to autoantigens during immune cell development is known to be a critical in establishing and maintaining tolerance towards self-antigens and therefore minimising autoimmunity. We have shown that irradiated mice transplanted with bone marrow stem cells transduced with the autoantigen myelin oligodendrocyte glycoprotein (MOG) are tolerant upon MOG peptide immunisation and do not develop the clinical symptoms associated with experimental autoimmune encephalomyelitis (EAE). However due to the large number of cell lineages that derive from bone marrow stem cells it is difficult to attribute the tolerance induced to a specific cell lineage or cell type. To overcome this difficulty I have established a novel method of generating cell type specific gene expression following retroviral transduction of bone marrow stem cells. This novel strategy utilises the Cre lox system to mediate a Cre-dependent DNA inversion event that drives expression of genes encoded within the retroviral provirus. By restricting MOG expression to specific cell lineages and utilising the EAE model of autoimmunity we can begin to identify cell types and key mechanisms that may be involved in this strategy of gene therapy mediated tolerance induction.

COMT and cognition in bipolar disorder: pilot findings (Poster C5)

Tamsyn Van Rheenen (CCS, MAPrc)

BACKGROUND: There is growing evidence that COMT, a gene involved in the transmission of dopamine, is implicated in cognition. The val allele manifests as higher COMT activity and diminished cognitive ability and is considered to be a risk allele, whereas the met allele manifests as reduced COMT activity and enhanced cognitive ability. The val allele has been linked with depression and suicide in bipolar disorder (BD). Hallmarks of BD are compromised cognitive and emotional abilities. It is plausible that COMT’s role in the disorder occurs via its effects on cognition and emotion.

METHODS: Participants with BD and healthy controls completed a battery of emotion processing and emotion regulation assessments that assessed their capacity to perceive and manage emotion appropriately, in addition participating in a neurocognitive assessment. Whole blood (10ml) was collected for each participant. A selected single-nucleotide polymorphisms (Val158Met[rs4680]) was genotyped using the high throughput MassArray system.

RESULTS: Preliminary analysis of the initial dataset showed that the genotype distribution of the BD group (N=18) was too small for meaningful analyses: N=3 Val/Val, N=11 Val/Met and N=4 Met/Met. The healthy controls (N= 45) had a distribution where preliminary analysis was possible: N=6 Val/Val, N=28 Val/Met and N=11 Met/Met. Analysis revealed that carriers homozygous for the val allele had significantly poorer neurocognition (NC), more difficulty in regulating emotions (ER) and were less accurate at identifying facial expressions (EP) (NC: M=43.5, SD=23; ER: M=72, SD=19; EP: M=69, SD=15) than carriers of the val/met alleles (NC: M=47, SD=16; ER: M=94, SD=13; EP: M=71, SD=11) and those homozygous for the met allele (NC: M=54, SD=8; ER: M=91, SD=9; EP: M=80, SD=12). Effect sizes stated in Cohen’s d were medium (NC: d=0.62; ER: d=0.3); EP: d=0.52).

CONCLUSION: Although not significant, the means observed at each genotype for COMT were in the predicted direction. This lack of significance was most likely due sample size restrictions. Interestingly, when effects sizes known to remove the influence of sample size were calculated, moderate effects of allele type on neurocognition, emotion processing and emotion regulation were observed. Given the small sample size, we suggest this pilot data is highly supportive of our hypotheses that a relationship exists between COMT, neurocognition and emotion abnormalities. Further testing will reveal whether this pattern of results holds in the BD sample.

Comparing Semantic Function in Schizophrenia to the Analogue Models of Ketamine and High Schizotypy (Poster C6)

Erica Neill (CCS, MAPrc)

BACKGROUND AND AIMS: This study investigated semantic memory function in psychosis by comparing two analogue groups, ketamine and schizotypy, to a schizophrenia group. The purpose was to determine (1) whether these analogue groups are associated with semantic dysfunction and (2) whether the patterns produced by each of the analogues is similar to that reported in schizophrenia.

METHODS: Semantic memory was examined both explicitly and implicitly using both direct and indirect stimulus pairs designed to evoke semantic priming. Four semantic tasks were administered: implicit/direct, implicit/indirect, explicit/direct and explicit/indirect. The related pairs in the direct task included association or category pairs (e.g. TIGER-STRIPES) the related condition in the indirect tasks included pairs related via a third concept (LION-STRIPES linked by TIGER). The implicit tasks were in a traditional semantic priming format where participants were given a distracter task and not explicitly told that some of the pairs were semantically related. The explicit tasks were designed so participants had more time to assess the pairs and they were explicitly told that they needed to classify pairs as related or unrelated based on their semantic relationship. Four groups were recruited: patients with schizophrenia, healthy controls administered an acute dose of ketamine, healthy controls scoring highly on a schizotypy questionnaire and healthy controls.
RESULTS: In terms of the implicit tasks, there were no significant group differences. Examination of performance on the explicit tasks showed that schizophrenia was associated with significantly more priming on the explicit indirect task (M = 403.23 ± 298.20) than the Ketamine group (M = 214.45 ± 247.69), high schizotypy (M = 125.62 ± 199.15) or control groups (M = 228.94 ± 195.34). This reflected a need for more time to correctly classify unrelated pairs. Differences on the explicit direct task were characterized by a greater priming in the schizophrenia group (M = 212.47 ± 237.18) and Ketamine group (M = 268.79 ± 292.20) compared to the control group (M = 123.07 ± 244.22, p=0.04). Once again, this increase in priming reflected slowed performance in correctly classifying unrelated material. This pattern suggests that schizophrenia was associated with greater benefit from instruction and the presence of a relationship between pairs (an access deficit). Additionally, the Ketamine group demonstrated a similar deficit on the direct explicit task which was actually the more difficult than the indirect explicit task. This difficulty arose because in this task, related pairs include examples of fruit (APPLE-BANANA) and unrelated pairs included other food pairs deemed unrelated (ORANGE-TUNA) which introduced an extra difficulty in deciding at what level they were related, a difficulty that did not exist in the indirect task.

CONCLUSIONS: This pattern of results suggests an access difficulty in schizophrenia generally and a more subtle access difficulty in the Ketamine group that presents itself only on more difficult tasks. This suggests that Ketamine can induce semantic access deficits similar, albeit less severe, than those seen in schizophrenia. These findings also support the notion of an access difficulty in schizophrenia which can be reduced with the introduction of explicit instruction. These results have direct implications for cognitive remediation of semantic memory difficulties in schizophrenia.

Shan Liu (CCS, Department of Epidemiology and Preventive Medicine)

Subtotal nephrectomy accelerates pathological cardiac remodeling post myocardial infarction: Implications for the cardiorenal syndrome (Poster C7)

Background: To understand the nature of concomitant cardiac and renal dysfunction, we investigated whether further pathophysiological changes occur when a kidney insult (induced by 5/6 nephrectomy-STNx) follows that of myocardial infarction (MI).

Methods: Male Sprague Dawley rats (n=43) were randomized into four groups: Sham-operated MI + Sham-operated STNx (Sham+Sham), MI + Sham-operated STNx (MI+Sham), Sham-operated MI + STNx (Sham+STNx) and MI+STNx. MI/Sham surgery (left anterior descending coronary artery ligation) was induced initially; STNx/Sham surgery performed 4 weeks post-MI. Echocardiography, glomerular filtration rate and blood pressure were assessed prior to the second surgery and 10 weeks later. Thereafter, hemodynamic parameters were measured and tissues collected for analysis.

Results: Survival rate was 100%, 59.7%, 91.7% and 44.1% in Sham+Sham, MI+Sham, Sham+STNx and MI+STNx animals, respectively. Left ventricular ejection fraction was further decreased in MI+STNx compared to MI+Sham animals (p<0.01) at week 14, despite no difference in infarct size. Tau logistic, the time constant of relaxation, was further increased in MI+STNx compared to MI+Sham animals (p<0.01). Heart weight (p<0.001) and lung weight (p<0.01) to body weight ratios were greater in MI+STNx compared to MI+Sham animals. In the non-infarct zone of the myocardium, MI+STNx animals demonstrated greater myocyte cross-sectional area (p<0.001), as well as increased cardiac interstitial fibrosis (p<0.01) and collagen I (p<0.01) compared to MI+Sham animals. Despite lower blood pressure in the MI+STNx animals (p<0.01), greater renal interstitial fibrosis in the non-infarct zone was observed in MI+STNx compared to Sham+STNx animals (p<0.001). No further deterioration in renal function was observed.

Conclusions: This study demonstrated STNx accelerates cardiac hypertrophy, fibrosis and cardiac dysfunction post-MI whilst MI accelerates STNx-induced renal fibrosis, supporting bidirectional interactions in cardiorenal syndrome.

Nhu-Y Nguyen (CCS, ACBD)

Induction of BIM leads to abrogation of acute myeloid leukemia in vitro and in vivo (Poster C8)

INTRODUCTION: Acute myeloid leukemia (AML) is currently the most common form of adult leukemia and remains one of the most difficult types of cancer to treat. AML is characterised by the proliferation of immature myeloid cells which is in part due to deregulated apoptotic pathway signalling.

AIM: To examine whether genetic modulation of the pro-apoptotic BIM protein leads to abrogation of leukemia.

METHODS: Lentiviral constructs containing various BIM isoforms (wildtype, 2A (Mcl specific), Bad (Bcl specific) and 4E (inert mutant)) were transduced into the MV4;11 (FLT3 ITD) AML cell line. For in vitro studies, these cells were treated with doxycycline for 72hr to induce expression of BIM genes and the levels of apoptosis assessed by flow cytometry. For in vivo studies, these cells were transplanted into sublethally irradiated immune-compromised NSG mice which were then given doxycycline supplemented water and monitored for Kaplan Meier analysis.

RESULTS: Induction of the wildtype, 2A and Bad isoforms following doxycycline treatment in vitro lead to apoptosis of MV4;11 cells (51.6%, 50.8% and 54.4% respectively). Transplantation of cells transduced with the inert 4E BIM isoform had no effect on NSG survival following treatment with doxycycline. However, mice transplanted with wildtype BIM transduced cells and given doxycycline water had
a significant survival advantage over mice not given doxycycline water (median 61.0 days versus 36.0 days). Furthermore, analysis of these mice upon illness showed a marked reduction of MV4;11 cells engrafted in the bone marrow.

**CONCLUSION AND FUTURE STUDIES:** Overexpression of BIM leads to an abrogation of disease and in leukemic mice, improves overall survival. This provides evidence that specifically targeting the apoptotic pathway in patients with AML may be of therapeutic benefit. Further transplantation studies using the ZA and Bad BIM isoforms may provide insights on whether these effects are predominantly due to Mcl-1 or Bcl inhibition.
### Presenters: Contact details

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<tr>
<th>Name</th>
<th>Email</th>
<th>Name</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megan Lim</td>
<td><a href="mailto:lim@burnet.edu.au">lim@burnet.edu.au</a></td>
<td>Hilary Veale</td>
<td><a href="mailto:hveale@burnet.edu.au">hveale@burnet.edu.au</a></td>
</tr>
<tr>
<td>William Figgett</td>
<td><a href="mailto:william.figgett@monash.edu">william.figgett@monash.edu</a></td>
<td>Mellissa Brown</td>
<td><a href="mailto:melliissa.brown@monash.edu">melliissa.brown@monash.edu</a></td>
</tr>
<tr>
<td>Kate Hoy</td>
<td><a href="mailto:Kate.Hoy@monash.edu">Kate.Hoy@monash.edu</a></td>
<td>Zeyad Nasa</td>
<td><a href="mailto:nasa.zeyad@monash.edu">nasa.zeyad@monash.edu</a></td>
</tr>
<tr>
<td>Roisin Worsley</td>
<td><a href="mailto:roisin.worsley@monash.edu">roisin.worsley@monash.edu</a></td>
<td>Amit Joglekar</td>
<td><a href="mailto:amit.joglekar@monash.edu">amit.joglekar@monash.edu</a></td>
</tr>
<tr>
<td>Emma Weaver</td>
<td><a href="mailto:eweaver@burnet.edu.au">eweaver@burnet.edu.au</a></td>
<td>Jie Yu Chung</td>
<td><a href="mailto:jieyu.chung@monash.edu">jieyu.chung@monash.edu</a></td>
</tr>
<tr>
<td>Renee Duncan</td>
<td><a href="mailto:rduncan@burnet.edu.au">rduncan@burnet.edu.au</a></td>
<td>Tamsyn Van Rheenen</td>
<td><a href="mailto:tamsyn.van-rheenen@monash.edu">tamsyn.van-rheenen@monash.edu</a></td>
</tr>
<tr>
<td>Elizabeth Moore</td>
<td><a href="mailto:elizabeth.moore@monash.edu">elizabeth.moore@monash.edu</a></td>
<td>Erica Neill</td>
<td><a href="mailto:Erica.Neill@monash.edu">Erica.Neill@monash.edu</a></td>
</tr>
<tr>
<td>Hao Lu</td>
<td><a href="mailto:hao.lu@monash.edu">hao.lu@monash.edu</a></td>
<td>Muriel Aldunate</td>
<td><a href="mailto:muriel@burnet.edu.au">muriel@burnet.edu.au</a></td>
</tr>
<tr>
<td>Smitha Rose Georgy</td>
<td><a href="mailto:smitha.georgy@monash.edu">smitha.georgy@monash.edu</a></td>
<td>Shan Liu</td>
<td><a href="mailto:shan.liu@monash.edu">shan.liu@monash.edu</a></td>
</tr>
<tr>
<td>Jo-Anne Chan</td>
<td><a href="mailto:jchan@burnet.edu.au">jchan@burnet.edu.au</a></td>
<td>Nhu-Y Nguyen</td>
<td><a href="mailto:nhu-y.nguyen@monash.edu">nhu-y.nguyen@monash.edu</a></td>
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We look forward to your continued support of the symposiums in 2013!