mHIVE

The network for Victorian HIV researchers

2022 WORLD AIDS DAY mHIVE SYMPOSIUM

HIV Prevention and Key Populations

WORLD AIDS DAY

1st December 2022

ABOUT MHIVE:

The Melbourne HIV Exchange (mHIVE) is a Victoria-wide consortium of over 150 HIV researchers from the Doherty Institute, Burnet Institute, RMIT University, Monash University, University of Melbourne, La Trobe University, Alfred hospital, NRL, VIDRL, Deakin, MSHC, AAHL and WEHI.

Our aim is to bring together all interested parties, once a month, for exchange of topical and relevant information on the broad range of basic, clinical and translational research currently being undertaken across the state. Every year we hold a special event to mark World AIDS Day. In past years, we have held hugely successful symposiums at the Doherty Institute (2015, 2017) and the Burnet Institute (2016, 2018) with over 150 delegates from the key scientific, affected community and political sectors. More recently, we have held virtual events in 2020 and 2021, however 2022 marks a return to an in-person event. The enthusiasm, communication and discussion that are fostered at these events is key to ultimately ending HIV.

We are always happy to add new names to our email distribution list and to welcome new faces to our monthly meetings over some light refreshments. Meetings are held once a month at 4 pm either at the Doherty Institute, Burnet Institute, RMIT University or online.

ORGANISING COMMITTEE:

Dr Lindi Masson, Burnet Institute (Co-Chair)
Dr Michael Roche, RMIT University (Co-Chair)
Dr Youry Kim, Doherty Institute
Dr Wei Zhao, Doherty Institute
Dr James McMahon, Alfred Hospital
Dr Tafireyi Marukutira, Burnet Institute
Dr Minh Pham, Burnet Institute
Ms Brianna Jesaveluk, Burnet Institute
Dr Thomas Angelovich, RMIT University
Dr Catherine Cochrane, RMIT University
Ms Sarah Byrnes, RMIT University

For more information contact: mHIVEcommittee@gmail.com

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mHIVE World AIDS Day Symposium 2022 Program

HIV Prevention and Key Populations

Thursday 1st December – Innovation and Education Hub, Alfred Precinct
(hosted by the Burnet Institute)

Official Symposium Opening		
13h00-13h10	**Welcome Address**	**Prof Gilda Tachedjian** (Life Sciences Discipline, Burnet Institute)
Session ONE: Chaired by Dr Lindi Masson		
13h10-14h10	**Keynote Presentation**	**Prof Linda-Gail Bekker** (Desmond Tutu Health Foundation, University of Cape Town, South Africa) ***Equalize! Young women and HIV - what is needed?***
14h10-14h40	**Clinical Perspective**	**Assoc Prof Edwina Wright** (Department of Infectious Diseases, The Alfred and Central Clinical School, Monash University, Australia) ***TBA***
14h40-15h10	**Social Perspective**	**Dr Kirsty Machon** (Positive Women Victoria, Australia) ***Letting the record show: women HIV, and the politics of change***
TEA BREAK (15h10-15h40) **Poster Viewing**		
Session TWO: Chaired by Dr Sushama Telwatte		
15h40-16h25	**Community Perspectives**	**Chair – Prof Jenny Hoy** (Alfred Hospital) **Panel – 3 women living with HIV** (Living Positive Victoria) ***Living well with HIV***
16h25-17h25	**Oral Abstract Session**	**Jenna Wilson** (Burnet Institute) ***Functional differences between cervicovaginal Lactobacillus species that protect against HIV infection*** **Priyank Rawat** (Peter MacCallum Cancer Centre) ***Suppression of HIV-1 RNA using novel CRISPR tools*** **Sarah Byrnes** (RMIT University) ***Chronic immune activation and barrier dysfunction in the gut are associated with persistent neuroinflammation in ART-suppressed SIV-infected rhesus macaques*** **Dr Youry Kim** (Doherty Institute) ***Romidepsin in combination with the BCL-2 antagonist venetoclax synergistically reduce the size of the HIV reservoir***
TEA BREAK (17h25-17h45) **Poster Viewing**		
Official Closing & Presentation of Awards		

17h45	**Award Presentation**	**Oral and Poster Prizes**
17h55	**Official Closing**	**Prof Sharon Lewin** Doherty Institute for Infection and Immunity

Proudly supported by:

Other events:

08h00-09h00: **WOMEN AND HIV BREAKFAST**
Innovation and Education Hub, Alfred Precinct

09h00-13h00: **COMMUNITY EVENT HOSTED BY LIVING POSITIVE VICTORIA**
Innovation and Education Hub, Alfred Precinct

mHIVE World AIDS DAY Awards

In 2022, mHIVE, in collaboration with its generous sponsors, are proud to present 4 prizes to Students and Early-Mid Career Researcher (EMCR) presentations selected from abstracts.

Awards will be presented at the end of the mHIVE World AIDS Day Symposium to the best oral and poster presentations including:

- ***ViiV Healthcare AIDS Scholarship ($5000)*** - **Best oral presentation** among finalists speaking at the symposium to fund travel to an international HIV/AIDS conference.

- ***mHIVE Student Poster Prize ($300)*** - **Best student poster presentation** at the symposium.

- ***mHIVE EMCR Poster Prize ($300)*** - **Best early-mid career researcher (EMCR) poster presentation** at the symposium.

VIIV HEALTHCARE YOUNG INVESTIGATOR AWARD FINALISTS

Jenna Wilson

Functional differences between cervicovaginal *Lactobacillus* species that protect against HIV infection

Jenna Wilson[1], Arghavan Alisoltani[2,3], Monalisa T. Manhanzva[2,3], Grace Androga[1], Matthys Potgieter[2,4,5], Liam Bell[6], Elizabeth Ross[6], Arash Iranzadeh[2,4], Imane Allali[4,7,8], Nicola Mulder[2,4,9], Smritee Dabee[2,3,10], Shaun L. Barnabas[3,11], Hoyam Gamieldien[2,3], Jonathan M. Blackburn[2,5], Linda-Gail Bekker[2,12], Heather B. Jaspan[2,10,13], Jo-Ann S. Passmore[2,3,14,15], Lindi Masson[1,2,3,14,16]

Affiliations: 1. Disease Elimination Program, Life Sciences Discipline, Burnet Institute, Australia; 2. Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa; 3. Division of Medical Virology, Department of Pathology, University of Cape Town, Cape Town 7925, South Africa; 4. Computational Biology Division, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town 7925, South Africa; 5. Division of Chemical and Systems Biology, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town 7925, South Africa; 6. Centre for Proteomic and Genomic Research, South Africa; 7. Faculty of Sciences, University Mohammed V in Rabat, Morocco; 8. Laboratory of Human Pathologies Biology, Department of Biology and Genomic Center of Human Pathologies, Mohammed V University in Rabat, Morocco; 9. Centre for Infectious Diseases Research (CIDRI) in Africa Wellcome Trust Centre, University of Cape Town, Cape Town 7925, South Africa; 10. Seattle Children's Research Institute, University of Washington, USA; 11. Famcru, University of Stellenbosch, Cape Town, South Africa; 12. Desmond Tutu HIV Centre, University of Cape Town, South Africa; 13. Division of Immunology, Department of Pathology, University of Cape Town, Cape Town 7925, South Africa; 14. Centre for the AIDS Programme of Research in South Africa, Durban 4013, South Africa; 15. National Health Laboratory Service, South Africa; 16. Central Clinical School, Monash University, Melbourne 3004, Australia.

Background: Young South African women are at disproportionate risk of bacterial vaginosis (BV) and HIV infection. Cervicovaginal *Lactobacillus* species such as *L. crispatus* protect against HIV, while *L. iners* is considered less protective. To understand this difference, this study compared the protein and functional profiles of cervicovaginal *L. iners* and *L. crispatus* communities in young South African women.

Methods: Vaginal swab samples collected from women (aged 16-22 years) with cervicovaginal microbiota dominated by either *L. crispatus* (n=19) or *L. iners* (n=50), were analysed using liquid chromatography-tandem mass spectrometry, a custom cervicovaginal database and MaxQuant.

Results: A total of 218 *L. iners* and 276 *L. crispatus* proteins were identified, and 39 of the 105 proteins shared by both species were significantly differentially abundant. Proteins including glucose-6-phosphate isomerase and LPXTG-motif anchor-domain protein were overabundant in *L. crispatus* communities, and enolase and L-lactate dehydrogenase were overabundant in *L. iners*. Additionally, several *L. iners* proteins involved in pathogenesis and carbohydrate metabolism were significantly upregulated in women with non-optimal microbiota. After excluding participants with BV, *L. iners* dominance was correlated with human immune markers (IgG H chain, clusterin and calpain small-subunit-1), while *L. crispatus* cytokeratin-8 overabundance suggests greater host epithelial barrier integrity.

Conclusion: Metaproteomic analyses provided valuable insight into the function of *Lactobacillus* spp. *in vivo*, demonstrating significant differences in the metabolic activities of *L. iners* versus *L. crispatus*. The functional activities of *L. iners* were linked to host BV status, suggesting that bacterial gene expression is influenced by environmental factors or strain level differences.

Priyank Rawat

Suppression of HIV-1 RNA using novel CRISPR tools

Rawat P[1], JA Trapani[1], SR Lewin[2,3], Roche M[2,4], Fareh M[1]

Affiliations: 1. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia; 2. Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia; 3. Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia; 4. STEM College, RMIT University, Melbourne, Australia

Background: Prolonged persistence of latent viral infections serves as a major roadblock towards developing a functional cure for HIV-infections. Current treatment regimens including highly active retroviral therapy (HAART) have been the gold standards for treatments, however, are not a functional cure, largely due to their inability to eradicate the latent reservoir of infected cells. We hypothesise that the programmable, RNA-targeting CRISPR-Cas13 system can be used to manipulate the HIV transcriptome by knocking down essential viral transcripts, with high efficiency and specificity and thus, locking HIV into deep latency.

Methods: In a transient transfection model, we targeted the HIV-1 tat transcript using the RfxCas13d and PspCas13b orthologs of Cas13 in HEK293T cells to validate the efficacy of the crRNAs. Knockdown of the transcript was determined via fluorescence microscopy which was further confirmed by western blot and RT-qPCR. Statistical analysis was performed using the one-way ANOVA test followed by Dunnett's multiple comparison.

Results: All rfxCas13d crRNA targeting the tat transcript showed over 90% reduction in mCherry signal indicative of knockdown of target transcript. Similarly, for pspCas13b statistically significant silencing was observed for all crRNAs (>80%), with crRNA3 and crRNA5 showing the greatest level of silencing (>90%) amongst the crRNA tested. To further confirm the knockdown of Tat protein, we performed western blots and observed drastic reduction in the expression of the Tat protein with the highly efficient crRNA relative to the non-targeting crRNA for both RfxCas13d and PspCas13b. Furthermore, we performed RT-qPCR to determine mRNA knockdown and observed a statistically significant decline in the tat mRNA expression for both Cas13 orthologs.

Conclusion: In this project, we demonstrate the ability of the programmable CRISPR-Cas13 system to effectively knockdown HIV-1 transcripts associated with latent infections in the transient transfection model. Further experiments are required to knockdown the tat transcript endogenously expressed in HIV latency models as well as ex vivo in samples from people infected with HIV. Furthermore, methods to deliver the CRISPR-Cas13 components including stable transduction and lipid nanoparticles is also being investigated.

Sarah Byrnes

Chronic immune activation and barrier dysfunction in the gut are associated with persistent neuroinflammation in ART-suppressed SIV-infected rhesus macaques

Byrnes SJ[1], Busman-Sahay K[2], Angelovich TA[1,3,4], Younger S[2], Taylor-Brill S[2], Nekorchuk M[2], Bondoc S[2], Dannay R[2], Terry M[2], Cochrane CR[1], Roche M[1,4], Deleage C[5], Brew B[6], Estes JD[1,2,5] and Churchill MJ[1,3,7]

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Background: HIV-associated neurocognitive disorders (HAND) affect ~30% of virally suppressed people with HIV, suggesting that HAND pathogenesis may be driven by mechanisms other than viral replication in the brain, including chronic inflammation. However, the precise viral dependent and independent changes to the brain of virally suppressed people with HIV remains unclear.

Methods: We characterized the CNS reservoir and immune environment of SIV-infected macaques during acute (n=4), chronic (n=12) or ART-suppressed SIV-infection (n=11). Multiplex immunofluorescence for SIV-infection (RNA/DNAscope) and immune activation were performed on matched brain and gut tissue. Additionally, immune activation was measured in an SIV-uninfected model of chronic colitis, validated to mimic SIV-induced gut damage, to determine the effect of gut damage on neuroinflammation independent of SIV.

Results: SIV-infected animals contained viral DNA+ cells that were not reduced in the brain or gut by ART ($P<0.05$), supporting the presence of a stable viral reservoir in these compartments. SIV-infected animals had heightened levels of activated astrocytes and microglia producing antiviral (Mx1 and/or TGF-β1) and oxidative stress markers (SOD1) as well as reduced blood-brain barrier (BBB) integrity compared to uninfected animals, and these dysfunctions were not abrogated by ART ($P<0.05$ for all). Furthermore, SIV-uninfected animals with experimentally induced gut damage showed a similar immune activation profile in the brain to animals with SIV, supporting the role of chronic gut damage as an independent source of neuroinflammation.

Conclusion: We show that ART-suppressed SIV-infected rhesus macaques exhibit impaired BBB integrity and heightened microglial and astrocyte activation which is associated in part with viral reservoirs and immune activation in the gut.

Dr Youry Kim

Romidepsin in combination with the BCL-2 antagonist venetoclax synergistically reduce the size of the HIV reservoir

Youry Kim[1], Carolin Tumpach[1], Jesslyn Ong[1], Ajantha Solomon[1], James McMahon[2], Phil Arandjelovic[3], Marc Pelligrini[3], Michael Roche[1] and Sharon Lewin[1,2]

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[2]Department of Infectious Disease, The Alfred Hospital and Monash University
[3]The Walter and Eliza Hall Institute of Medical Research

Background: Latency reversing agents (LRAs) can successfully induce HIV transcription in people living with HIV (PLWH) on antiretroviral therapy (ART), however, a reduction in the HIV reservoir has not been observed. One potential explanation is that latently infected cells over express pro-survival proteins such as BCL-2. Given that the HIV proteins protease and envelope, can induce apoptosis in CD4+ T-cells, we hypothesised that the combination of a potent LRA with the BCL-2 antagonist, venetoclax, will enhance elimination of latently infected cells.

Methods: Total CD4+ T-cells were isolated from blood collected by leukapheresis from PLWH on ART (n=6). Venetoclax (5nM, 10nM and 100nM) was added for 24-hours, followed by treatment with the LRA romidepsin (20nM) for 4-hours. Cells were washed and cultured for an additional 20-hours. Total integrated HIV DNA, intact and defective DNA, cell-associated unspliced and multiply spliced HIV RNA were quantified by qPCR. Changes in T-cell subsets were quantified using flow cytometry.

Results: Venetoclax alone induced dose dependent cell toxicity in total CD4+ T-cells however, viability was >80% with all concentrations. Naive and effector memory CD4+ T-cells compared to other T-cell subsets were more susceptible to cell death following venetoclax. The combination of romidepsin together with venetoclax at 5nM, 10nM and 100nM, significantly reduced integrated HIV DNA ($p<0.0005$, $p<0.005$ and $p<0.05$, respectively), and this combination was synergistic compared to either drug alone (Bliss independence scores for each concentration of venetoclax was of 0.36, 0.59 and 0.20 respectively). Using the intact proviral DNA assay in 3 donors, we observed a reduction in intact HIV DNA in 3 of 3 donors with 10nM venetoclax and in 2 of 3 donors with 5nM and 10nM venetoclax with romidepsin. This combination also induced a significant increase in unspliced HIV RNA ($p=0.03$). Unexpectedly, venetoclax alone induced expression of both unspliced and multiply spliced HIV RNA.

Conclusion: Reductions in integrated HIV DNA in CD4+ T-cells from PLWH on ART *ex vivo* was enhanced using the pro-apoptotic drug venetoclax together with the LRA romidepsin. Venetoclax may also directly activate HIV transcription. Romidepsin together with venetoclax is an effective combination in reducing the HIV reservoir and should be further explored in additional pre-clinical models.

POSTER ABSTRACTS:

	NAME	SURNAME	AFFILIATION	POSTER TITLE
1	Jillian	Lau	Alfred Hospital	HIV cure research: Assessing understandings and perceptions of HIV Cure among Peer Navigators and Treatment Officers in Australia
2	Jess	O'Bryan	Monash University	Uptake of COVID vaccination in a diverse cohort of PLWHIV in Melbourne: how can we do better?
3	Rory	Shepherd	University of Melbourne	Methyseleninic Acid (MSA) synergises with SMAC mimetic AZD5582 to reactivate a cell line model of HIV latency
4	Kiho	Tanaka	University of Melbourne	Eradication of HIV-1 reservoirs using SMAC mimetics
5	Abdalla	Ali	University of Melbourne	Nanoparticle delivery of romidepsin to reverse HIV latency
6	Sushama	Telwatte	University of Melbourne	Single-cell analysis of HIV reservoir cells from virally-suppressed individuals living with HIV
7	Brianna	Jesaveluk	Burnet Institute	Modulation of ICAM-1 on cervicovaginal epithelial cells by a microbiome bioactive and HIV transmission
8	Janna	Jamal Eddine	RMIT University	Transcriptional activity of brain-derived HIV long terminal repeat sequences from virally suppressed people with HIV
9	Celine	Gubser	University of Melbourne	GITR impairs CD8 T-cell response and drives CD4 T-cell proliferation in chronic HIV infection

POSTER 1

HIV cure research: Assessing understandings and perceptions of HIV Cure among Peer Navigators and Treatment Officers in Australia

Lau JSY[1,2], Clifton B[3], Rule J[3], Ellard J[3], McMahon J[1,2]

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Background: Community understanding of HIV cure science is critical for the meaningful engagement of people with HIV (PHIV) in clinical trials aimed at curing HIV. The National Association of People Living with HIV Australia (NAPWHA) Treatment Outreach Network (TON) is a group of peer educators from the HIV community sector workforce who engage with PHIV to provide health and treatment information. A previously conducted survey of TON members found limited knowledge of HIV cure science and varying interest in trial participation. This study aims to evaluate understanding of HIV cure research, following an educational workshop.

Methods: Attendees of the NAPWHA TON meeting will participate in a 75-minute workshop focused on HIV cure research. This will include a presentation on the basics of HIV cure science and a discussion with a participant who has completed an HIV cure trial. After a question-and-answer session, participants will be invited to complete an anonymous online survey about their perspectives on HIV cure research. This survey is identical to the previously conducted survey.

Results: The TON meeting is being held on the 9th November and thus no results are available from the repeat survey yet. We hypothesise that following the educational workshop, there will be increased understanding of cure science and increased willingness to participate in cure-related research. Results will be presented at World AIDS Day if selected.

Conclusion: Treatment officers and peer educators are critical sources of information about HIV cure research for PHIV, and efforts must be made to engage them in research activities.

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POSTER 2

Uptake of COVID vaccination in a diverse cohort of PLWHIV in Melbourne: how can we do better?

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Background: The Australian Government commenced its COVID-19 vaccination program on February 22nd, 2021. People Living With HIV (PLWHIV) were assigned Phase 1b, commencing March 22nd, 2021. On October 1st, 2021, Victorian Premier Andrews announced all authorised workers would need to be vaccinated against COVID-19 to continue working onsite (the vaccine mandate).

Methods: Adult PLWHIV were identified through the Monash Infectious Diseases HIV database. Only PLWHIV engaged with care (at least one clinic visit and/or one HIV viral load taken in both 2019 and 2020) were included. Demographic information was collected from medical records .COVID-19 vaccination status as of 1st October 2021 was collected from AIR (Australian Immunisation Register). For PLWHIV without a COVID-19 vaccine, a chart review was undertaken for a documented discussion with their HIV clinician about COVID-19 vaccination.

Results: A total of 290 participants were identified as eligible. 55 (19%) PLWHIV had not received a COVID-19 vaccine prior to 1st October. 71% of the unvaccinated group were male and Australian born (47%), compared with 73% of vaccinated group being male and 37% Australian born.
Only 7 unvaccinated people had a documented conversation with their treating clinician regarding their concerns. The most common reason was fear of side effects and a desire to have the vaccine of their choice.

Conclusion: There was lower uptake of COVID vaccines amongst PLWHIV at our institution compared to the general population. While clinicians may have had conversations with vaccine hesitant PLWHIV, these were poorly documented. This represents a lost opportunity to identify the reasons for vaccine hesitancy amongst PLWHIV and help inform further public health measures.

POSTER 3

Methyseleninic Acid (MSA) synergises with SMAC mimetic AZD5582 to reactivate a cell line model of HIV latency

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Background: The latent reservoir of HIV persists due to low immune activation and enhanced cell survival pathways. Eradication of these cells requires both stimulation of the quiescent virus and enhancement of the host cell's apoptotic pathways. During prior work on circadian rhythm modulating compounds, we identified the organic selenium compound methylseleninic acid (MSA) as a potential latency reversal agent. Having established this, we sought to determine if MSA could synergise with other latency reversing agents (LRAs) of known and varied modes of action: SMAC mimetics, BCL2 antagonists, HDAC inhibitors, Bromodomain inhibitors and PKC agonists.

Methods: JLAT A2 cells, which contain an integrated LTR-Tat-IRES-GFP-LTR construct, were stimulated with MSA alone and in combination with AZD5582, Xevinapant, Panabinostat, Venetoclax, Pep005 and JQ1 for 24 hours continuous culture. Synergy was calculated via the Bliss Independence model of synergy, with significance determined via a paired T-test.

Results: We confirmed all LRAs were able to reactivate latent HIV with varying potency. AZD5582 in combination with MSA was able to reactivate 73% of live cells, compared to 4% and 14% alone respectively. The Bliss Independence score was >0 (0.65, p=0.0028) indicating synergy.

Conclusion: AZD5582 and MSA synergise to reactivate the JLAT A2 model of latent HIV, with the mechanism of MSA mediated latency reversal to be further investigated. In addition to LRA properties, MSA has demonstrated pro-apoptotic killing of cancer cells. Thus, we intend to explore the actions of MSA as a dual LRA and apoptotic sensitiser. MSA presents as a novel therapeutic which will inform further "Shock and Kill" focused research.

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POSTER 4

Eradication of HIV-1 reservoirs using SMAC mimetics

Kiho Tanaka[1], Youry Kim[1], Michael Roche[1], Sharon Lewin[1,2,3]

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Background: 'Shock and kill' is an approach to eradicate the HIV reservoir in people living with HIV on ART through reactivation of the latent provirus by latency reversal agents (LRAs). However, latently infected CD4+ T-cells are primed for survival due to the over-expression of factors that regulate apoptosis such as inhibitors of apoptosis proteins (IAPs). This may explain why 'shock and kill' efforts to date have largely failed. SMAC mimetics (SMACm) are a new class of LRA that induce IAP degradation which leads to the binding of non-canonical NfkB complex to HIV-1 LTR to activate the provirus. Interestingly, this may also simultaneously render the cell more susceptible to apoptosis. We investigated the latency reversal effects of two classes of SMACm, bivalent and monovalent.

Methods: Jlat10.6 cells, encoding a full-length HIV genome and GFP, and primary CD4+ T-cells were treated with a range of SMACm concentrations for 48h with proviral reactivation and cell toxicity measured using flow cytometry.

Results: The bivalent SMACm (AZD5582, BV6 and Birinapant) showed higher levels of reactivation compared to monovalent SMACm (GDC0152, GDC0917, xevinapant and LCL161). Both bivalent and monovalent SMACm demonstrated similar toxicity profiles in CD4+ T-cells isolated from uninfected PBMC.

Conclusion: SMACm induces latency reversal in HIV infected cells. Although bivalent SMACm are more potent, they have demonstrated significant side effects in clinical trials including induction of Bells Palsy. As such monovalent SMACm are thought to be a more attractive option clinically. Altogether, SMACm poses as a novel therapeutic approach to eradicate HIV latently infected cells to achieve ART remission in people living with HIV.

POSTER 5

Nanoparticle delivery of romidepsin to reverse HIV latency

Abdalla Ali[1], Michael Roche[1], Christina Jugo-Cortez[2], Paula Cevaal[1], Damian Purcell[1], Frank Caruso[2], Jori Symons[3], Sharon R Lewin[1]

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Background: One strategy to eliminate latently infected CD4+ T cells in people living with HIV on antiretroviral therapy (ART) is to reactivate virus expression using latency reversing agents (LRAs). However, this strategy (often called "shock and kill") has demonstrated limited efficacy both in vitro and in vivo based on high toxicity combined with low potency and the failure to reduce reservoir size. One approach to increase the potency and reduce toxicity of LRAs is to use a nanoparticle (NP) delivery system.

Methods: We developed a biopolymer nanocarrier system using polymethacrylic acid (PMASH) to encapsulate the hydrophobic LRA romidepsin (RMD) (RMD-NPs). The latency reversal potential and toxicity profile of RMD-NPs was compared to free RMD in T cell-line models of HIV latency, including J-Lat A2 and J-Lat 10.6. Activation of the LTR was measured by green fluorescent protein (GFP) expression using flow cytometry. Cell viability was quantified using an ATP-based chemiluminescent assay.

Results: In the J-Lat A2 cell line, RMD-NPs compared to free RMD displayed a similar potency (mean GFP expression = 73 and 82% respectively) and toxicity profile (mean viability = 36 and 29% respectively). In contrast, in the HIV J-Lat cell line 10.6, which contains near full length HIV, RMDNPs compared to free RMD significantly enhanced potency of LTR activation [mean GFP expression = 53 and 22% respectively, p= 0.0253 unpaired t test] and markedly reduced toxicity [mean viability = 100% and 1.4%, respectively, p= 0.0001 unpaired t test].

Conclusion: We have demonstrated in a T cell line model of HIV latency that encapsulation of the LRA romidepsin can lead to enhanced latency reversal with reduced toxicity. Further studies are necessary to investigate the mechanism behind this effect and whether similar results are found in CD4+ T cells from people living with HIV *ex vivo*.

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POSTER 6

Single-cell analysis of HIV reservoir cells from virally-suppressed individuals living with HIV

Telwatte S[1,2 3], Frouard J[4], Luo X[4], Martin HA[3], Kadiyala GN[3], Wedrychowski A[3], Hoh R[2], Deeks SG[2], Lee SA[2], Roan N[4], Yukl SA[2,3]

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Background: Latently infected CD4+ T cells are widely considered to be the critical barrier to a cure for HIV-1. The frequency of latently infected CD4+ T cells that contain replicative viral forms is very low, and a large proportion reside in lymphoid tissues such as the gut, which can be more challenging to study in-depth. An additional obstacle is that there are currently no known biomarkers that reliably distinguish latently-infected cells from uninfected cell populations in vivo.

Methods: We developed "HIV-Seq", a new single-cell (sc)RNAseq approach that enables simultaneous characterization of the transcriptome and surface proteome of unstimulated HIV-infected cells from blood and gut tissue from effectively treated people living with HIV (PWH). we analysed HIV reservoir cells from longitudinal blood samples from in antiretroviral therapy (ART)-suppressed PWH, including Week 0 (prior to commencing ART) during acute infection and Week 24 or 45 (on ART) during viral suppression [n=4]. We additionally characterised CD3+ and CD45+ T cells from the blood and gut of one ART-suppressed individual. Sequences were aligned to a constructed subtype B consensus reference sequence and scRNAseq and CITE-seq analyses were performed.

Results: We identified 1232 HIV RNA+ cells from viremic timepoints and 26 HIV RNA+ cells from the ART-suppressed timepoints representing the transcriptionally-active reservoir. Based on viremic sample data, HIV-seq enables 32-72% increased capture of HIV RNA+ cells.

Conclusion: This innovative HIV-seq method enables increased capability to identify and characterize HIV-infected cells from ART-suppressed individuals to provide in-depth transcriptomic and surface phenotypic analysis of transcriptionally-active reservoir cells.

POSTER 7

Modulation of ICAM-1 on cervicovaginal epithelial cells by a microbiome bioactive and HIV transmission

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Introduction: The genital epithelium plays a critical role in modulating the sexual transmission of HIV. Expression of intercellular adhesion molecule-1 (ICAM-1) facilitates cell-to-cell virus transmission and is increased by inflammation. Women with *Lactobacillus* spp.-dominated vaginal microbiota have a decreased risk of HIV acquisition. Lactic acid (LA) is a key metabolite (or bioactive product) of *Lactobacillus* spp. with antimicrobial and anti-inflammatory properties that is differentially produced by *Lactobacillus* spp. as L- and D- isomers. However, the mechanisms for LA reducing mucosal HIV-1 transmission are unknown.

Methods: Immortalised ectocervical (Ect), endocervical (End) and vaginal (VK2) epithelial cells were cultured in a transwell system and treated apically for 1 h with 0.3% L-LA or D-LA (pH 3.9) L- or D-lactate (pH 7.0), or acidified media (pH 3.9, HCl adjusted) simultaneously with toll-like receptor (TLR) agonists poly I:C (PIC, TLR3), FSL-1 (TLR2/6), or PAM3CSK4 (TLR1/2). Expression of ICAM-1 mRNA was determined by RNASeq and surface protein expression by flow cytometry.

Results: ICAM-1 mRNA was increased by PIC stimulation of Ect cells, which was found to be reduced 2.2-fold in the presence of L-LA (n=3, FDR<0.05). Stimulation of Ect cells with PIC significantly increased ICAM-1 protein expression by 1.6-fold (n=4, $p<0.05$) compared to unstimulated cells. However, this increase was abrogated 1.4-fold by treatment with L- or D-LA, but not HCl-acidified media or L- or D-lactate at a neutral pH, indicating that inhibition of ICAM-1 upregulation is specifically mediated by the uncharged form of LA. Similar findings were observed for Ect cells treated with FSL-1 and PAM3CSK4, as well as TLR-stimulated End and VK2 cells.

Conclusions: Downmodulation of ICAM-1 expression in the context of inflammation suggests that LA potentially mitigates mucosal HIV-1 transmission. Future studies will investigate direct cell-to-cell transmission of virus sequestered in epithelial cells to HIV-1 target cells and the effect of LA treatment.

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POSTER 8

Transcriptional activity of brain-derived HIV long terminal repeat sequences from virally suppressed people with HIV

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Background: The CNS is a reservoir of HIV. Recently we demonstrated that the HIV reservoir in the brain of virally suppressed people with HIV (PWH) consists of a pool of both intact and defective HIV proviruses. We and others have demonstrated that HIV long terminal repeats (LTR) isolated from the CNS of viremic PWH are functional and are distinct to those isolated from matched peripheral tissues of the same individual. Further, CNS-derived LTRs from viremic PWH demonstrated a lower basal transcriptional activity to lymphoid-derived LTRs. Despite the presence of intact potentially replication competent proviral genomes, whether the LTRs are functional and transcriptionally active is unclear.

Methods: To determine if LTRs from the CNS of virally suppressed PWH are potentially functional, LTRs have been isolated from CNS tissue of virally suppressed PWH by single genome amplification. Phylogenetic analysis and transcription factor binding site analysis was performed under basal and activated conditions.

Results: While we have previously demonstrated clear compartmentalisation of sequences between the CNS and lymphoid compartments for viremic PWH, compartmentalisation was not observed in virally suppressed PWH. Additionally, sequence analysis of LTRs from the CNS of virally suppressed PWH have intact core promoter regions (TATA, Sp1, NF-κB) which differed from that observed in viremic PWH. LTRs isolated from virally suppressed PWH were inducible with HIV tat, PMA and current latency reversal agents.

Conclusion: These findings support the presence of a potentially transcriptionally competent inducible pool of HIV in the brain of virally suppressed PWH. Understanding the function of HIV LTRs derived from intact proviral genomes in CNS may inform the role of the CNS reservoir in ongoing disease and as a barrier to HIV cure.

POSTER 9

GITR impairs CD8 T-cell response and drives CD4 T-cell proliferation in chronic HIV infection

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Background: Over the last 10 years a body of well accepted literature emerged showing that agonistic mouse anti-GITR antibody clone DTA-1 has potent immunotherapeutic effects owing to its capacity to concurrently promote effector T-cell function and abrogate Treg mediated suppression in mouse models of cancer and chronic infection. However most clinical trials with GITR monotherapy or GITR/PD-1 combination therapy to date could not translate these promising findings into humans. Here we investigate if there is a role for GITR in HIV persistence under ART. We characterize GITR expression in acute and chronic HIV infection and identify the impact of GITR-GITR-L interaction on HIV-specific T-cell function in aviremic participants.

Methods: Participants in this study include people with HIV off ART (viremic; n=11) and on suppressive ART (aviremic; n=10) and uninfected controls (n=12), matched for age, sex, ethnicity, hepatitis B and C virus infection status. In addition, we used PBMC from HLA-typed people with HIV on ART together with a panel of MHC class I HIV tetramers to investigate the role of GITR on HIV-specific CD8 T-cells.

Results: We identified multiple persistent changes in GITR expression of viremic participants that did not normalise in aviremic participants consistent with ongoing immune dysfunction. We further show that GITR stimulation does not reinvigorate functionality of HIV-specific CD8 T-cells from HIV aviremic donors, but instead results in significant hindrance of their cytotoxic capacities ex vivo. In contrast we see an increased proliferation of anti-CD3/GITRL co-stimulated CD4 T conventional cells and abrogation of Treg suppression.

Conclusion: We conclude that immunomodulatory GITR treatment of antigen specific CD8 T-cells further decreases their functionality and has no implications in the setting of chronic HIV. The effects of GITR stimulation on the latent HIV reservoir and on productively infected CD4 T-cells are unknown and need to be carefully assessed in a future study.

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