

**Theme B: Managing HIV.**

This theme highlighted the clinical management of HIV, through presenting latest research findings relating to the diagnosis and treatment of HIV and the experience of living with HIV.

**Rapporteur:** Mark Kelly, Queensland Health

**Antiretroviral Guidelines Consensus Discussion****Management of Treatment-Experienced Patients**

Professor Stephen Deeks addressed the management of the treatment experienced patient in the first session of the 'consensus conference'. He made four key points. Firstly, he cautioned against the recommendation to change to a more 'simple' regimen in virologically suppressed patients. There is a great temptation to change off older 'less convenient' antiretroviral agents onto the newer more 'convenient' novel agents such as raltegravir and etravirine. However the relatively low genetic barrier to resistance of these agents may prove problematic. Switching enfuvirtide to raltegravir in virologically suppressed patients, many of whom have other fully active agents including boosted protease inhibitors in their regimen, was shown to be safe and associated with sustained virological suppression in the ANRS 138 study. On the other hand changing off kaletra to raltegravir proved to be harmful to patients participating in the SWITCHMK studies particularly in those with past virological failure. Secondly, Professor Deeks proposed that a definition of virological failure may be changed to 'persistently detectable viral load above 200cpm'. Newer HIV viral load assays employing real time PCR technology are inaccurate at the lower end of the dynamic range often reporting low positive results (see later summary). This finding clearly has implications in the management of patients suspected of having virological failure. The AIDS Clinical Trial Group (ACTG) in the USA is expected to adopt this definition for clinical trials. Thirdly, Professor Deeks was unable to add further information to dissect the current recommendation to use two, or preferably three, fully active drugs to achieve virological suppression in persons with prior treatment failure. He pointed out that luckily, even in North America, rates of second antiretroviral regimen failure are decreasing. The good news was that approximately 90% of patients being treated with the RED regimen (raltegravir, etravirine and darunavir/r) achieved virological suppression at 24 weeks in the TRIO study. The bad news was that 29/79 raltegravir exposed patients in a cohort of very treatment experienced who possibly commenced raltegravir without other active agents had confirmed failure and no apparent treatment options. Finally Professor Deeks summarized some of the experimental approaches to the treatment of immunological failure on antiretroviral therapy. He demonstrated that some of the following agents have been associated with increased CD4 count in persons on ART: protease inhibitors, maraviroc, growth hormone, and IL-7. However in light of the finding of IL-2 studies (see later) it is unlikely that these therapies will be recommended as treatment for immunological failure until clinical endpoint studies have been conducted.

**How should cardiovascular disease affect antiretroviral therapy?**

Professor Andrew Carr delivered a long awaited analysis of the impact on the risk of cardiovascular disease on the choice of antiretroviral therapy. Is it the drugs or HIV? Are the guidelines appropriate? The ASHM-endorsed DHHS guidelines demoted abacavir last year to an 'alternate' first line nucleoside based on evidence at the time which suggested that recent use of the drug was associated with increased cardiovascular events. Three key points from his talk draw into question this development and he suggested that we may be looking in the wrong direction. Professor Carr began by reminding us of the data that cumulative use of ritonavir boosted protease inhibitor therapy is associated with increased risk of myocardial infarction as demonstrated in the DAD study. The same study indicated that myocardial infarction was almost twice as likely to occur in patients with 'recent use' of abacavir (cf cumulative or past > 6 months ). Abacavir use was significantly more likely to be associated with AMI in those with higher baseline cardiovascular risk (hence the 2008 ART guideline change). However, emerging data muddy this picture. The French Database study was initially reported as agreeing with the DAD study and showed an association between recent use of abacavir (< 1yr or stop < 6/12) and myocardial infarction. However in an embarrassing reanalysis of the data the association between abacavir and myocardial infarction was not significant after adjustment for cardiovascular risk factors. Multiple other cohorts and RCT have not found an association between abacavir use and myocardial infarction or CVD. Professor Carr drew our attention to a fascinating study presented at IAS which retrospectively analysed 278 patients with myocardial infarction and 19,416 control patients from the VA cohort in the USA. MI and CVA were more likely to occur in patients with eGFR < 60. Patients with eGFR 60-89 were also tended to have more MI and CVA than patients with eGFR > 90. Abacavir use was more common in persons with eGFR < 90. Recent or cumulative abacavir use was not associated with increased MI or CVA in the VA study especially when other risk factors were taken into account. The STEAL study remains the only randomised study to demonstrate an association between abacavir and serious non-AIDS events including cardiovascular disease. Professor Carr concluded his remarks about the association between abacavir and myocardial infarction with the C word – 'channelling bias'. The DAD study authors did all they could to remove any channelling bias. They proposed that as the effect of abacavir persisted despite adjustment for known cardiac risk factors but was no longer present after abacavir discontinuation and that similar findings were found in one randomised study that channelling bias is not the explanation for this observation. However the findings from the French Hospital Database and the VA cohort studies suggesting that abacavir use may have been more common in persons of higher cardiovascular risk and impaired renal function support the notion that some channelling bias accounts for the findings. Where does that leave us??

Changing tack, Professor Carr reviewed the evidence that increased CVD may be secondary to HIV rather than ART. It is well established that HIV associated immune deficiency (as reflected by low CD4 counts) correlates with increased risk of CVD. HIV infection has its own peculiar effect on serum lipids and endothelial function which are only partly corrected by antiretroviral therapy. No overarching consensus re the interpretation of biomarkers and cardiovascular disease in persons with HIV +/- treatment is able to be reached at this point. There may be an antiretroviral therapy class effect, for the protease inhibitors, which is independent of changes in lipids. Professor Carr pointed out that the effect of PIs on increased CVD events persisted even after adjustment for lipids (including triglycerides?). He also drew our attention to the ACTG 5142 study which compared lopinavir/r and efavirenz. Changes in total, HDL and non-HDL cholesterol were almost

identical suggesting that differential changes in lipids *per se* may not be the complete explanation for these findings. There were however significant changes in triglycerides which were more marked in patients receiving kaletra. Increases in a variety of biomarkers have been demonstrated in persons with HIV and these elevations have been shown to be associated with CVD. There remains significant conflict as to whether these changes in biomarkers are increased in patients taking abacavir. What it all means – no body knows! In closing Professor Carr reviewed the current commentary in the guidelines about abacavir and CVD risk. He agreed with the Australian commentaries caution regarding the fact that the DAD study findings may not apply to naïve patients and the fact that channelling bias could not be excluded. He pointed out that PIs may have an effect beyond their adverse effect on lipids. He suggested that the commentary should consider recommending avoiding protease inhibitor therapy in persons with high baseline cardiovascular risks.

### **Should CNS penetration influence the choice of an antiretroviral regimen?**

Neurocognitive impairment (NCI) remains a significant concern for patients on ART. Different antiretroviral agents penetrate the CNS with differing efficiencies therefore many ask the following questions: is there a prophylactic benefit of using neuropenetrative ART to prevent NCI? Does neuropenetrative ART result in better outcomes for patients with established NCI? There exists a silent band clinicians known as the ‘cognitive change sceptics’ (CCS). This group find it difficult to reconcile the reported rates of neurocognitive impairment (NCI) in persons with viral suppression on ART with what they see in clinical practice. They also question the value of so-called neuropenetrative ART. Professor Brew addressed the CCS in five ways. Firstly he examined the evidence that cognitive impairment occurs despite apparent virological suppression on ART. He quoted a number of different studies which found remarkably similar findings: 35-40% of patients with undetectable viral loads on antiretroviral therapy have some degree of neurocognitive impairment (NCI). The possibility of selection bias was not clearly excluded nor were the studies able to dissect incident versus prevalent NCI. Current viral load in blood or CSF did not correlate with NCI. The studies conflicted over whether current or nadir CD4 count was associated with NCI. Professor Brew’s own study demonstrated that NCI was more common in persons with shorter duration of ART. Secondly Professor Brew reviewed the data that the central nervous system was a virological ‘sanctuary site’. He presented some recent data which suggests that astrocyte infection accounts for a proportion of the HIV viral burden within the central nervous system. Thirdly the evidence that the CNS was an antiretroviral therapy ‘exclusion zone’ was reviewed. Professor Brew highlighted that some antiretroviral agents penetrate the CNS better than others: abacavir better than tenofovir; kaletra better than atazanavir and nevirapine better than efavirenz. Fourthly Professor Brew reviewed the evidence of the benefit of neuroHAART. He outlined the methods he and Dr Cystue have used to attempt to make some sense of the data. After selecting 21 possible studies which provided conflicting results the analysis found four adequately powered studies which suggested that patients with NCI may benefit from neuropenetrative ART. It is important to state that no randomized controlled trials, powerful observational or case-controlled study have been conducted to confirm these preliminary findings. These studies are eagerly awaited by the CCS. Professor Brew concluded by stating that he agreed with current guidelines. There is no proven benefit of the prophylactic benefit of neuropenetrative ART in either naive or treatment experienced patients. However in patients with clinical evidence of

cognitive impairment Professor Brew suggests that neuropenetrative ART should be used (All). His final comment that he would 'moderately' recommend neuropenetrative ART in advanced patients without clinical evidence of cognitive impairment who harbour (yet to be validated) predictors of cognitive impairment (see later). An algorithm to identify these patients was presented by Dr Cysique earlier in the conference.

### **Antiretrovirals in pregnancy and in women considering pregnancy**

Dr Giles based her talk on a very engaging and interactive case. She began by advising that a discussion about 'reproductive intent' should be a routine part of care for women with HIV. Dr Giles outlined the principles underlying contraception in women on antiretroviral therapy which is generally an area in which most clinicians looking after women with HIV lack confidence. She reviewed the evidence concerning four modes of contraception including oral contraceptive pill (OCP); depot injections; intrauterine devices (IUD) and implanon. Use of OCP in women on cART is problematic due to the potential of drug drug interactions. A brief summary is provided here. Efavirenz and unboosted atazanavir will increase estrogen estradiol (EE) levels while ritonavir boosted protease inhibitors and nevirapine will decrease EE levels and result in clinical failure. There are no interactions between the OCP and nucleoside analogues. While there is a potential for drug-drug interactions to occur with depot medroxyprogesterone acetate and drugs affecting the activity of the CYP 3A4 system there have been no reports of reduced activity in women on cART. However long term use of depot medroxyprogesterone acetate is associated with osteopenia. Intrauterine devices (IUDs) can be used in women with HIV without increased complications. Levonorgestrel impregnated IUDs are effective in women taking antiretroviral therapy. Implanon, the subcutaneous injection of a pellet of etonogestrel for long term contraception, may interact with drugs which induce hepatic enzymes and Dr Giles drew our attention to a case report of failure of implanon in an HIV infected woman taking efavirenz. Dr Giles then focused her attention on women who actually intend to become pregnant. Firstly the 'pre-conception checklist' which includes advice re folic acid, vaccination and sexually transmitted infection screening amongst other things should be conducted by someone competent to do so. Women who are not on antiretroviral therapy but want to become pregnant are advised to defer ART initiation until after the first trimester. Dr Giles presented emerging data which suggests that ART should be commenced as early as possible in the second trimester as the duration of maternal ART is inversely proportional to the risk of transmission even in women who achieve an undetectable viral load at the time of delivery. The choice of antiretroviral agents in pregnancy relies on experience in pregnancy to date and the known teratogenic potential of the agent. Dr Giles preferred combivir to be the nucleoside backbone of choice citing greater experience and the fact that the guidelines say that zidovudine should be part of ART during pregnancy. Tenofovir is not favoured as monkey models revealed decreased fetal growth and reductions in bone density. Furthermore chronic use in children is also associated with reduced bone density. The use of tenofovir in pregnancy is advised 'only after careful considerations of the alternatives'. Turning to the non-nucleosides Dr Giles pointed out that nevirapine was safe but only in women with CD4 cells less than 250/  
Efavirenz remains a FDA category D drug. 15% of monkeys exposed to this drug *in utero* experienced significant malformations. There have been at least 7 children develop neural tube defects following exposure to efavirenz *in utero*. This teratogenic potential has not been borne out however in a registry of safety of antiretroviral therapy in pregnancy which is accessed at [www.APREgistry.com](http://www.APREgistry.com). This is a public register funded by the pharmaceutical

industry. The background birth defect is 2.6% in the general population. The registry has an 80% power to detect a two fold increase in the 'birth defect' rate caused by a drug. In this registry only 2.7% of children exposed to efavirenz developed a 'birth defect'. Nonetheless efavirenz remains an FDA category D drug and should not be commenced in the first trimester. Turning to the protease inhibitors Dr Giles pointed out that Kaletra must be used twice daily instead of once daily. Multiple pharmacokinetic studies indicated that lopinavir levels decrease in the third trimester. Therefore the dose should either be empirically increased to 3 BD in the third trimester or increased according to lopinavir levels. Concerns relating to atazanavir induced hyperbilirubinaemia compounding neonatal jaundice remain speculative. Atazanavir should be given with ritonavir as there is little to no data guiding the use of unboosted atazanavir in pregnancy. Currently there is no data to support increased doses of atazanavir in the third trimester of pregnancy (cf Kaletra). Antiretroviral therapy should be continued unchanged in women who conceive on suppressive therapy. There are three exceptions to this rule. Firstly, consideration to switching off efavirenz in the first trimester should be undertaken. Secondly, atazanavir should be boosted. Thirdly, lopinavir should be dosed twice daily and not once daily. Considerations to increase the dose of Kaletra and the addition of zidovudine have been outlined above. Vaginal delivery is preferred in women who achieve undetectable viral load whilst on antiretroviral therapy at term. Intrapartum zidovudine is recommended in all cases. Post-partum antiretroviral discontinuation should occur with due consideration of the differential half lives of individual agents. Most clinicians felt that their capacity to address the reproductive issues of their female patients had been significantly increased by Dr Giles' presentation.

### **Pathogenesis of non-AIDS morbidity and mortality:**

#### ***Are patients on HAART ageing too quickly, and if so, why?***

Illnesses that are associated with ageing occur in HIV infected individuals at a younger age. This 'accelerated ageing' may have an immunological basis?

Professor Deeks countered optimism about the prognosis of our patients on HAART by reminding us that the life expectancy of our patients was reduced by 10-30 years despite ART. This reduction in life expectancy was greater in patients commencing ART at lower CD4 counts. This gap is caused more by SNAEs rather than AIDS events. In the ART era our clinical, epidemiological, psychosocial and now immunological focus has shifted from AIDS events to the so-called serious non-AIDS events (SNAE). These illnesses include: cardiac, oncology, bone, liver, kidney disease, cognitive decline and 'frailty'. Why should this be so? Even after increasing age, increasing duration exposure to ART and increased prevalence of traditional health related risk factors (especially low HDL, high TG and smoking) have been taken into account, SNAEs remain more common in patients with HIV than in the general population.

Is there an immunological basis? So far we can only point to associations. There are two immunological factors that have been associated with SNAEs in persons on ART: inflammatory markers and 'immunodeficiency' as reflected by low CD4 T cell counts. Firstly many inflammatory markers, including hsCRP, IL-6 and D-dimer have been demonstrated to be increased in persons on ART even after accounting to traditional health related risk

factors. Elevation of these markers in patients on ART have been shown to predict mortality in the SMART study. Immunodeficient patients (CD4 < 500) on antiretroviral therapy are more likely to develop SNAEs than ART-treated patients with higher CD4 counts. Approximately 40% of patients who commence ART with a CD4 count of less than 200/L fail to reach a CD4 count above 500/even after a decade of ART induced viral suppression. What is the cause of this failure to fully immune reconstitute? Many HIV-induced immune disorders fail to normalise in the majority of patients taking ART. These include: T cell activation; defective T cell proliferation, microbial translocation and collagen deposition in the T cell zone of lymph nodes. Residual abnormalities of each of these processes is associated with poorer CD4 rises on ART. Additional immunological deficits have been demonstrated in persons on ART including: increased CMV-specific CD8 cells (which predict the development of atherosclerosis in persons with HIV) and persistent expansion of terminally differentiated CD8 T cells. Many of these changes are also seen in ageing. Currently there does not exist a unifying model but Professor Deeks proposed that these persistent immunological deficits lead to residual inflammation and reduced CD4 gains on ART. These later phenomenon also influence each other in a bidirectional manner. Residual inflammation may also lead to a hypercoagulable state. These later three phenomenon may contribute to the observed increased in SNAEs and the premature mortality observed in patients treated with HIV. Professor Deeks concluded by summarising experimental approaches to the management of immunological failure on ART (see his consensus conference summary). He finished by suggesting that statins, aspirin and immunomodulators may be the way forward – watch this space.

### **Interleukin-2 : the post mortem**

The results of three interleukin-2 trials were presented at ASHM after initially being presented at the IAS conference in Capetown in July of this year. It had been well established for almost a decade that subcutaneous administration of IL-2 resulted in significant increases in CD4 counts in persons taking antiretroviral therapy. It was not known if these increases in CD4 counts translated into clinical benefits. The first of these trials, SILCAAT, recruited patients with CD4 counts less than 300/ $\mu$ L on antiretroviral therapy. 1971 patients were recruited to this trial. The second trial, ESPRIT, recruited 4,110 patients with CD4 counts > 300/ $\mu$ L on antiretroviral therapy. In each trial patients were randomised to receive rhIL-2 on a 1:1 basis. Significant increases in CD4 T cells were observed in subjects receiving IL-2 in both trials. The average increase in CD4 T cells over the course of the ESPRIT study was 160/ $\mu$ L. A smaller increase was observed in the SILCAAT study in the first 3-4 years following randomization. This difference did not persist and by the end of the study CD4 cells were similar in both groups. This may have been explained by the reduction in IL-2 cycling towards the end of the SILCAAT study. These increases in CD4 cells unfortunately did not translate into any clinical benefit. Was the rise in CD4 cells observed in both trial enough to show a clinical benefit? Professor Law presented an analysis which combined both studies. The observed reduction in event rates associated with known increases in CD4 counts was determined in the control groups. This rate was then used to calculate the 'expected' reduction in event rate for patients receiving IL-2. The 'expected' reduction in event rate in patients achieving CD4 count increases was then compared with the 'observed' reduction in event rate in patients receiving IL-2. The observed reduction in event rate was much smaller than the expected reduction. The expected reduction, in

events, expressed as a hazard ratio, was 0.75 to 0.8 however what was observed was 0.92 (non-significant). This analysis confirmed the previous suggestions that the IL-2 expanded CD4 cells were not functioning in the same way as the antiretroviral therapy expanded CD4 cells. The results of the STALWART study were also presented. This trial followed preliminary studies which suggested that IL-2 could increase CD4 T cells in persons not taking antiretroviral therapy and therefore may be a useful strategy to defer antiretroviral therapy. This trial recruited 269 subjects. Again subjects who received IL-2 experienced significant increases in CD4 cells of approximately 110 cells at week 32. These increases were associated with deferral in initiation of antiretroviral therapy. Unexpectedly however subjects who received IL-2 experienced more clinical events than control subjects (12 v 1). These results again raised concerns about the benefit of IL-2 expanded CD4 cells. Thus IL-2 as a therapeutic agent for patients with HIV was laid to rest.

### **Markers of Inflammation, Coagulation and Renal Function in HIV-infected Adults in SMART and in two Large Population-Based Studies, CARDIA and MESA**

HIV-induced immune activation is believed to increase inflammatory and coagulation markers which are associated with CVD and mortality. Elevation of baseline markers of inflammation in participants in the SMART study has previously been shown to predict all-cause mortality in that study. Fraser Drummond from the National Centre for HIV Epidemiology and Clinical Research presented a study which extended these findings by comparing baseline serological markers predicting CVD and death in SMART participants with the general population. Levels of these serological markers in patients in the SMART study were compared with those of two other cohorts. These comparative cohorts looked at different age groups. The first comparative cohort, CARDIA – coronary artery risk development in young adults, looked at subjects of a younger age bracket whereas the second cohort, MESA – multi-ethnic study of atherosclerosis, looked at a cohort of an older age. 287 SMART participants from the USA aged between 33 – 44 were compared with 3,231 participants in CARDIA (aged 33-44). 494 SMART participants in the US aged between 45-76 were compared with 5,386 participants from the MESA cohort. Three adjustments were made: firstly – age, race and gender; secondly – known CVD risk factors; thirdly – known CVD risk factors in those with ART-controlled virus. The study's key findings were that levels of the four markers examined (hsCRP, IL-6, D-dimer and cystatin-C) were elevated in SMART participants even those with controlled viral replication on antiretroviral therapy. These differences persisted even after controlling for known CVD risk factors. These findings add to the notion that HIV drives increased serological markers which are associated with increased CVD and mortality. No attempt was made to examine the impact of different antiretroviral agents on these inflammatory markers in this presentation.

### **Predicting the short term risk of diabetes in HIV infected patients: The D:A:D study group**

The incidence of diabetes mellitus (DM) in the DAD study is 5.7/1000 py. Modifying risk factors reduces the risk of development DM in the general population. While there are well established DM prediction models which have been developed for the general population it

is not known how well these models perform in HIV infected populations. One salient point is that these models take triglycerides into account and little data currently exist to indicate if ART-induced changes in triglycerides carry the same predictive value of elevated triglycerides in other settings. Kathy Petoumenos from NCEHCR presented an interesting analysis from the DAD study which may prove useful in HIV clinical management. In this study all patients without known DM, MI or other CVD recruited to the DAD study and had follow-up data and a complete diabetic risk profile were followed for the development of DM. Factors considered risks for DM were classified as general or HIV-specific. The former included age, sex, glucose BP, HDL, BMI, triglycerides, family history of CHD (as a surrogate for family history of DM). The latter included: mode of HIV exposure, duration since HIV positive test, prior AIDS, CD4 cell count, HIV viral load, lipodystrophy, duration of ART exposure, ART class exposure, individual ART as well as HCV and HBV status. A prediction model was developed which was then tested for accuracy using validation datasets. 13,609 patients were included in the analysis. There were 251 new onset DM during the follow-up period which was a mean of 3.5 years. While a number of HIV factors were significant in univariate analysis they did not remain significant after multivariate analysis. The following six general factors remained significant after multivariate analysis: glucose, gender, age, BMI, triglyceride and HDL. The DAD model identified the same predictive factors as the Framingham model. This suggests that the risk factors identified can be interpreted in the same way in HIV infected populations as they are in HIV uninfected populations. Approximately 30% of the cohort had a predicted annual risk of new onset DM of 1% or greater, representing approximately a predicted 10% risk after 10 years. These patients may benefit from risk factor modification such as reduction of BMI and triglycerides. Metabolic assessment should be conducted prior to and three months following the initiation of ART and annually thereafter. This model is soon to be publically available on the DAD website: <http://www.cphiv.dk>.

### **Predictors of Initial Episode Bacterial Pneumonia in ESPRIT**

The association with IL-2 therapy and bacterial pneumonia is an interesting one and remains incompletely understood. Patients receiving IL-2 as part of clinical trials were warned about the potential association. Single episode bacterial pneumonia is not AIDS defining but was the most common infection observed in patients both in the ESPRIT trials and STALWART trials. E. Lin from the National Centre for HIV Epidemiology and Clinical Research presented a study which examined the predictors of initial episode bacterial pneumonia (BP) in patients participating in ESPRIT. 179 of the 4,111 patients participating in ESPRIT developed an initial BP. There was in fact no overall difference in the rate of BP in the IL-2 arm versus the control arm. There was however a tantalizing association between recent use of IL-2 and the development of initial BP. The receipt of IL-2 within 60 days was associated with the development of initial BP (HR 1.91 p=0.03). The mechanisms underlying this observation remain speculative. The following factors were also associated with the development of initial BP: older age, higher HIV viral load (baseline and on study), prior recurrent BP as an ADI and IVDU.

## **A screening for HIV-Associated Neurocognitive Disorders A 3 minutes tool to aid neurological referrals in HIV Clinics**

The development of a screening tool for those who are likely to develop 'subclinical' HIV-associated neurocognitive impairment (NCI) would be a welcomed tool in our current arsenal. Dr Cysique presented a paper which may be the first step in that direction. A group of 97 advanced patients who were recruited to a prospective study of neurological and neuropsychological complications were analysed in great depth. 93 of these patients were CDC category C3. All were stable on antiretroviral therapy. Using detailed neuroclinical and neuropsychological measurements neurocognitive impairment was assessed. The following formula was developed to predict NCI in advanced patients:  $NP\text{-impairment} = 0.377 \times \text{Age} - 0.004 \times \text{current CD4} + 2.502 \times \text{CNS disease} - 0.165 \times \text{CART duration} - 14.990 \geq 0$  This screening formula may help identify asymptomatic patients who may benefit from neuropsychometric analysis to diagnose 'subclinical' NCI.

## **Immune-based therapies (IBT) for HIV – where might this be heading now?**

Professor Emery delivered a characteristically straight forward and witty plenary session. In general it seemed as though he was not very hopeful about the future of IBT for HIV. He bluntly stated that we really have little idea about protective immunity against HIV. This makes the design and validation of IBT for HIV problematic. Professor Emery suggested that the gap in current therapeutic strategies needs to be defined. If this gap exists because of persistent immunological deficits despite 'successful' ART then perhaps well-targetted IBT may fill this gap. Perhaps...

Professor Emery described the development of IL-2 therapy for HIV. He outlined the preliminary data which suggested that IL-2 may be beneficial. He stated that 'everything measured in IL-2 expanded T cells suggested there were no reasons for them to fail.' However as outlined just a couple of days before although IL-2 was associated with increases in CD4 cells it was not associated with any clinical benefit. While other cytokine therapies are being studied such as interleukins-7, 12 and 15 there is some lack of enthusiasm. A critical question is what is the appropriate surrogate marker of immune based therapy – clearly CD4 counts can not be in the post-IL-2 era.

Therapeutic vaccines to date have been underwhelming. While some *in vitro* responses have been generated they have been 'disappointing both in breadth and magnitude'. Actually Australia has some runs on the board here. The most promising therapeutic vaccine trial was actually conducted in Australia. Viral loads were lower following antiretroviral therapy interruption in patients who had received HIV vaccine via a recombinant fowl pox vector which expressed HIV genes and interferon gamma. No immunological correlate could be detected by the assays used in this study. In a novel strategy pioneered by Professor Stephen Kent's OPAL vaccine where lymphocytes are exposed to overlapping HIV peptides *ex vivo* and then reinfused. This intervention resulted in virological benefits and the induction of demonstrable favourable immunological responses.

Professor Emery then turned his attention to chronic immune activation which is now clearly recognised to be reduced but not normalised by 'successful' ART and to be associated with serious non-AIDS events and AIDS events in persons on ART. The two lead aetiological

contenders for this persistent immune activation are residual viral replication and bacterial translocation. Professor Emery alluded to the CORAL study which is an Australian initiative examining interventions aimed at tackling persistent immune activation on both of these fronts. The study is fully recruited and results are expected in May of next year. He noted that recent studies designed to reduce residual viral replication by ART intensification in this context have proven disappointing. Professor Emery ended with stating that IBT remain experimental – ‘nothing has worked to date!’. On an even more depressing note he stated that the compulsion to trial IBT is not as strong nor is it as forgiving as it once was. I guess its all about that unmet clinical need.

### **Immunology of Co-infection in HIV-infected Persons**

Professor Ruxrungtham discussed HIV/tuberculosis and HIV/malaria co-infection. His talk dealt with the immunological and the clinical aspects of these conditions. The later will be discussed here. Exactly when to commence antiretroviral therapy in persons with tuberculosis remains controversial. Data from two trials was presented. The first trial, SAPiT, randomized 642 South African patients with tuberculosis in a 2:1 basis to receive early ART (commencing during tuberculosis treatment – randomised to commence within 2 months or after two months of TB Rx) or late ART (commencing after tuberculosis therapy was complete). There was a 56% reduction in death rate in the early ART group. IRIS was higher amongst the early ART patients (12% v 3%) but most cases were self-limiting and did not require treatment discontinuation. The second trial, CIPRA HT 001, randomised patients with TB to commence ART when their CD4 count was 200-350 versus < 200/ $\mu$ L. It was ceased prematurely due to excess mortality in the group deferring ART until CD4 cells fell below 200/ $\mu$ L. The prevalence of tuberculosis has decreased in populations following the introduction of HAART. Professor Ruxrungtham showed a study from South Africa where the prevalence prior to the introduction of HAART was 9.2% in 2002 decreased to 2.9% in 2008 when 30% of the HIV population were on ART. He also showed data that the incidence of malaria also decreased following the introduction of ART (along with cotrimoxazole and insecticide treated bed nets) in Uganda. Professor Ruxrungtham finished by examining some of the immunological mechanisms underlying the inflammatory immune restoration syndrome (IRIS). This occurs in 10-15% of patients in North America and Europe but up to 25% of patients in resource limited settings. He concluded that early diagnosis of HIV, TB and malaria with early ART was the key strategy to manage HIV co-infection. He finished with a mathematical model which suggested that universal testing for HIV and immediate initiation of ART would result in the elimination of HIV. This strategy would be expected to decrease HIV incidence /mortality to <1 per 1000 /yr by 2016 and to decrease HIV prevalence to <1% within 50 years.

### **Lack of correlation at the Lower Limit of Detection of three commercially available viral load assays for the Quantitation of HIV-1 RNA**

Two broadly different methodologies underlie current HIV viral load PCR assays. The ‘end-point’ assay detects and quantifies the amplified product only after the reaction is complete. The ‘real-time’ assay detects and quantifies the amplified product during the PCR reaction.

While this results in both a wider range of detection and a lower limit of detection it comes at the cost of inaccuracies at the lower limit of detection. These inaccuracies are manifested by viral blips. Viral blips are anxiety provoking experiences for both patient and doctor. The performance of three different PCR assays the end-point ROCHE Amplicor; the real-time ROCHE Taqman and the real-time ABBOTT m2000 were compared in the 'blip study' performed at Saint Vincent's Hospital, Sydney. Over a six month period viral load samples which read between the lower limit of detection and 400 cpm were repeated using all three assays. 143 samples were tested in all. 46.9% of these when repeated were below the limit of detection. Intra-assay precision was then assessed using approximately 40 samples. The Roche Amplicor (end-point) PCR assay was found to be more precise than the other two assays (% CV 5.79 v 11.44 [Taqman] and 10.33 [Abbott m2000]) While these assays correlate well across the range from 50 – 100,000 there were substantial discrepancies at the lower end of detection.

### **Using dried plasma spots to measure HIV-1 viral load with the Cavid ExaVir Load assay**

The development of less expensive HIV viral load assays would be a tremendous step forward especially for resource-limited settings. The Burnet Institute is pioneering research development in this arena. Ms Vicki Edourd presented the trials and tribulations experienced to date in the development of an assay which uses dried blood to measure HIV viral load. Ms Edourd's group is developing an assay which detects reverse transcriptase activity in dried plasma samples. This was the first time this technology had been employed. This commercially available assay quantifies HIV viral load by measuring HIV-1 reverse transcriptase activity in a simple ELISA based format. It correlates well with RT PCR viral load assays. Preliminary experiments found that RT activity could be detected using dried plasma samples but the results were lower than that observed with other viral load assays. The scientists then set about elucidating the reason for the observed decrease in viral load seen with this assay. Variations in the input volume of plasma used or variations in the duration of storage of the liquid plasma did not alter the results. The key determinant was the dessication of the plasma onto the filter paper. Once plasma is allowed to dry on the filter paper, the decline in viral load is nearing significance ( $p = 0.06$ ) The addition of the sugar trehalose does not prevent the loss of RT activity. There is still a little way to go.

### **Low-cost HIV-1 genotyping from dried blood spots**

Dr Anna Hearps continued the theme of using dried blood spot (DBS) in the surveillance of and the clinical management of HIV. She presented further work at the Burnet Institute which is pioneering the development of a genotype resistance assay employing DBS as part of its work with the World Health Organisation (WHO). Firstly her team developed and validated a low-cost drug resistance assay. This assay has been adapted for use with DBS. The lower limit of detection is a viral load of 5,000 cpm. In an elegant series of experiments Dr Hearps examined a series of field conditions which may influence the performance of the assay. The assay was sensitive to temperature and humidity so therefore samples should not be stored at ambient temperature. The assay performance was not appreciably affected by repeated

freeze thaw cycles. Therefore Dr Hearps suggested that storage at -20°C was preferable to ambient temperature even if repeat freeze-thaw cycles were necessary. Dr Hearps concluded that the assay was suitable for use as surveillance of drug resistance in resource constrained settings and that further work was needed prior to it being validated for use in individual patient care.

### **An HIV-1 integrase genotyping assay for the detection of drug resistance mutations.**

Dr Anna Hearps' marathon effort continued in the next presentation where she outlined the development of an in-house assay to detect integrase inhibitor resistance mutations. The dynamics and consequences of integrase inhibitor resistance are yet to be fully determined. Dr Hearps succinctly outlined the mode of action of the integrase enzyme and integrase inhibitors and reviewed the genetic pathways of integrase inhibitor resistance. She then described the development of an assay which amplifies the entire integrase gene and is subtype independent. Currently a viral load cut-off of 2,000 cpm is recommended. It also detects polymorphisms the clinical significance of which is yet to be defined. This is the only assay of its type available in Australia to the knowledge of the author.