ANNUAL CONFERENCE

15th Annual Conference
4 – 6 November 2020

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HIV: Jennifer Juno & Andrew Harman

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We acknowledge and thank

Australian Government
Department of Health

GUEST SPEAKER

HIV - Professor Sharon Lewin

Professor Sharon Lewin is the inaugural director of The Peter Doherty Institute for Infection and Immunity, a joint venture between The University of Melbourne and Royal Melbourne Hospital; Professor of Infectious Diseases, The University of Melbourne; consultant infectious diseases physician, Alfred Hospital and Royal Melbourne Hospital and a National Health and Medical Research Council (NHMRC) Practitioner Fellow. She is based in Melbourne, Australia.

She is an infectious diseases physician and basic scientist. She completed her medical degree and PhD in virology at Monash University in 1996 and her post-doctoral fellowship with Dr David Ho at Rockefeller University in 1999. Her research focuses on understanding why HIV persists on treatment and developing clinical trials aimed at ultimately finding a cure for HIV infection.

In 2014, she was named Melburnian of the Year and awarded the Peter Wills Medal from Research Australia. In 2019 she was named an Officer of the Order of Australia and a Clarivate Web of Science high citation researcher.

She leads a large national network funded by the National Health and Medical Research Council of Australia called APPRISE which focuses on pandemic preparedness and started in Australia in 2016. Scientists from the Doherty Institute were the first to isolate and share the SARS COV2 virus outside of China and have a broad program of research focused on testing, treatments and vaccines for COVID19.

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GUEST SPEAKER

HBV - Professor Scott Bowden

Professor Scott Bowden is the former Head of the VIDRL Molecular Microbiology Laboratory at The Doherty Institute and senior scientist in the WHO Regional Reference Laboratory for Hepatitis B.

Scott holds a number of academic positions, including as an adjunct Professor in the Department of Microbiology at Monash University and Honorary Professor in the School of Health and Biomedical Sciences at RMIT University. He has served on several Government committees developing the National Strategies for Hepatitis B and Hepatitis C and chaired the expert reference committee developing the National Hepatitis B Testing Policy. Scott has published over 180 papers in the international literature as well as several book chapters and is an expert on applications of molecular technology to the diagnosis of the hepatitis viruses.

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GUEST SPEAKER

HBV - Dr Thomas Tu

Dr. Thomas Tu is a molecular biologist who has researched the Hepatitis B virus and the associated liver disease for the last >13 years.

He currently leads a research group at The Westmead Institute for Medical Research (Sydney), focusing on how a chronic hepatitis B infection is maintained and, over time, causes liver cancer. He also lives with chronic hepatitis B himself, now acting as a public advocate for the affected community.

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GUEST SPEAKER

HTLV-1 - Professor Damian Purcell

Professor Damian Purcell is Professor of Virology at The University of Melbourne and theme leader for viral infectious diseases at the Peter Doherty Institute. After receiving a PhD from The University of Melbourne in 1987 he was a CJ Martin traveling fellow of the NHMRC working at the Laboratory for Molecular Microbiology of the NIAID, NIH in Bethesda, MD, USA. He returned to Melbourne's Burnet Institute in 1995 before moving to The University of Melbourne in 2001.

His research has focused on molecular virology of human retroviruses HIV, HTLV-1 but this year he has led pre-clinical research into the virology of SARS-CoV-2 and development and testing of vaccines for COVID-19. His research on the intrinsic, innate and adaptive immune responses to viral infection and molecular events in the interplay between the host and persistent HIV and HTLV viruses have provided strategies for new vaccine candidates and therapeutic approaches. He has developed and patented several vaccine and immunotherapeutic candidates and led pre-clinical studies for human trials.

He was President of the Australasian Virology Society from 2011-2015, an Executive Committee Member of the Australian Centres for HIV and Hepatitis Virology since 2000 and is the regional Governing Councillor of the International Retrovirology Association since 2017.

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PROGRAM

Day One: Wednesday 4 November 2020

10:00 – 10:15 **Welcome: Tony Cunningham and Heidi Drummer**

10:15 – 12:30 **Session 1: HIV**
Chair: Bethany Horsburgh

10:15 – 10:30 **Jennifer Currenti** **[1]**
Cross-reactivity to mutated viral immune targets can influence $CD8^+$ T cell functionality: an alternative viral adaptation strategy

10:30 – 10:45 **Eric Alves** **[2]**
Investigation of viral adaptation dynamics during early HIV infection yields important considerations for vaccine design

10:45 – 11:00 **Anna Hearps** **[3]**
Early induction of innate immune cell activation in acute HIV infection; implications for persistent immune dysfunction following antiretroviral therapy

11:00 – 11:15 **Hans Kek** **[4]**
Differential Expression of HIV Envelope Epitopes on the Surface of HIV-Infected Macrophages and CD4+ T Cells

11:15 – 11:30 **Kevin Hu** **[5]**
Investigating the Immunology of Leaky Guts in HIV Infection

11:30 – 11:45 **Li Li** **[6]**
Development of a vaginal administration of anti-HIV microbicide nanosystem

11:45 – 12:00 **Brianna Jesaveluk** **[7]**
Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission

12:00 – 12:15 **Erica Longmuir-Vine** **[8]**
HIV interactions with colorectal macrophages in early infection

12:15 – 12:30 **Thomas O'Neil** **[9]**
Investigating CD4+ Tissue resident T cells in human anogenital tissues as targets for HIV

12:30 – 13:30 **Lunch**

13:30 – 15:00 **Session 2: HIV**
Chair: Kerrie Sandgren

13:30 – 13:45 **Silvana Gaudieri** **[10]**
The different adaptation strategies adopted by RNA viruses to escape the host's T cell immune response: an update

13:45 – 14:00 **Andy Poumbourios** **[11]**
Immunogenicity of novel polyvalent HIV glycoprotein vaccines

14:00 – 15:00 **Presentation**

Chair: Andrew Harman

Sharon Lewin
Director, The Peter Doherty Institute for Infection and Immunity
Professor of Infectious Diseases, The University of Melbourne
Consultant Infectious Diseases Physician, Alfred Hospital and Royal Melbourne Hospital
National Health and Medical Research Council (NHMRC) Practitioner Fellow

Title: Advances towards an HIV cure: role of immune checkpoint blockers: good or bad for latent infection?

15:00 – 15:30 **Break**

15:30 – 17:00 **Session 3: HIV**
Chair: Caroline Royle

15:30 – 15:45 **Celine Gubser** **[12]**
Using combination immune checkpoint blockade to reinvigorate exhausted T cells in people living with HIV (PLWH) on antiretroviral therapy (ART)

15:45 – 16:00 **Bethany Horsburgh** **[13]**
Cellular proliferation influences the dynamics of genetically-intact proviruses over time

16:00 – 16:15 **Michelle Wong** **[14]**
Modulation of HIV-1 transcription by cellular factors and therapeutic small molecules using an in vitro macrophage HIV-1 latency mode

16:15 – 16:30 **Jillian Lau** **[15]**
Effects of immune checkpoint therapy on latent HIV in people with HIV and malignancy

16:30 – 16:45 **Bonnie Hiener** **[16]**
Understanding the mechanism for persistence of intact HIV-1 proviruses within effector memory CD4+ T-cells

16:45 – 17:00 **Thomas Rasmussen** **[17]**
HIV persists preferentially in memory CD4+ T cells that co-express PD1 and CTLA4 and are less inducible

17:00 – 17:15 **Lindi Masson** **[18]**
Microbial function and genital inflammation in young South African women at high risk of HIV infection

17:15 – 17:30 **Lindi Masson** **[19]**
Suppression of HIV pseudovirus infectivity by cervicovaginal *Lactobacillus*-conditioned medium

17:30 **Conference Day 1 Close**

Day Two: Thursday 5 November 2020

10:00 – 12:15 **Session 1: HCV/HBV**
Chair: Chaturaka Rodrigo

10:00 – 10:15 **Monica Pinkerton** **[20]**
Do mutations in Hepatitis B integrations contribute to hepatocellular cancer?

10:15 – 10:30 **Thomas Tu** **[21]**
Covalently closed circular DNA in Hepatitis B infected cells is not efficiently eliminated by targeting capsid formation

10:30 – 10:45 **Laura McCoullough** **[22]**
Analysing the effects of HBV splice variants on wildtype HBV replication

10:45 – 11:00 **Vaishnavi Veeraraghavan** **[23]**
The role of host factor TM6SF2 in HDV secretion: A potential therapeutic target

11:00 – 11:15 **Yianni Droungas** **[24]**
Assessing the immunogenicity of chimeric HBeAg-epitope bio-nanoparticles

11:15 – 11:30 **Rifqiyah Nur Umami** **[25]**
Developing a functional cure for chronic Hepatitis B by targeting human transmembrane 6 superfamily member 2 (TM6SF2)

11:30 – 11:45 **Jessica Howell** **[26]**
Validation of a novel rapid point-of-care ALT test in patients with viral hepatitis

11:45 – 12:00 **Jessica Howell** **[27]**
Validation of a novel rapid point-of-care ALT test to determine treatment eligibility in hepatitis B patients: a pilot cohort study

12:00 – 12:15 **Priyanka Srinivasan** **[28]**
Development of a point-of-care assay for quantification of HBV

12:15 – 13:15 **Lunch**

13:15 – 15:15 **Session 2: HCV/HBV**
Chair: Nadia Warner

13:15 – 14:15 **Presentation**

Chair: Nadia Warner

Thomas Tu
Senior Scientist/Senior Lecturer
The Westmead Institute for Medical Research and The University of Sydney

Title: ACHV – Early Career Research Grant
Followed by additional presentations, chaired by Thomas Tu:
Yianni Droungas, Yuanyuan Liu, Makutiro Masavuli, Harikrishnan Balachandran, Fan Jia, Joey McGregor, Beth Catlett, Vaishnavi Veeraraghavan, Rifqiyah Nur Umami

14:15 – 15:15	**Presentation** **Chair: Nadia Warner** **Scott Bowden** Former Head of the VIDRL Molecular Microbiology Laboratory, The Peter Doherty Institute for Infection and Immunity and senior scientist in the WHO Regional Reference Laboratory for Hepatitis B **Title:** Career and Journey – Viral Hepatitis	
15:15 – 15:30	**Break**	
15:30 – 17:15	**Session 3: HCV/HBV** **Chair: Nadia Warner**	
15:30 – 15:45	**Michael Beard** Assessing HBV Infection and Application Using LGR5+ Human Liver Organoids Culture	**[29]**
15:45 – 16:00	**Julie Nigro** Donor-derived liver organoids as a model of hepatitis B virus infection	**[30]**
16:00 – 16:15	**Kerry Breheney** Validation of functional assays to measure the activity of immunotherapeutic antivirals	**[31]**
16:15 – 16:30	**Julie Nigro** Refinement of culture conditions to improve the growth of donor-derived hepatocellular carcinoma tumouroids	**[32]**
16:30 – 16:45	**Olivia Maslac** Characterisation of hepatitis B virus spliced variants in the sera of individuals across different genotypes in three phases of chronic infection	**[33]**
16:45 – 17:00	**Samuel Hall** HBV STOP Study – Large HBV Flares off NA Therapy cause Large Innate Immune Response	**[34]**
17:00 – 17:15	**Xiu Yu** Functional cure of chronic hepatitis B is associated with co-occurrence of HBsAg/anti-HBs immune complex peaks with ALT flares, and seroconversion to potently neutralising anti-HBs	**[35]**
17:15	**Conference Day 2 Close**	

Day Three: Friday 6 November 2020

10:00 – 12:00 **Session 1: HIV**
Chair: Kirstie Bertram

10:00 – 10:15 **Jarrod York** **[36]**
A new strategy for eradication of HIV using a combination of interferon and CAR T cells

10:15 – 10:30 **Eunok Lee** **[37]**
Identifying CTL Epitopes for Immunotherapy Candidates Against Replication-competent and defective HIV

10:30 – 10:45 **Min-Hsuan Lin** **[38]**
Proof-of-concept that LDH Nanoparticle facilitates delivery and effectiveness of antiretroviral drugs to combat HIV-1 replication in the brain and central nervous system

10:45 – 11:00 **Bruce D Wines** **[39]**
Harnessing the glycan reactivity of PGT121-like bNAbs for potent FcγRIII mediated killing of Env expressing target cells

11:00 – 12:00 **Presentation**

Chair: Kirstie Bertram

Thomas Tu
Senior Scientist/Senior Lecturer
The Westmead Institute for Medical Research and The University of Sydney

Title: Person with Lived Experience

12:00 – 13:00 **Lunch**

13:00 – 15:00 **Session 2: HCV/HBV**
Chair: Thomas Tu

13:00 – 13:15 **Dao Sen (Sam) Wang** **[40]**
Hepatitis C resistance-associated substitutions and antiviral salvage therapy outcomes across Australia

13:15 – 13:30 **Nick Brasher** **[41]**
Identification and analysis of a public antibody repertoire among Hepatitis C Virus infected patients

13:30 – 13:45 **Yanran Zhao** **[42]**
Functional characterisation of antigen-specific CD8 TSCM during hepatitis C virus reinfection and clearance

13:45 – 14:00	**Rob J Center**	**[43]**
	Immunosilencing of the non-neutralizing face of HCV E2 glycoprotein for improved vaccine responses	
14:00 – 14:15	**Joey McGregor**	**[44]**
	Establishment of a VLP based Hepatitis C virus vaccine	
14:15 – 13:30	**Felicia Schlotthauer**	**[45]**
	Characterization of a monoclonal antibody towards the N-terminal hypervariable region 1 (HVR1) and epitope I of Hepatitis C Virus Glycoprotein E2	
14:30 – 14:45	**Joey McGregor**	**[46]**
	Investigation of the optimal strategy to deliver a B cell immunogen for a HCV vaccine	

14:45 – 15:45

HTLVl-1 Presentation

Chair: Thomas Tu

Damian Purcell
Professor of Virology, The University of Melbourne
Theme Leader for Viral Infectious Diseases, The Peter Doherty Institute for Infection and Immunity

Title: Neutralising antibody to HTLV-1: lessons from HIV

15:45 – 16:15

Conference Close and Thank You:
Tony Cunningham and Heidi Drummer

THANK YOU FOR ATTENDING THE 2020 ACH2 CONFERENCE

ABSTRACTS

Cross-reactivity to mutated viral immune targets can influence CD8+ T cell functionality: an alternative viral adaptation strategy

Jennifer Currenti[1*], Becker M.P. Law[1*], Kai Qin[2*], Mina John[3,4], Mark A. Pilkinton[5], Anju Bansal[2], Shay Leary[3], Ramesh Ram[3], Abha Chopra[3], Rama Gangula[5], Ling Yue[6], Christian Warren[5], Louise Barnett[5], Eric Alves[1], Wyatt J. McDonnell[5&], Anuradha Sooda[3], Sonya Heath[2], Simon Mallal[3,5], Paul Goepfert[2†], Spyros A. Kalams[5†], Silvana Gaudieri[1,3,5†]
[*,†] Equal contribution

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[4]Department of Clinical Immunology, Royal Perth Hospital, Perth, Western Australia, Australia
[5]Division of Infectious Diseases, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA
[6]Emory Vaccine Center at Yerkes National Primate Research Center and Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA

Introduction: The prototypic anti-viral immune response requires the actions of naïve T cells, which differentiate into a specific population of clonotypes with an optimal T cell receptor (TCR) repertoire for viral clearance and anti-viral memory. In HIV, this process is subverted by viral escape (adaptation) from T cells. However, viral mutations that disrupt the HLA-peptide-TCR complex leading to loss of antigen recognition represent only one strategy of adaptation. In many instances adapted viral strains can still be recognised by the host's T cells.

Methods: We developed a single-cell TCR analysis pipeline to delineate whether responses to the adapted form of the T cell epitope are mediated by the recruitment of new clonotypes or by selection of particular clonotypes with more cross-reactive TCRs. Furthermore, we assessed the effect of these different TCR-Ag-HLA complexes at the scRNA transcriptome level. To isolate single antigen-specific T cells to a select set of HIV CD8+ T cell epitopes, we utilised HLA class I tetramers (for the adapted and non-adapted form) and activation markers (CD69 and CD137) following peptide stimulation in acutely (n=4) and chronically (n=8) infected individuals. TCR and RNA sequencing analyses were performed using the Illumina platform. The affinity of specific TCR combinations with adapted and non-adapted forms of the epitopes was assessed using a Jurkat transfectoma cell line with luciferase activity as the measurable output.

Results: CD8+ T cells were predominantly dual tetramer+, confirming the large proportion of cross-reactive TCR clonotypes between non-adapted- and adapted-activated T cells, although differences in clonality were observed in chronic and acute infection. The function of cross-reactive tetramer+ T cells differed based on the autologous virus and evidence of immune pressure: acutely and chronically infected subjects with the non-adapted form that transitioned to the adapted form at a later time point (not studied) demonstrated a more 'effective' immune response.

Discussion/conclusions: These data suggest that viral adaptation at a single amino acid residue can provide an alternative strategy for viral survival by modulating the transcriptome of cross-reactive CD8+ T cells and potentially selecting for less effective T cell clones from the acute to chronic phase. Understanding how the TCR diversity of an immune response can be altered or exploited by a pathogen is a fundamental question for HIV vaccine design and for many other pathogens for which natural, vaccine or cell therapy-based immunity is not currently effective or available.

Investigation of viral adaptation dynamics during early HIV infection yields important considerations for vaccine design

Eric Alves[1], Niamh Keane[2], Mina John[2,3], Shay Leary[2], Coral-Ann Almeida[2], Marwah Al-Kaabi[1], Jennifer Currenti[1], Pooja Deshpande[1,2], Abha Chopra[2], Rita Smith[4], Simon Mallal[2,4], Spyros Kalams[4], Silvana Gaudieri[1,2,4]

[1]School of Human Sciences, University of Western Australia, Australia.
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[3]Department of Clinical Immunology, Royal Perth Hospital, Australia.
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Introduction: HIV has been shown to adapt to an individual's anti-HIV immune response via the selection of random mutations, resulting in immune evasion. These viral adaptations are specific to the host's human leukocyte antigen (HLA) alleles, which encode for molecules that determine what viral fragments are presented to T cells. As HLA molecules are highly polymorphic, horizontal transmission events are often between HLA-mismatched donor/recipient pairs, representing novel immune selection environments for the incoming virus. Previous research has shown that chronic-stage individuals with viruses harbouring high levels of adaptation upon transmission exhibit poor disease outcome. However, research clarifying the extent to which the adaptation level changes over time post-infection within an individual remains limited, particularly during the acute and early stages of infection, despite this being the key establishment stage of the latent reservoir.

Methods: Deep plasma sequencing of HIV quasispecies for the *gag*, *pol* and *nef* genes was utilised for 12 semi-controllers with longitudinal timepoints during early HIV infection. Autologous adaptation (adaptation to the host's HLA alleles) and circulating adaptation (adaptation to other HLA alleles in the population) values were calculated for each timepoint. The average change over time in these values for each HLA class-I locus (*HLA-A*, *HLA-B* & *HLA-C*) was compared within and between both adaptation types (autologous or circulating). In addition to the overall adaptation level, the degree of reversion, maintenance and *de novo* adaptations were measured longitudinally in the context of each gene (*gag*, *pol* & *nef)* and HLA class-I locus (*HLA-A*, *HLA-B* & *HLA-C*).

Results: The level of autologous adaptation increases over time during early HIV infection, whilst the circulating adaptation level is maintained. Across early HIV infection, *HLA-B* mediated immune pressure appears to drive the overall increase in autologous adaptation. Additionally, more *HLA-B* associated autologous adaptations appear to arise and revert over time, compared to *HLA-A* and *HLA-C*, suggesting greater immune pressure is exerted by this particular locus. *Nef* and *gag* show significantly greater adaptation dynamics over time, however this is not seen within *pol* – reflecting this gene's evolutionary constraint.

Discussion: Our data indicate that upon transmission to a new immune environment, circulating adaptations are largely maintained, rather than revert over time. In addition, these circulating adaptations are likely to combine with a proportion of the accumulating autologous adaptations to increase the overall adaptation level of the virus within an infected individual. These data provide a potential mechanism to support recent findings that overall adaptation levels within populations are increasing, particularly in populations with high seroprevalence, limited HLA diversity and/or geographical isolation. Furthermore, our work highlights that *gag* and *nef* undergo substantial genetic changes due to HLA-restricted immune pressures during early infection, thereby highlighting the contribution of viral adaptations in altering the HIV genome.

Conclusions: Understanding early HIV adaptation has become fundamental as individuals are being placed on treatment as soon as infection is suspected. Our research will aid current vaccine design prospects for HIV by clarifying how quickly the virus can adapt in acute/early infection, which genomic regions are more likely to adapt, and how these adaptations can alter the genetic landscape of the virus – the basis from which a vaccine is likely to de designed.

Early induction of innate immune cell activation in acute HIV infection; implications for persistent immune dysfunction following antiretroviral therapy.

Anna C Hearps[1,2], Jingling Zhou[3], Michelle Wong[1,2], Eugene Kroon[4], Siriwat Akapirat[5], Alexandra Schuetz[5,6,7], Sandhya Vasan[6,7], Jintanat Ananworanich[8], Anthony Jaworowski[1,3] on behalf of the SEARCH010/RV254 Study Team

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Introduction: People living with HIV (PLWH) exhibit persistent immune dysfunction despite long term viral suppression with antiretroviral therapy (ART). Immune dysfunction and related chronic inflammation are thought to contribute to heightened risk of inflammatory comorbidities in PLWH. Current treatment guidelines recommend ART initiation upon HIV diagnosis irrespective of CD4 T cell count, and this is associated with reduced incidence of comorbid disease and mortality in PLWH. However, the differential effects of early ART initiation on various innate and adaptive cellular compartments has not been fully defined, and it remains unclear how 'early' ART must be initiated to prevent ongoing immune dysfunction.

Methods: This study utilised samples from the SEARCH10/RV254 trial in Bangkok, Thailand, which enrolled individuals who were diagnosed with HIV at Fiebig stages I to V and initiated ART within 1-2 days of diagnosis. We analysed longitudinal samples from individuals diagnosed with HIV in various Fiebig stages (n=10 each for Fiebig stages I, II and III and n=8 for stage IV/V) and measured cellular biomarkers of NK cell, monocyte and T cell activation/dysfunction at time of diagnosis (prior to ART initiation) and 4, 12 and 48 weeks after ART-initiation by flow cytometry. Participants were all male, had an overall median age of 26.0 (IQR: 22.8-30.0) years all and exhibited viral suppression after 48 weeks of ART.

Results: The majority of NK cells at diagnosis (median 66%, IQR: 53-79%) exhibited an adaptive-like phenotype lacking the intracellular signal transduction factor FcR chain, irrespective of stage of diagnosis. Suppressive ART had no impact on proportions of either FcR^{-}, adaptive-like NK cells or an analogous population of adaptive-like NK cells (defined as $CD56^{dim}CD57^{+}NKG2C^{+}$). The cellular activation state of monocytes and NK cells was elevated at the time of diagnosis in all individuals, including those diagnosed at Fiebig I. This was in contrast to T cells, where heightened expression of early ($CD69^{+}$) and late ($HLA\text{-}DR^{+}/CD38^{+}$) activation markers were evident in individuals diagnosed in Fiebig stages II or III respectively, but not earlier. Levels of T cell activation were significantly reduced by ART in all participants with no differences in residual activation due to stage of diagnosis, whilst PLWH who initiated ART in Fiebig III or IV/V tended to show higher expression of NK activating receptors (eg NKp46) after 48 weeks on ART than those who initiated in Fiebig I or II.

Discussion: These data confirm innate immune cells including monocytes and NK cells are activated at earlier stages of acute HIV infection than T cells, but imply that heightened cellular activation prior to ART initiation may result in higher levels of some biomarkers of innate immune dysfunction persisting after 48 weeks of ART. Individuals in this cohort exhibited high levels of adaptive-like NK cells, which show enhanced cytokine secretion and antibody-dependent cellular cytotoxicity potential. The persistence of high proportions of these NK cells in ART treated individuals may have implications for NK cell function and inflammatory status.

Conclusions: Whilst ART initiation during acute infection prevents a substantial degree of immune activation and dysfunction associated with viremic HIV infection, which is likely beneficial for the long-term health and wellbeing of PLWH, some aspects of NK cell dysfunction persist in ART-treated individuals despite early ART initiation.

Differential Expression of HIV Envelope Epitopes on the Surface of HIV-Infected Macrophages and CD4+ T Cells

Hans Kek[1,2], Annemarie Laumaea[2], Srihari Parise[2], Andy Poumbourios[2,3], Anna C Hearps[2,4], Anthony Jaworowski[2,5]

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[2] Life Sciences Discipline, Burnet Institute, Melbourne, VIC, Australia
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[4] Department of Infectious Diseases, Monash University, Melbourne, VIC, Australia
[5] School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia

Introduction: HIV-infected macrophages contribute to the persistence of HIV reservoirs in the tissues of people living with HIV on antiretroviral therapy. One potential targeting strategy is the use of antibody-dependent cellular cytotoxicity (ADCC) against infected cells expressing the HIV envelope (Env) protein on the surface. ADCC strategies require antibodies capable of opsonising exposed Env epitopes, yet little is known regarding the epitopes expressed on HIV-infected macrophages to predict which antibodies will be useful .

Methods: Monocytes purified from HIV-seronegative donors were cultured into monocyte-derived macrophages (MDM) for 5 days. MDM and activated peripheral blood mononuclear cells (PBMC, + phytohaemagglutinin/IL-2) were then infected *in vitro* with the R5-tropic HIV_{BaL} strain for 7-10 days and 3-4 days respectively. Flow cytometry and fluorescence microscopy was used to assess productive infection (intracellular HIV p24) and surface expression of Env (using a panel of antibodies specific for separate epitopes) on MDM and CD4+ T cells in PBMCs.

Results: Our results are consistent with differences in Env epitope expression on the surface of HIV-infected MDM and T cells. Notably, a greater proportion of HIV_{BaL}-infected MDM were recognised by NIH45-46 and 17b antibodies (median=37.2% and 28.2% respectively) compared to infected T cells (median=15.4% and 2%; p=0.002 and 0.004 respectively). In contrast, a greater proportion of HIV-infected T cells were recognised by PG16 (median=27.2%) as compared to MDM (median=7.9%, p=0.004). Furthermore, the neutralising antibodies 10E8 and PGT145 were ineffective at recognising cell-surface Env on MDM and CD4+T cells in PBMCs, indicating it may be presented differently to Env on cell-free virions.

Discussion and Conclusions: Here we show that HIV-infected macrophages may display a distinct surface Env epitope expression profile as compared to infected T cells. The differential Env epitope expression between MDM and T cells suggest that cell-type dependent differences alter the conformation of Env on the cell surface, which affects binding of anti-Env antibodies. These findings have implications for antibody-mediated approaches to target HIV-infected cells and highlight the need to consider all relevant cellular reservoirs of HIV in future cure strategies.

[5]

Investigating the Immunology of Leaky Guts in HIV Infection

Kevin Hu[1,2], Heeva Baharlou[1,2], Nicolas P. Canete[1,2], Osaretin E. Asowata[3], Henrik N. Kløverpris[3], Anthony L. Cunningham[1,2] and Andrew N. Harman[1,2]

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Introduction: In HIV infection, perturbations associated with the integrity of the gut mucosal barrier result in a leaky gut that is characterised by increased microbial translocation and chronic inflammation. Antiretroviral therapy (ART) can suppress HIV replication, but it does not restore full health as residual inflammation persists in the gut in infected individuals and the reason for this remains unclear.

Methods: Duodenal sections derived from HIV^+ and HIV^- individuals in South Africa were imaged by multiplexed cyclic immunofluorescence for $CD4^+$ $CD103^+$ T cells and $CD8^+$ $CD103^+$ T cells. These cells were quantified in the epithelial and lamina propria compartments of the duodenal mucosa. $CD4^+$ $CD103^+$ T cells were further assessed for the expression of CCR5 and the proportion of $CCR5^+$ cells was determined.

Results: No significant difference was observed in the frequency of $CD8^+$ $CD103^+$ T cells in the duodenal mucosa between HIV^+ and HIV^- individuals. $CD4^+$ $CD103^+$ T cells however were significantly depleted in HIV^+ individuals in both the epithelial and lamina propria compartments, and this depletion was more pronounced in viremic individuals compared to ART-suppressed individuals. It was further found that in HIV^- individuals, between 70-80% of duodenal $CD4^+$ $CD103^+$ T cells expressed CCR5.

Discussion: These findings suggest that the depletion of $CD4^+$ $CD103^+$ T cells may contribute towards the chronic gut inflammation in HIV^+ individuals which does not resolve even after ART intervention. As these cells express CCR5, they are susceptible towards HIV infection and this may mediate their depletion.

Conclusions: Overall, $CD4^+$ $CD103^+$ T cells are depleted in the duodenum of HIV^+ individuals in comparison to HIV^- individuals. Further studies are required to clarify their role in the gut and the consequences of their depletion.

Development of a vaginal administration of anti-HIV microbicide nanosystem

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Introduction: Prevention strategies play an important role in fighting against HIV/AIDS. Vaginal and rectal microbicides have great promise in tackling sexual transmission of HIV-1, but effective and safe products are yet to be approved and made available to those in need. Nano-carrier systems have been shown considerable advances in the enhancement of mucosal distribution and retention of promising antiretroviral compounds, therefore enhance the performance of current drugs in protecting from HIV infection. Layered double hydroxide nanoparticles (LDHs) are made of inorganic matrices and have shown excellent capability and good adjuvant function as a nanocarrier. Furthermore, LDHs have good biocompatibility and low cytotoxicity. Tenofovir (TFV) and emtricitabine (FTC) have been used for the development of a liposomal hydrogel and will be used for LDH delivery. The formulation of this nanoparticle delivery system will be optimized in vitro for loading capacity, drug release, and permeation studies and then will be test in vivo with an EcoHIV mouse model.

Methods: TFV was formulated with LDH as LDH-TFV. The size of the LDH-TFV was measured with a nano ZetaSizer. The release profile of LDH-TFV at PH 4 was characterised. The permeability of the LDH-TFV was tested using a Franze cell system. The anti-EcoHIV activity of TFV and LDH-TFV was tested *in vitro* and in an EcoHIV mouse model.

Results: LDH-TFV has been formulated and tested *in vitro*. Anti-EcoHIV

Lactic acid produced by an optimal vaginal microbiota promotes cervicovaginal epithelial barrier integrity: implications for HIV transmission

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Introduction: Women with a *Lactobacillus* spp.-dominated vaginal microbiota have a decreased risk of HIV acquisition compared to women colonized with 'non-optimal' vaginal microbiota, the latter being associated with decreased cervicovaginal epithelial barrier integrity. In an optimal vaginal environment, paracellular penetration of the epithelium by HIV is blocked by tight junctions. Lactic acid (LA) is a key metabolite of *Lactobacillus* spp. with antimicrobial and anti-inflammatory properties that is differentially produced by *Lactobacillus* spp. as L- and D-isoforms. However, the impact of LA in promoting epithelial barrier integrity through modulation of junctional molecules is unknown.

Methods: Cervicovaginal epithelial (Ect) cells were cultured in a transwell system and treated apically for 1 h with 0.3% L-LA or D-LA (pH 3.9), or acidity alone (pH 3.9, HCl adjusted). Transepithelial electrical resistance (TEER) across the cell monolayer was determined prior to and 24 h post-treatment to measure epithelial barrier integrity. Expression of junctional molecule mRNA after L or D-LA treatment was determined by RNASeq and qRT-PCR, and protein levels were determined by Western blot.

Results: Treatment of Ect cells with L- or D-LA significantly increased TEER by 1.5-fold ($n= 4$; $p<0.05$), in contrast to the pH 3.9 (HCl) control treatment. RNASeq and gene ontology enrichment analysis were consistent with the TEER functional data demonstrating that L- and D-LA caused significant differential expression (FDR<0.05) compared to untreated cells of at least 11 genes associated with intracellular junctions and barrier function, including claudin-1 (CLDN1, L-LA Fold change [FC] 2.17 and D-LA 2.3-fold), claudin-4 (CLDN4, 2.8 and 3.1-fold) and occludin (OCLN, 1.4 and 1.5-fold), with no differential gene expression between isoforms. These findings were confirmed by qRT-PCR. In addition, tight junction protein levels were significantly increased by L-LA treatment (CLDN1 FC = 1.56, TJP2 FC = 1.42) but not with the pH control ($n= 5$; $p<0.05$).

Discussion: Treatment with physiological concentrations of LA was found to significantly increase epithelial barrier integrity independently of pH as demonstrated by TEER, with a potential mechanism being the upregulation of key barrier function genes and their encoded proteins, including CLDN1, CLDN4 and TJP2. Experiments are currently underway to measure the paracellular penetration by HIV and similarly sized molecules of cervicovaginal epithelial monolayers in the presence and absence of L- and D-LA to further characterize the role of LA in modulating epithelial barrier integrity.

Conclusions: LA significantly increases cervicovaginal epithelial barrier integrity by increasing the expression of junctional molecules, which has implications for the paracellular penetration of HIV through cervicovaginal tissue and subsequent HIV acquisition.

HIV interactions with colorectal macrophages in early infection

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Introduction: New HIV infections remain a significant global health issue despite antiretroviral therapy and pre-exposure prophylactics helping to reduce transmission rates. In Australia, as in most of the developed world, the majority of new infections occurs in men who have sex with men through anal intercourse. Despite this, little is known of the early events that follow HIV entry into the colorectal mucosa, particularly the role that macrophages play in early infection. This constitutes a fundamental gap in our knowledge of early HIV targets and understanding HIV-macrophage interactions may provide a basis for macrophage-specific HIV studies as well as microbicide design.

Methods: Human colorectal tissue discarded from surgery was digested to liberate immune cells. Macrophage subsets, the expression of HIV binding receptors, and HIV uptake were determined by flow cytometry. Macrophage subsets will be FACS sorted and incubated for 96 hours in the presence of HIV to determine level of infection.

Results: We have found distinct subsets of macrophages in the colorectum that have recently been defined in the human small intestine. Moreover, we have shown that these macrophage subsets have varied expression of key HIV binding receptors. Interestingly, these macrophage subsets take up significantly differing amounts of HIV 2 hours post-exposure. We are currently investigating HIV productive infection of these macrophages subsets and their ability to transfer HIV to CD4 T cells.

Discussion: These results indicate that HIV can be taken up very quickly by colorectal macrophages. The observation that macrophage subsets have differing levels of HIV uptake and expression of key HIV binding receptors suggests that HIV-macrophage interactions in the colorectum are subset specific. We believe that blocking these HIV binding receptors may offer insight into the preferential uptake that has been observed, as well as highlighting the hierarchy of binding for HIV on these cells. By further investigating productive infection in these macrophage subsets, we aim to understand if HIV infection can be associated with specific macrophage subsets and how these subsets are contributing to early infection in the colorectum.

Conclusions: Here we have characterised macrophage subsets in colorectal tissue and have defined their expression of key HIV binding receptors. We have shown that HIV uptake by these macrophage subsets is varied. We will continue to investigate these macrophages ability to become productively infected by HIV, as well as their ability to transfer HIV to CD4 T cells, further characterising HIV interactions at this critical transmission site.

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Investigating CD4+ Tissue resident T cells in human anogenital tissues as targets for HIV

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Introduction: Sexual transmission is the predominant mode of HIV acquisition. Inflammation at the sites of transmission increases the risk of HIV acquisition by providing HIV better access to its target cells, However, the early immune events in HIV transmission are not fully understood. It has been recently appreciated that human tissues contain a large reservoir of CD4+ T cells, termed **Tissue Resident Memory T cells**, which are separate from their recirculating/blood counterparts. The human anogenital tissues may also contain unique populations of TRM that may differ between healthy and inflamed tissues. In 2019, Cantero-Perez et al. implicate the CD4+ Tissue resident T cell as having a role in HIV infection and latency. It is therefore paramount that we investigate tissue resident T cells in human tissues to understand their role in early events of HIV transmission.

Methods: Immunofluorescent microscopy was used to determine the localisation and density of CD4+CD69+ Tissue resident T cells across a range of anogenital tissues. Single-cell RNA sequencing of CD4+ T cells isolated from a human foreskin and a rectum was performed, and this data is currently guiding the development of a high-parameter flow cytometry panel to investigate the unique populations of TRM CD4+ T cell subsets in different human anogenital tissues.

Results: There was an enrichment of CD3+ cells in all healthy anogenital tissues compared to abdominal skin. Interestingly, TRM CD4+ T cells were found within epidermis, but at lower frequencies compared to dermis/lamina propria. However, they increased in numbers in the dermis/lamina propria, specifically between 0-60µm from the basement membrane. Contrasting previous flow cytometry data, the proportions of tissue resident CD4+ T cells was higher in dermis and lamina propria compared to epidermis.

Discussion: CD4+ T cells are found in the epidermis of human anogenital tissues, but at a lower frequency than CD4- T cells. This was a crucial observation to make, given the general misconception that CD4+ T cells are absent in epidermal layers. We connote this misconception to mice studies, or human studies which receive rare and/or small human biopsies. Frequencies of CD4+CD69+ T cells were found to be higher in dermis/lamina propria between 0-60µm, then 60-120µm and least so in epidermis. The role of the CD4+ T cells in HIV infection may vary across these two regions, as the density of other immune cells such as macrophages and dendritic cells also vary with distance from basement membrane.

Conclusions: The localisation of CD4+ T cell subsets may aid the understanding of sexual HIV transmisson. While the expression of CD69 was proportionally high in all anogenital tissues, future studies will investigate i) which CD4 T cell subsets are susceptible to productive or latent HIV infection, and ii) the localisation of T cells expressing preferential HIV infection markers, such as CCR6 and CCR5, from healthy and inflamed tissues. This will allow us to understand how the inflammation intended to protect the host, favours HIV transmission and will guide better blocking strategies against HIV.

The different adaptation strategies adopted by RNA viruses to escape the host's T cell immune response: an update

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Introduction: Highly mutable pathogens such as HIV and HCV can evade the host's anti-viral $CD4_+$ and $CD8_+$ T cell responses via mutations in the genome. Such viral mutations can disrupt the HLA-peptide-TCR complex leading to loss of antigen recognition and represent one strategy of adaptation ('classical' adaptation). However, in some instances adapted viral strains can still be recognised by the host's T cells or indeed generate new epitopes that may be beneficial for the virus ('non-classical' adaptation). Understanding these different strategies of adaptation is important to inform vaccine design.

Methods: We have utilised large-scale host and viral genomic typing of HIV- and HCV-infected subjects to identify these different forms of adaptation in both HIV and HCV. For HIV, we have also utilised a single-cell T cell analysis pipeline to delineate how 'non-classical' viral adaptations impact T cell receptor diversity and the transcriptome.

Results: Both HIV and HCV exhibit extensive adaptation to both $CD4_+$ and $CD8_+$ anti-viral T cell responses. These adaptations are often maintained even in individuals that do not have the relevant immune selective environment. Furthermore, 'non-classical' adaptation was not associated with major changes in TCR repertoire and was associated with cross-reactivity to both the adapted and non-adapted form of the epitope. Differences for the adapted and non-adapted form were observed in the single cell transcriptomic profile reflecting changes in polyfunctionality and cytotoxicity.

Conclusions: These data suggest that viral adaptation at a single amino acid residue can provide multiple strategies for viral survival, including selecting for less effective T cell clones from the acute to chronic phase. Furthermore, the lack of reversion for many of these adaptations is likely to result in the accumulation of adaptations in the circulating strains that may affect clinical outcomes.

Immunogenicity of novel polyvalent HIV glycoprotein vaccines

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Introduction: Despite 25 million people receiving antiretroviral therapy in 2019, the global HIV transmission rate was 4,700 new infections/day. A protective vaccine would have a profound impact on the HIV pandemic by blocking the chain of viral transmission and is therefore a global priority. Neutralizing antibodies (NAbs) that block infection are a strong correlate of protection for many antiviral vaccines. The HIV-1 envelope glycoprotein (**gp120-gp41**) trimer is the sole target of NAbs and thus represents an essential vaccine component. However, experimental HIV-1 glycoprotein vaccine candidates have thus far had limited success in inducing broadly reactive NAbs (**bNAbs**) effective against diverse circulating strains. We produced a novel subtype B soluble gp120-gp41 vaccine with enhanced presentation of bNAb epitopes that was able to elicit bNAbs in small animals. In this study, we ask whether a multi-subtype polyvalent bNAb epitope-enhanced/non-NAb epitope occluded SOSIP vaccine will elicit broader and more potent NAb responses than the prototype single-isolate vaccine.

Methods: We engineered, expressed, purified and characterised a panel of gp120-gp41 trimeric vaccine candidates, derived from HIV-1 subtypes A, B, and C. The vaccines were derived from the difficult to neutralize “Tier 2" isolates AD8 (subtype B), 459c (subtype C) and Q168env2 (subtype A). To enable expression of the latter 2 subtypes, their gp120 domains were chimerized with the gp41 domain of a highly stable and highly expressed isolate, SC45 (subtype B). 2 groups of guinea pigs were immunised with the candidate vaccines to compare responses to the prototype subtype B vaccine *versus* the multi-subtype ABC vaccine, using Addavax as the adjuvant. An extended immunisation protocol was followed involving boosts at 4-, 14- and 24-weeks post-priming. The immune responses were analysed by ELISA and will be analysed in neutralization assays.

Results: Subtype B and chimeric subtype A/B and C/B gp120 gp41 trimers were obtained in multi-milligram quantities by size exclusion chromatography. A thermofluor assay revealed highly stable trimers with melting temperatures ranging from 60°-64°C. Biolayer interferometry indicated strong bNAb (PGT121, PGT145, PGT151, VRC01) binding but low non-NAb (17b, 447-52D and F105) binding to the vaccine trimers, consistent with gp120 molecules adopting a closed, “Tier-2-like” conformation. ELISA indicated strong antibody responses with gp120-gp41 trimer binding titres in the range 10^5-10^6. Importantly, the polyvalent subtype ABC vaccine elicited consistently higher titres against a multi-subtype trimer panel than the prototype subtype B vaccine. Neutralization assays employing a reference panel of global Tier 2 isolates are underway.

Conclusions: A polyvalent subtype ABC polyvalent vaccine elicits broader and more potent antibody responses than the prototype single subtype B vaccine.

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Using combination immune checkpoint blockade to reinvigorate exhausted T cells in people living with HIV (PLWH) on antiretroviral therapy (ART)

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Introduction: ART has dramatically improved life expectancy for PLWH but it needs to be taken lifelong and immune dysfunction persists with elevated expression of immune checkpoints (IC) including programmed death (PD-1) and cytotoxic T lymphocyte Antigen (CTLA-4). In individuals with cancer, immune checkpoint blockade (ICB) augments tumour-directed T cell responses resulting in significant clinical cures. Recently, a subset of transcription factor T cell factor 1 (TCF1) expressing precursor exhausted T cells (Tpex) were shown to be responsible for the proliferative burst and increased effector functions of CD8 T cells after ICB. Given these results, there is high interest in whether blockade of PD-1 and/or CTLA-4 can drive recovery of HIV-specific T-cell cytolytic function in PLWH on suppressive ART.

Methods: In a phase one clinical trial, where PLWH on ART with cancer received ICB (anti-CTLA-4 and anti-PD1 or anti-PD1 alone), we quantified HIV-specific T-cell function prior to and on at least 2 time points during treatment. We stimulated peripheral blood mononuclear cells (PBMC) from six participants who received anti-PD1 alone and in one case anti-CTLA-4 in combination with anti-PD1 with pools of overlapping HIV peptides (Gag and Nef) and measured the percentage of TNFa and IFNg producing CD8 T cells to identify polyfunctional T-cells

Results: We identified two participants who had a >2 fold increase in the frequency of HIV-specific cytokine-producing CD8 T cells following ICB, which we defined as a ''responder''. Next we determined the frequency of Tpex cells (defined as CD8+PD1+TCF1+GzyB-CD45RA-) at baseline in these six participants and found that Tpex frequency was highest in the two treatment responders (3.1% and 1.7%) compared to non-responders (mean <0.9%).

Conclusions: Taken together we identified two participants who had an increase in polyfunctional HIV specific CD8 T cells following ICB. These same participants had a high frequency of Tpex cells at baseline which potentially predicts an effective response to ICB. ICB can enhance HIV-specific T-cell function and should be further explored as a component of cure strategies.
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Cellular proliferation influences the dynamics of genetically-intact proviruses over time

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Introduction: Human immunodeficiency virus (HIV) persists in cells despite antiretroviral therapy. However, the influence of cellular mechanisms such as activation and proliferation upon the levels and distribution of proviruses over time is unclear. As such, we aimed to define the stability of genetically-intact proviruses within CD4+ T-cell subsets during effective antiretroviral therapy.

Methods: Naïve, central (CM), transitional (TM) and effector (EM) memory CD4+ T-cells, as well as memory cells displaying or not displaying HLA-DR, were sorted from the peripheral blood of eight participants on long-term ART. At a second time-point approximately four years later, we co-sorted memory CD4+ T cell subsets with or without the HLA-DR receptor from a leukapheresis from the same eight participants. Full-length individual proviral sequencing was used to characterise proviruses as intact or defective. Clusters of identical sequences were identified as ≥2 100% identical proviral sequences.

Results: At the first time-point, a total of 1124 genomes were isolated from six CD4+ T-cell subsets, and at the second time-point, 1654 genomes were isolated from seven cell subsets. Only 48 (4.3%) and 104 (6.3%) genomes were genetically-intact at time-points one and two respectively. We found that the odds of finding any HIV provirus was 1.28 times greater in EM cells after four years of therapy ($p=0.002$), but was nearly halved in TM cells (odds ratio: 0.53, $p<0.0001$). The odds of identifying a genetically-intact provirus was increased in all cell subsets after four years of therapy, however not significantly. In all cell subsets, HLA-DR+ cells had a higher overall infection frequency with intact provirus than HLA-DR- cells. We then investigated the role of cellular proliferation in maintaining HIV proviruses, and found that the proportion of 100% identical proviruses increased in all subsets with time, though this wasn't statistically significant. However, the number of clusters of identical sequences within a cell subset did not change with time, except in TM cells, where the number of clusters was found to increase. While the overall number of clusters within each cell subset was relatively stable with time, the frequency and makeup of these clusters within each cell subset changed between time points

Discussion: Cell proliferation is increased in differentiated cell subsets and in cells displaying HLA-DR, as is the infection frequency with intact proviruses. However, the cells undergoing this proliferation change with time, likely as a result of the unique clinical history of a participant. The role of cell proliferation in shaping the reservoir underscores the importance of limiting proliferation in any curative strategy for HIV.

Modulation of HIV-1 transcription by cellular factors and therapeutic small molecules using an *in vitro* macrophage HIV-1 latency model.

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Background: HIV-infected macrophages persist despite viral suppression, yet little is known regarding the establishment and control of latency in this important HIV reservoir. Physiologically-relevant *in vitro* systems which provide a robust, quantitative model of latent infection and reactivation are required to investigate factors which govern latency in macrophages.

Methods: Primary human monocyte-derived macrophages (MDM) were infected *in vitro* with a GFP-HIV reporter virus and FACS sorted 7-days post-infection, to purify GFP- populations consisting of uninfected bystander and non-productively infected MDM. GFP- MDM were cultured for a further 9 days in media containing entry inhibitor T20, to prevent *de novo* infection, and potential latency modulating agents. Reactivation of HIV transcription was quantified by live cell fluorescent microscopy via expression of GFP in reactivated cells.

Results: Spontaneous reactivation of HIV transcription within macrophages was observed in all donors with a linear rate of 0.22% ± 0.04% (mean ± SEM, n=10) GFP+ cells per day, slower than rates of HIV transcription following initial infection (0.91% ± 0.12% GFP+ cells per day), indicating the presence of a population of potentially latently infected macrophages. Reactivated MDM produced replication competent virus, demonstrated by infection of heterologous PBMC in co-culture and in a cell-free infection system. Polarization of MDM to M1 or M2 significantly inhibited or enhanced HIV reactivation rates, to a mean of 71% or 198% of the matched reactivation rate in unpolarised MDM, respectively. HIV reactivation was increased in unpolarised MDM by latency reversing agents including PKC agonist, bryostatin-1, and HDAC inhibitors, vorinostat and panobinostat.

Conclusion: We have developed a robust and quantitative model of latently infected primary MDM which can be used to advance cure strategies targeting the latent HIV reservoir. Our data suggest the potential of MDM to harbour latent HIV infection and contribute to viral rebound. The modulation of reactivation rates by polarization and latency reversing agents suggests latent macrophage reservoirs are sensitive to local environments *in vivo*, and may be therapeutically modulated.

Effects of immune checkpoint therapy on latent HIV in people with HIV and malignancy

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Introduction: Immune checkpoint blockade (ICB) is highly effective for the management of some malignancies and can potentially perturb HIV persistence in people living with HIV (PLWH) on antiretroviral therapy (ART) by enhancing HIV-specific CD8+ T cells and/or reversing HIV latency. We established a prospective cohort of PLWH on ART with malignancy who received any ICB and quantified immunological and virological changes in three participants.

Methods: Blood was collected around the first 4 cycles of ICB at days -1,+1 and +7 relative to ICB administration. We quantified cell associated (CA) unspliced (US) RNA and HIV DNA from peripheral blood CD4+ T cells, the proportion of cells with inducible multiply spliced (MS) HIV RNA by the Tat/rev Induced Limiting Dilution Assay (TILDA) and HIV RNA in plasma by single copy assay (SCA). Gag specific immune responses were measured by intracellular cytokine staining (ICS) for IFN-γ, TNF-α, and CD107a in memory T-cell subsets defined by expression of CD45RA and CCR7. The frequencies of precursor exhausted T cells (Tpex) and exhausted T cells (Tex) were measured using flow cytometry as their relative frequency and dynamics during therapy may be associated with expansion of functional T cells.

Results: Participant (P)1 received avelumab (anti-PD-L1) 2 weekly for chest wall Merkel cell carcinoma. P2 and P3 received ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) 3 weekly for metastatic melanoma. P1 demonstrated partial response to ICB, before relapse and progression of disease. P2 had disease progression on ICB and died before study completion. P3 responded to ICB and remains on maintenance anti-PD-1.
An increase in CA-US RNA following each infusion was noted in all 3 participants. There was a gradual increase in the mean fold change relative to baseline in CA-US RNA from cycle 1 to 4 of 1.3, 3.1, 6.8 and 8.6 respectively. There were no consistent changes overall in in HIV DNA or the proportion of cells with inducible MS HIV RNA. However, P3 had an increase in plasma viremia from 4 c/mL at baseline up to 16 following cycle 2, a 55% reduction in HIV DNA and a 33% reduction in inducible MS RNA as measured by TILDA. There were no changes in plasma viremia or inducible MS RNA in P1 or P2. P2 demonstrated a striking increase in the frequency of gag-specific central and effector memory CD8+ T cells producing IFN-γ, TNF-α, and CD107a, which were not demonstrated in P1 and P3.
The frequency of CD8+ Tpex cells pre-ICB was highest in P2, the same participant who had a dramatic increase in HIV-specific T cells following anti-PD1 and anti-CTLA-4. In addition, the frequency of CD8+ Tex cells increased during ICB therapy for all three participants.

Discussion: Our findings of enhanced effects with combined blockade is consistent with findings in ART-treated SIV-infected monkeys, where the combination of anti-PD1 and anti-CTLA-4 reversed latency more potently than either antibody alone. Our finding of a higher Tpex frequency pre-ICB in P2, may be predictive of enhanced HIV-specific T cell function after blocking PD1, however this individual did not have a favourable anti-tumour response to ICB.

Conclusion: We observed HIV latency reversal in response to ICB and a significant enhancement of HIV-specific T cell function in one participant receiving combined blockade of PD-1 and CTLA-4. The frequency of Tpex cells before ICB may have a predictive role for expansion of polyfunctional T cells, but further work is needed to understand or predict these responses, and who may best benefit from this therapy.

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Understanding the mechanism for persistence of intact HIV-1 proviruses within effector memory CD4+ T-cells

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Introduction: HIV-1 persists despite effective antiretroviral therapy (ART) due to a small population (2-12%) of latent replication-competent proviruses that contribute to rebound viraemia upon treatment interruption. Previously we showed genetically intact and potentially replication-competent HIV-1 proviruses to be enriched within effector memory (TEM) CD4+ T-cells. Although the vast majority of persistent HIV-1 proviruses are genetically defective, many produce viral proteins, making them vulnerable to CD8+ T-cell clearance. Viral proteins, such as *gag* are immunogenic, whereas others, such as *nef,* may protect against CD8+ T-cell clearance by MHC I downregulation. Immune pressure likely contributes to the observed dynamics of the genetic landscape of persistent HIV-1 in individuals on ART. Investigating the proviral landscape in naïve and memory CD4+ T-cells in individuals on ART, including the dynamics of the landscape with ART duration, may give clues as to why TEM cells contain more genetically intact HIV-1 proviruses.

Methods: We conducted Full-Length Individual Proviral Sequencing on naïve (TN), central (TCM), transitional (TTM) and TEM memory CD4+ T-cells isolated from 25 HIV-1 positive individuals on suppressive ART (2-22 years), obtaining 4400 sequences. Each proviral sequence was categorised as full-length (>8800 bp and subcategorised as intact or defective) or containing deletions (and subcategorised as 5' deleted (*gag to pol*), or 3' deleted (*vif to env*)). For each sequence, the presence of genetically intact open reading frames (ORFs) for all HIV-1 proteins was determined. The proportion of sequences within each category and containing intact ORFs for each viral protein was compared between cell subsets and a cross-sectional analysis conducted to determine how these proportions change with time on ART.

Results: When comparing the genetic landscape of proviral sequences between cell subsets we found, compared to TCM and TTM, TEM contained a higher proportion of intact ($p=0.001$ and 0.004 respectively) and full-length proviruses ($p=0.001$ and 0.003 respectively). A higher proportion of proviruses within the TEM subset had deletions spanning the 5' half of the genome compared to TN ($p=0.03$) and TCM ($p=0.007$) and a lower proportion of deletions spanning the 3' half of the genome compared to TCM ($p=0.02$). Unlike other cell subsets, the proportion of full-length proviruses within the TEM subset increased with time on ART ($p=0.03$), with a trend towards an increase in the proportion of proviruses with a 5' deletion ($p=0.08$). As these results suggested a protein encoded within the 3' half of the genome may be responsible for the persistence of full-length proviruses within the TEM subset, we compared the proportion of sequences containing intact ORFs across cell subsets. Compared to TCM and TTM, TEM contained a higher proportion of proviruses with intact ORFs for all viral genes except *gag* ($p<0.0001$ to $p=0.003$) and this proportion increased with time on ART in the TEM subset only ($p=0.007$ to $p=0.04$). We then investigated a role for *nef* in the persistence of intact and full-length proviruses. Compared to TCM and TTM cells, TEM cells contained a higher proportion of gag+nef+ proviruses ($p=0.003$ and $p=0.002$ respectively), but a lower proportion of gag+nef- proviruses ($p=0.002$ and $p=0.009$ respectively), with the proportion of gag+nef+ proviruses increasing with time on ART ($p=0.03$).

Discussion/conclusion: Intact and full-length HIV-1 proviruses are likely maintained in the TEM subset through the expression of *nef* which may protect proviruses expressing immunogenic proteins, such as *gag*, from clearance by the host immune system through downregulation of MHC I. Future studies will aim to elucidate this mechanism *in vitro* through CD8+ T-cell killing assays.

HIV persists preferentially in memory CD4+ T cells that co-express PD1 and CTLA4 and are less inducible

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Introduction
HIV persists on antiretroviral therapy (ART) in long-lived latently infected cells, which constitute the main barrier to a cure. Identifying cellular subsets that preferentially contain HIV DNA and/or harbour persistent expression of HIV RNA is important for curative strategies. We aimed to address the role of the immune checkpoints, programmed cell death 1 (PD1) and cytotoxic T-lymphocyte associated protein 4 (CTLA4) for HIV persistence on ART, as both these receptors can be targeted therapeutically.

Methods
We collected peripheral blood mononuclear cells (PBMCs) and lymph node (LN) mononuclear cells by performing leukapheresis and excisional biopsies of inguinal LNs in people living with HIV (PLWH) on suppressive ART. We then sorted memory CD4+ T cells into four subsets based on their expression of PD1 and CTLA4 to obtain four memory CD4+ T cell subsets defined as: double-positive (PD1+CTLA4+), PD1 single positive (PD1+CTLA4-), CTLA4 single positive (PD1-CTLA4+) and double-negative (CTLA4-). We used flow cytometry on unsorted cells to assess the distribution of PD1/CTLA4 subsets in blood and LN. Within each sorted subset from both blood and LN we then quantified the level of HIV DNA and cell-associated unspliced HIV RNA (CA-US HIV RNA). To measure the frequency of cells containing inducible HIV, we stimulated cells with PMA and ionomycin and quantified the proportion of cells with inducible CA-MS HIV RNA. We tested for differences across all 4 subsets using mixed-effect analysis with the Geisser-Greenhouse correction or Friedman test depending on data distribution. We also performed pairwise comparisons across the individual subsets using paired t-test or Wilcoxon matched-pairs signed rank test, depending on data distribution.

Results
We enrolled 21 PLWH on suppressive ART at clinical trial sites in San Francisco, USA and Melbourne, Australia. We obtained both LN biopsies and leukapheresis samples in 8 participants and leukapheresis only in 13 participants. The frequency of cells co-expressing PD1 and CTLA4 was higher in lymph node tissue compared to blood. We found enrichment of total HIV DNA within memory CD4+ T cells co-expressing PD1 and CTLA4 in blood but not in LN cells. There was no difference across PD1/CTLA4 subsets in the level of CA-US HIV RNA in blood or LN. The frequency of cells containing HIV DNA within PD1/CTLA4 subsets correlated with CD8+ T cell count and CD8+ T cell percentages at study entry. Despite their enrichment for total HIV DNA, a lower proportion of blood memory CD4+ T cells co-expressing CTLA4 and PD1 produced multiply spliced HIV RNA upon stimulation.

Discussion
The frequency of HIV-infected cells was moderately higher in blood memory CD4+ T cells co-expressing PD1 and CTLA4 compared to their double-negative counterpart, but despite this double-positive cell had a lower frequency of inducible virus. This indicates that despite being enriched for HIV, memory CD4+ T cells co-expressing PD1 and CTLA4 are characterised by their negative signalling and a limited susceptibility to induction of latent HIV. Targeting PD-1 and CTLA-4 through immune checkpoint blockade might therefor have a significant impact on latency reversal and virus elimination.

[18]

Microbial function and genital inflammation in young South African women at high risk of HIV infection

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Introduction: Female genital tract (FGT) inflammation is an important risk factor for HIV acquisition. The FGT microbiome is closely associated with inflammatory profile, however, the relative importance of microbial activities has not been established.
Methods: Since proteins are key elements representing actual microbial functions, this study utilized metaproteomics to evaluate the relationship between FGT microbial function and inflammation in 113 young and adolescent South African women at high risk of HIV infection. Women were grouped as having low, medium or high FGT inflammation by K-means clustering according to pro-inflammatory cytokine concentrations.

Results: A total of 3,186 microbial and human proteins were identified in lateral vaginal wall swabs using liquid chromatography-tandem mass spectrometry, while 94 microbial taxa were included in the taxonomic analysis. Both metaproteomics and 16S rRNA gene sequencing analyses showed increased non-optimal bacteria and decreased lactobacilli in women with FGT inflammatory profiles. However, differences in the predicted relative abundance of most bacteria were observed between 16S rRNA and metaproteomics analyses. Bacterial protein functional annotations (gene ontology) predicted inflammatory cytokine profiles more accurately than bacterial relative abundance determined by 16S rRNA gene sequence analysis, as well as functional predictions based on 16S rRNA gene sequencing data ($p<0.0001$). The majority of microbial biological processes were underrepresented in women with high inflammation compared to those with low inflammation, including a *Lactobacillus*-associated signature of reduced cell wall organization and peptidoglycan biosynthesis. This signature remained associated with high FGT inflammation in a subset of 74 women nine weeks later, was upheld after adjusting for *Lactobacillus* relative abundance, and was associated with *in vitro* inflammatory cytokine responses to *Lactobacillus* isolates from the same women.

Discussion: The link between FGT microbial function and local inflammatory responses suggests that both the presence of specific microbial taxa in the FGT and their properties and activities likely play a critical role in modulating inflammation. Our findings support those of previous studies suggesting that peptidoglycan is directly immunosuppressive, and identify a possible avenue for biotherapeutic development to reduce inflammation in the FGT.

Conclusions: The present study contributes to our understanding of the mechanisms by which the microbiota may influence local immunity, and in turn alter the risk of HIV infection.

[19]

Suppression of HIV pseudovirus infectivity by cervicovaginal *Lactobacillus*-conditioned medium

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Introduction: Heterosexual HIV transmission accounts for the majority of incident HIV infections in women. Previous studies have shown that the cervicovaginal microbiome likely plays a major role in HIV acquisition. Non-optimal microbiota is associated with increased risk of HIV infection, while a *Lactobacillus*-dominated microbiota has been linked to reduced susceptibility to HIV acquisition in women. *L. crispatus*, in particular, has been associated with lowest risk of HIV, compared to *L. iners*-dominated and non-optimal microbiota. However, the relative roles of other non-*iners* lactobacilli that are also common in the female genital tract are unknown. The aim of this study was to compare the effects of different cervicovaginal *Lactobacillus* species on HIV pseudovirus infectivity and to evaluate possible underlying mechanisms.

Methods: Vaginal *Lactobacillus* species (n=16), including *L. crispatus* (n=4), *L. jensenii* (n=4), *L. mucosae* (n=4) and *L. vaginalis* (n=4), were isolated from young and adolescent South African women. *Lactobacillus* isolates were standardised to $4.18x10_6$ colony forming units per ml and incubated for 24 hours in de Man Rogosa and Sharpe (MRS) at 37°C under anaerobic conditions. Using a Luciferase Reporter Gene Assay, we evaluated the influence of *Lactobacillus*-conditioned culture medium on HIV infectivity in TZM-bl cells in three independent assays. D- and L-Lactate concentrations were measured using colorimetric kits and total lactic acid was calculated using the Henderson-Hasselbalch equation. Mann Whitney-U test was used for comparisons and Spearman Rank test was used for correlations. P-values were adjusted for multiple comparisons using a false discovery rate step-down procedure and adjusted $p<0.05$ were considered statistically significant.

Results: We showed that conditioned medium from *L. crispatus* (adjusted p=0.0176) and *L. vaginalis* (adjusted p=0.0176) cultures significantly suppressed HIV pseudovirus infectivity in TZM-bl cells. Although both *L. jensenii* and *L. mucosae* also reduced HIV infectivity relative to MRS only controls, these changes were not statistically significant. Strain-level variation in HIV inhibition was substantial, however the two isolates that showed the greatest suppression of HIV infectivity were both *L.crispatus*. HIV infectivity correlated directly with culture pH (rho=0.55; p=0.0283), which in turn correlated inversely with total lactic acid concentrations (rho=-0.84; $p<0.0001$).

Discussion: These findings suggest that *Lactobacillus* species suppress HIV infectivity in a strain-specific manner, with *L.crispatus* isolates showing the greatest inhibition. However, other species were also able to inhibit HIV infectivity. This inhibition was associated with culture acidification, which was largely determined by lactic acid production.

Conclusions: This study suggests that multiple common *Lactobacillus* species have the capacity to suppress HIV infectivity, with *L. crispatus* isolates showing greater inhibition than *L. vaginalis, L. jensenii* and *L. mucosae*.

[20]

Do mutations in Hepatitis B integrations contribute to hepatocellular cancer?

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Introduction: Chronic Hepatitis B infection (CHBV) is a major cause of Hepatocellular cancer (HCC). In CHBV patients, HCC typically occurs late in infection (>50 years). HBV integration into the host genome is associated with HCC, occurring in the majority of tumour cells but only in 1 out of every ~1000 infected liver cells. The mechanism behind this association remains unclear. While insertional mutagenesis & HBx fusion protein expression have been described as possible mechanisms, integrations in tumour samples generally show no specific site preference, thereby they are unlikely to be involved in HCC initiation. Another theory involves the persistent expression of mutant proteins from integrated HBV DNA. Integrations sequenced from HBV patient tumour samples and in the human hepatoma cell line PLC/PRF/5 contain mutations such as duplications, deletions and inversions within the HBsAg PreS1 and PreS2 ORF. The expression of these HBsAg mutants can cause ER stress, a HCC risk factor. Knowing when these mutations occur in CHBV will determine if/how they contribute to HCC. Therefore, this project aims to determine the identity of integrated sequences at different stages during chronic infection. We hypothesise that mutations in the HBsAg are selected for in clonal expansion prior to HCC, therefore will be present in clonally expanded cell populations in both tumour and non-tumour tissue.

Methods: To determine the identity of viral integrations directly post-infection, cell culture models of integration using HuH7-NTCP and HepG2-NTCP cells have been designed. The cells were transfected with a plasmid encoding a CRISPR/Cas9 construct to cleave a safe-harbour site in the host genome and then infected with HBV to allow HBV to integrate at the cellular dsDNA break. Host and virus-specific primers were designed to PCR amplify and sequence integrated HBV DNA forms. To determine the sequences of integrated HBV DNA forms in patient tissues (tumour and non-tumour), inverse nested PCR was used to amplify virus-cell junctions. Using these sequences, primers specific for the host sequence were designed to sequence the entire integrated HBV DNA form.

Results: The CRISPR plasmid with AAVS1 gRNA has been cloned successfully, and results show transfection and HBV infection efficiency is high in HuH7-NTCP and HepG2-NTCP cell lines. Sequencing results from 5 non-tumour samples thus far indicate the ORFs of the S ORF in integrations do not contain stop mutations in the HBsAg ORF, data from the PreS region has not yet been generated. 11 integrations from tumour samples have been detected by inverse-nested PCR. All integrations detected terminate at or slightly upstream of the dslDNA form of HBV, consistent with previous studies showing that it is the substrate for integration.

Discussion:
This study represents the first characterization of HBV integration sequences in non-tumour tissue and at time points prior to carcinogenesis. If the sequenced HBV integrations indicate a potential role in HCC, this suggests removing HBV integrations is necessary for HBV treatment to reduce HBV-HCC (a major cause of mortality in CHBV patients). If our preliminary results are indicative of a broader pattern, then integrated surface antigen mutations may only be present in tumour samples, indicating a possible direct oncogenic role.

Conclusions:
Integrations have been identified in tumour and non-tumour samples and a model for sequencing integrations in early infection has been established. If results indicate an oncogenic role, this may inform clinical treatment of HBV and HBVrelated HCC. For example, silencing RNA could be designed to directly target and stop production of integrated mutants, or drugs to target the ER stress pathway could reduce the carcinogenic potential of these mutants.
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Covalently closed circular DNA in Hepatitis B infected cells is not efficiently eliminated by targeting capsid formation

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Introduction: Hepatitis B virus (HBV) infection causes the majority of liver cancer cases worldwide. The infection cannot be cured by current therapeutics due to the persistence of covalently closed circular (ccc)DNA in the liver of infected individuals. It is unclear whether cccDNA persistence during antiviral therapy is 1) due to inefficient targeting of viral replication (e.g. by current generation nucleoside analogue therapy) or 2) due to a very low turnover rate of cccDNA. We aimed to answer this question by precisely measuring cccDNA using a novel PCR method in cell culture models infected with wild-type or replication-incompetent HBV.

Methods: We developed a novel assay called cccDNA inversion quantitative (cinq)PCR to specifically quantify cccDNA. The cinqPCR assay gives values consistent to previous approaches, but importantly allows normalisation to cellular DNA and therefore dramatically improves precision. We also engineered a replication-deficient HBV mutant containing a genome with a stop codon at position T67 in the HBV core ORF (ΔHBc HBV). This virus is packaged with a nucleocapsid composed of wild-type HBV core protein, but is does not produce nucleocapsids.

Results: Infection of HepG2-NTCP cells with ΔHBc and WT HBV resulted in comparable secretion of HBV surface antigen (HBsAg), suggesting bona fide cccDNA formation and transcription. Immunofluorescence for HBsAg also showed that ΔHBc and WT HBV were equally infectious. Using cinqPCR, we found that almost identical numbers of cccDNA were formed by equivalent inoculating doses of WT and ΔHBc HBV in cell lines (Huh7-NTCP, HepG2-NTCP, and HepaRG-NTCP) and primary human hepatocytes. Levels of cccDNA were stably maintained for WT and ΔHBc HBV for at least 9 weeks post-infection.

Discussion: We show that the reverse transcription step of HBV replication is not required to maintain cccDNA levels in infected cells. This suggests that no matter how well reverse transcription is suppressed (e.g. by current nucleoside analogues), cccDNA levels would not be reduced.

Conclusion: Thus, a multi-pronged approach targeting several factors (e.g. viral replication, virus entry and antiviral immune responses) is necessary to induce cure of chronic HBV infection.

[22]

Analysing the effects of HBV splice variants on wildtype HBV replication

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Introduction: Chronic hepatitis B virus (HBV) affects over 257 million people worldwide and an infection can lead to liver cirrhosis and hepatocellular carcinoma (HCC). Upon infection, the relaxed circular DNA (rcDNA) genome is released into the nucleus where it is converted into a covalently closed circular DNA (cccDNA) minichromosome. This cccDNA is then transcribed into pregenomic RNA (pgRNA) which is normally packaged into core particles in an unspliced form, and reverse transcribed by the viral polymerase to from rcDNA. However, prior to nuclear export, the pregenomic RNA can be spliced by the host cell spliceosome to form shorter RNA sequences, known as splice variants. This splicing is not essential for HBV replication, however their role still remains largely unknown. To date, 21 splice variants have been identified and an increased proportion of splice variants in patient sera has been associated with the development of HCC. However, it is unclear whether the production of splice variants increases the risk of HCC development or whether splice variants are produced as a by-product of HCC. Furthermore, different splice variants have been shown to have different effects on wildtype replication. Sp1, the most common splice variant, has been shown to decrease wildtype replication by producing a protein derived from the precore protein that interferes with capsid formation. Sp10 and Sp13 have also been shown to decrease wildtype replication, whereas Sp7 has been shown to increase replication. It remains unknown how other common splice variants, Sp3 and Sp9, affect wildtype replication.

Methods: 1.3mer Sp3 and Sp9 DNA clones will be co-transfected with a wildtype 1.3mer HBV DNA clone in Huh7 and HepG2 hepatoma cells and the replication phenotype of wildtype HBV will be analysed. Intracellular and extracellular DNA will be measured via densitometry of Southern blotting on core-associated DNA and immunoprecipitated virions respectively to determine the effect on wildtype DNA production. Intracellular and extracellular E and S antigen will be measured via the Elecsys quantitative assay. Intracellular core, S and X protein production will be measured via western blotting. Intracellular RNA expression will be measured via northern blotting and RT-qPCR. Cell viability assays will also be performed to determine whether Sp3 or Sp9 has any apoptotic or cytopathic effect.

Results: Determining the effects of Sp3 and Sp9 on wildtype replication will provide new insights into the roles that splice variants play during HBV pathogenesis. Even though splice variants are not essential for replication, their presence may influence the rate of replication as a mechanism to promote viral persistence or evade the host cell immune response.

Conclusion: Different splice variants may have different effects on wildtype replication to promote HBV pathogenesis.

The role of host factor TM6SF2 in HDV secretion: A potential therapeutic target

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Introduction: Hepatitis D virus (HDV) is a satellite virus that is dependent on co-infection with Hepatitis B virus to propagate. Chronic Hepatitis D leads to cirrhosis in around 80% of individuals within 5-10 years and has a mortality rate of 20% among infected patients, the highest of all hepatitis viruses, yet there are no effective treatments. Our group has shown that Transmembrane 6 superfamily 2 (*TM6SF2*) plays a role in HBV replication; lower levels of TM6SF2 are associated with lower HBsAg secretion during HBV infection in both patients and *in vitro* models. As HDV uses the same secretion pathway as HBsAg through the ER and Golgi, we hypothesise that inhibiting TM6SF2 will reduce trafficking and secretion of HDV.

Methods: Two hepatoma cell lines were used to model HDV infection. Huh7-END cells stably express the NTCP entry receptor, HBsAg and the HDV genome, secreting HDV virions continuously. HepG2-NB2.7 cells express NTCP and HBsAg, so are susceptible to HDV infection. HepG2-NB2.7 were infected with the supernatant of Huh7-END cells to model true HDV infection. As Huh7-END cells express high levels of TM6SF2, siRNA was used to knock-down the gene. As HepG2-NB2.7 cells have low TM6SF2 expression, they were transfected with a plasmid expressing TM6SF2-GFP fusion proteins. A siRNA-resistant TM6SF2-GFP was designed with silent mutations at the siRNA binding site, to ensure knock-down specificity. RT-qPCR and western blotting was used to confirm TM6SF2 knock-down. To measure the trafficking of viral proteins, confocal microscopy was performed. Anti-calnexin, anti-HBsAg and anti-delta antigen antibodies and fluorophore-conjugated secondary antibodies were used to visualise the ER, HBV and HDV antigens respectively. Secreted HBsAg was measured by ELISA, and secreted HDV RNA was measured by RT-qPCR.

Results: siRNA treatment of Huh7-END cells reduced *TM6SF2* mRNA expression by 65%, which we confirmed at the protein level by western blot. Consistent with our hypothesis, *TM6SF2* knockdown caused a 2-fold reduction in secreted HBsAg. For ongoing experiments, immunofluorescent (IF) labelling and confocal microscopy have been optimised to visualise delta antigen, HBsAg and TM6SF2-GFP in the ER. *TM6SF2* over-expression in HepG2-NB2.7 cells has been improved 5-fold using a HepG2-specific transfection reagent. The silencing resistant TM6SF2-GFP construct has been successfully produced and is detectable by IF, qPCR and western blot.

Discussion: Here we reveal the relationship between HDV and the host factor *TM6SF2*. Our results suggest secretion of HBsAg is facilitated by *TM6SF2*, most likely through ER-mediated trafficking. HBsAg production and secretion are essential for HDV virion budding and replication. If *TM6SF2* is required for HBsAg secretion and trafficking of other HDV proteins, which we are confirming by colocalisation microscopy, it provides a novel host target to inhibit HDV secretion. *TM6SF2* knock-down *in vitro* mimics patients with the E167K variant, who have reduced TM6SF2 protein in the liver and lower levels of HBsAg in serum. This suggests that *TM6SF2* can be targeted to reduce HDV production in humans. Previous *in vitro* models have been limited to co-transfection of HBV and HDV plasmids, so exploring the effect of inhibiting host *TM6SF2* in a true HDV infection model will be more insightful and yield translatable results.

Conclusions: Our *in vitro* data show that targeting *TM6SF2* leads to reduced secretion of HBsAg. HBsAg is a clinically relevant marker for patients with HBV and HDV infection, so reducing HBsAg secretion by targeting *TM6SF2* provides a novel strategy for drug development to treat hepatitis D. This approach is consistent with global efforts to target HBsAg secretion to achieve a functional cure.

[24]

Assessing the immunogenicity of chimeric HBeAg-epitope bio-nanoparticles.

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Introduction: Chronic hepatitis B (CHB) contributes to more than 880,000 deaths each year, and despite existing prophylactic and therapeutic advances, we are still lacking a cure. An important but often overlooked viral protein, the hepatitis B e antigen (HBeAg) is a non-particulate, secreted viral component, essential for initial establishment of CHB by modulating the host's innate and adaptive immune responses. HBeAg seroconversion in CHB patients is a current treatment endpoint and a preceding step for hepatitis B surface antigen (HBsAg) seroclearance, also termed functional cure. However, spontaneous or treatment induced HBeAg seroconversion is generally associated with progression to later phases of CHB infection (HBeAg negative phases) and is accompanied by the gradual emergence of precore and basal core promoter mutations that prevent further HBeAg production. The prevalence of functional cure in these patients is extremely low. This project utilises HBeAg epitope expressing bio-nanoparticles (BNPs) aiming to specifically program the immune responses to detect and eliminate HBeAg at earlier phases of infection, as a groundwork step towards a potential curative approach.

Methods: Our BNPs are subviral particles composed of the HBsAg, which also serves as a structural component of the HBV outer envelope. BNPs are excellent vaccine candidates, as they are non-infectious, easy to modify and provide high antigenic density of exposed epitopes. Thus far, we have performed extensive biochemical design and characterisation of BNPs expressing different HBeAg epitopes and have investigated their immunogenicity. Firstly, we have bioengineered and cultured BNPs with variable HBeAg exposed epitopes, and assessed their ability to assemble, secrete and retain their antigenic capabilities. Next, we have immunised BALB/c mice subcutaneously and assessed BNP immunogenicity against the HBsAg and HBeAg proteins.

Results: In this project, HBeAg-epitope BNPs have been antigenically recognised *in vitro* via a panel of well characterised antibodies, and their assembly and secretion competency has been assessed via electron microscopy. Thereafter, the detection of antibodies against HBsAg in immunised BALB/c mice sera has demonstrated that chimeric BNPs still induce an anti-HBs antibody response, albeit slightly decreased compared to wt BNP. In addition, BALB/c mice immunised with chimeric HBeAg-epitope BNPs have also been able to produce antibodies against HBeAg. This is an important first step, prior to utilising chimeric BNPs in a CHB mouse model to investigate their ability to stimulate immune responses against HBeAg clearance.

Discussion: The immune factors and antibodies contributing to HBeAg seroconversion in CHB patients remain unclear. This approach is the first to describe assembly competent HBeAg-epitope BNPs, immunogenic against both the native HBsAg and HBeAg. BNPs are safe biomolecules with great bioengineering potential that enable the expression of inserted epitopes at high density in order to stimulate specific immune responses. Chimeric HBeAg-epitope BNPs could potentially trigger a targeted immune response against the native HBeAg that leads to the 'breaking' of immune tolerance and ultimately increased HBeAg and HBsAg seroclearance rates to CHB patients that currently do not respond to therapy.

Conclusions: With HBV being one of the deadliest viruses of this century and 257 million people worldwide still chronically infected, there is an urgent need to develop new therapeutic approaches towards HBV cure. Novel experimental approaches, such as bioengineering of BNP platforms for the delivery of medically relevant antigenic sequences, have shown great promise as a way of programming the immune system against pathogens that have otherwise developed mechanisms to avoid clearance. The successful production of HBeAg-epitope BNPs in this project forms the basis of initiating a new experimental approach to target HBeAg and ultimately promote HBsAg seroclearance and functional cure.

Developing a functional cure for chronic Hepatitis B by targeting human transmembrane 6 superfamily member 2 (*TM6SF2*)

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Introduction: Despite the availability of a vaccine against the Hepatitis B virus (HBV) and treatments that suppress virus replication, currently there is no cure. A 'functional cure' (defined as the sustained clearance of circulating HBV surface antigen, HBsAg) is considered the most achievable chronic Hepatitis B (CHB) endpoint and is the international consensus endpoint for most treatment trials. The human gene transmembrane 6 superfamily member 2 (*TM6SF2*) variant rs58542926 (E167K mutation) is a risk factor for fatty liver disease and we have shown previously that it also correlates with HBV viral load in people with CHB. *TM6SF2* plays a role in lipoprotein secretion through the endoplasmic reticulum (ER)/Golgi, the pathway used by HBV for HBsAg secretion. Therefore we decided to study the effect of targeting *TM6SF2* on HBV replication (particularly HBsAg secretion) as a novel strategy to induce functional cure.

Methods: To assess the effect of natural *TM6SF2* polymorphisms on HBV in humans, circulating HBsAg from 65 CHB patients was measured and the effect of *TM6SF2* polymorphism was analysed. To determine the role of *TM6SF2* in HBV expression, *TM6SF2* was knocked down in Huh7 cells followed by transient transfection of an over-length HBV construct. HBsAg secretion and expression were measured by ELISA and Western Blot.

Results: HBV patients with the CT/TT genotype of *TM6SF2* (an unstable variant causing E167K mutation and a decrease in liver protein levels) had ~25% less circulating HBsAg than the CC genotype. siRNA knock-down of *TM6SF2* in Huh7 cells achieved a 75% reduction in *TM6SF2* mRNA (compared to the non-targeting control) which induced a 50% decline in secreted HBsAg.

Discussion: *TM6SF2* plays a role in hepatic cholesterol metabolism, particularly lipoprotein secretion through ER/Golgi. HBsAg particles are also secreted through the ER/Golgi pathway, likely explaining the effect of *TM6SF2* knock-down on HBV secretion. However, the detailed mechanisms on the inhibition of HBsAg secretion needs further investigation and is the focus of ongoing studies.

Conclusions: We have shown that *TM6SF2* affects secretion of HBsAg both in HBV patients and *in vitro*. HBsAg is a potent suppressor of host antiviral immunity, so targeting host *TM6SF2* could be a novel approach to reduce HBsAg secretion and facilitate functional cure of chronic HBV infection.

Validation of a novel rapid point-of-care ALT test in patients with viral hepatitis

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Introduction: Alanine aminotransferase (ALT) level is an important marker of liver inflammation. Limited access to laboratory resources makes ALT measurement challenging in low resource and remote regions. ALT1 protein antigen is cleared slowly from plasma than enzymatic ALT and is more stable at ambient temperature when stored for long periods outside cold chain. We validated a novel point-of-care (POC) ALT1 test in patients with viral hepatitis.

Methods: The BioPoint® POC ALT1 test is an antigen immunoassay-based lateral flow test, which uses 40µL whole blood or 15µL plasma to provide ALT measurement two ways: 1. quantitative ALT result (Axxin handheld lateral flow reader) or 2. visual semi-quantitative result (cutoff 40IU/mL), within 20 minutes. Quantitative POC ALT1 results were compared to standard laboratory assay ALT using Spearman correlation. A linear regression model was used to derive comparable values from POC ALT1 results (Axxin reader, arbitrary units) to standard ALT. Accuracy of POC ALT1 to detect ALT > upper limit of normal (ULN) was calculated by ROC analysis and agreement determined by Bland-Altman plot.

Results: 240 patients were included: 74 (31%) with hepatitis B and 166 (69%) with hepatitis C. 168 (70%) were male, mean age was 39 +/- 7.9 years and 18 (11.5%) had cirrhosis. Median ALT was 32 IU/mL (IQR 19-50). Quantitative (Axxin Reader): There was moderate correlation between quantitative POC ALT1 results and ALT measured by laboratory assay (R_2=0.68; p<0.0001). Derived POC ALT1 values had excellent accuracy for laboratorybased ALT > ULN in males and females. Bland-Altman plot showed a small mean difference 0.039 (95% CI -4.54-4.62) and significant variance difference (Pitman's test <0.0001).

Derived quantitative ALT results (Axxin reader)	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV
ALT > ULN*					
All	0.927 (95% CI 0.901-0.953)	92%	71%	80%	88%
Men	0.909 (95% CI 0.871-0.946)	92%	74%	80%	89%
Women	0.916 (95% CI 0.862-0.971)	93%	62%	79%	86%
ALT > 2 x ULN*					
All	0.927 (95% CI 0.901-0.953)	87%	85%	69%	95%
Men	0.923 (95% CI 0.895-0.959)				
Women	0.955 (95% CI 0.917-0.992)				
ALT > 40IU/mL**	0.929 (95% CI 0.901-0.956)	91%	84%	77%	93%

**ULN men 30IU/mL, women 19IU/mL; AASLD guidelines*
***ULN 40IU/mL; EASL 2017 guidelines*
Semi-quantitative (visual read): Visual POC ALT1 results (cutoff 40IU/mL) had good accuracy for assay measured ALT > 40IU/mL (sensitivity 80%, specificity 82%, PPV 73%, NPV 87%).

Discussion: The BioPointâ POC ALT1 test had excellent correlation with laboratory assay ALT using the quantitative hand reader., while the visual read semi-quantitative test had good accuracy for identifying hep B patients with ALT levels greater than 40IU/L. Importantly, the sensitivity and negative predictive value were high, ensuring significant liver inflammation was detected.

Conclusion: The BioPointâ POC ALT1 test had good correlation with laboratory assay ALT and good accuracy for identifying hep B patients with ALT levels > ULN using AASLD and EASL criteria. Further validation of the POC ALT1 test in prospective clinical trials is needed.

[27]

Validation of a novel point-of-care ALT test to determine treatment eligibility in hepatitis B patients: a pilot cohort study

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Introduction: Hepatitis B guidelines determine treatment eligibility by blood tests for inflammation (ALT), HBeAg and HBV viral load, however these tests are costly and difficult to access in low resource settings. The TREAT-B algorithm(1) was developed to determine treatment eligibility in low-resource settings using ALT and HBeAg only and is validated against EASL criteria. Our aim was to validate a novel point of care (POC) ALT test and commercial POC HBeAg test against standard laboratory assays for determining hepatitis B treatment eligibility by 1. EASL 2017 guidelines and 2. the TREAT B algorithm.

Methods: The BioPointR POC ALT1 test is an antigen immunoassay-based lateral flow test, which uses 40µL whole blood or 15µL plasma to provide ALT measurement within 20 minutes. Stored plasma samples from hepatitis B patients were added to POC test cartridges (BioPointR ALT1 test and AlereR HBeAg test). Clinical data was recorded concurrently with sample collection. TREAT-B criteria for nucleos(t)ide analogue treatment are 1. HBeAg +ve and ALT >=20IU/L, or 2. HBeAg -ve and ALT>=40IU/L. EASL 2017 treatment criteria are 1. Cirrhosis and detectable HBV DNA; 2. ALT >80IU/L and HBV DNA > 20,000IU/mL; 3. HBV DNA > 2000IU/mL, ALT > 40IU/L and/ or > F2 stage fibrosis; and 4. HBeAg +ve, HBV DNA > 2000IU/mL and Age > 30 years. We determined the sensitivity, specificity, PPV and NPV of the POC ALT and POC HBeAg tests compared to standard laboratory ALT and HBeAg assays for determining treatment eligibility per EASL 2017 guidelines (combined with HBV DNA level and Fibroscanâ) and per the TREAT B algorithm (based on ALT and HBeAg only).

Results: 77 hepatitis B patients were recruited. 68% were Asian, 61% were male and the median age was 47 +/- 15 years. 23% were HBeAg positive, median ALT level was 32IU/mL (IQR 21-48IU/mL) and median viral load was low (531 IU/mL, IQR 24-57,400IU/mL). Median FibroscanR result was 5.7kPa (IQR 4.5-6.3); 15% had cirrhosis. The POC ALT1 test had excellent accuracy for ALT > 40IU/mL (AUROC 0.92 95% CI 0.84-0.99, sensitivity 75%, specificity 97%, PPV 95%, NPV 84%). The POC HBeAg test had poor sensitivity (55%) and high specificity (100%) for HBeAg detection (PPV 100%, NPV 89%). A subset of 59 treatment-naive hepatitis B patients were used to determine accuracy of treatment eligibility using POC ALT1 and POC HBeAg tests. The TREAT-B algorithm had 77% sensitivity and 64% specificity for EASL 2017 treatment criteria. POC ALT1 and POC HBeAg tests combined with HBV DNA levels and Fibroscanâ had 100% sensitivity, 78% specificity and 100% NPV for EASL 2017 treatment criteria compared to standard assays. POC ALT1 and POC HBeAg tests had 81% sensitivity, 84% specifity, PPV 81% and NPV 84% compared with laboratory assays for treatment eligibility by the TREAT-B algorithm based on ALT and HBeAg alone.

Discussion: Compared with standard laboratory assay, the POC ALT1 test had excellent accuracy for laboratory ALT. Accuracy of the POC ALT and POC HBeAg tests combined for determining treatment eligibility by EASL guidelines and TREAT-B algorithm was good, but limited by low accuracy of the POC HBeAg test.

Conclusion: The POC ALT test combined with POC HBeAg test had moderate accuracy for determining EASL 2017 hepatitis B treatment eligibility when used with the TREAT B algorithm. Further prospective trials are needed to validate use and cost-effectiveness of the POC ALT1 test to manage hepatitis B.

Reference: Shimakawa Y, Njie R, Ndow G, et al. J Hepatol 2018; 69(4): 776-84.

Development of a point-of-care assay for quantification of HBV

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Background: Hepatitis B virus (HBV) infection results in cirrhosis and development of hepatocellular carcinoma in up to 50% of infected people. Timely treatment can reduce the progression to cirrhosis and the risk of HCC. While treatments are gradually reducing in price and becoming increasingly accessible, identification of treatment-eligible patients remains a major challenge. The EASL guidelines for commencing treatment for HBV require the detection of persistently elevated ALT and elevated viral DNA titres. Thus optimal treatment eligibility screening still depends on access to expensive and specialized quantitative laboratory assays. A point-of-care (POC) test for HBV treatment eligibility could provide a cheaper, more readily available assay. The Burnet Institute and Nanjing BioPoint Diagnostics have developed a POC test for ALT1 that is currently under evaluation for clinical use, however determining HBV DNA titre at POC remains a challenge. The HBV capsid is formed by core protein (HBcAg) in a predictable relationship with viral DNA, with an estimated 180 copies of HBcAg per genome. Commercial ELISA for HBV core-related antigen (HBcrAg) are available, however, detection depends on heat and chemical denaturation of competing patient antibody along with the HBcAg, which precludes its adaptation for POC tests.

Method/Results: Our approach to developing a POC test involves a three step process to (1) *capture* HBV, (2) *lysis* to release HBcAg and (3) *detection* of HBcAg using lateral flow to wash away patient antibodies. We are developing these three steps in a deconstructed manner. *Capture:* ELISA plates were coated with anti-HBs, and then incubated with HBV in human serum, before washing. Captured virus was quantified by PCR, revealing that our system could capture 100% of HBV. Binding of HBV to anti-HBs was reduced in serum and plasma compared with virus in buffer, suggesting interference by plasma proteins. While this was readily overcome by dilution, it implies immediate reduction in the sensitivity of a proposed test. *Lysis*: We aimed to develop a lysis buffer that could release the viral capsid from its outer membrane without degradation of HBcAg immune reactivity. Candidate buffers were initially screened by incubation with recombinant HBcAg and detected by ELISA. Successful buffers were then selected for trial with serum HBV, with the captured virus lysed then layered onto anti-HBc. The methodology developed has streamlined the screening of buffers, and further work is need to achieve lysis of HBV while retaining HBcAg detection. *Detection:* Antibodies were screened for binding and detection of HBcAg utilising both lateral flow and ELISA before trialling with plasma and serum. Plasma proteins bound non-specifically to several of the screened antibodies, but a successful pair were identified that could capture and detect HBcAg by both lateral flow and ELISA. Further work to improve sensitivity of detection is needed to achieve clinical utility.

Conclusion: Development of a lateral flow POC test to replace HBV DNA quantification is a challenging process with the currently available technologies. We have developed several key methodologies using a deconstructed approach that permits optimisation of each step of viral capture, lysis and detection independently. Further work is needed before moving to a lateral flow platform.

Assessing HBV Infection and Application Using LGR5+ Human Liver Organoids Culture

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Background: HBV *in vitro* model systems in general do not recapitulate the complexity and individuality of HBV infection *in vivo*, thus limiting their application in predicting clinical response. The LGR5+ human liver organoid culture system developed by *Huch* and colleagues demonstrate mature hepatocyte function and long-term expansion capability. Most importantly, organoids retain the genetic background of the donor with inter-individual variability. We aim to use this physiological relevant culture system as a model to study the HBV life cycle, cellular response and its application for antiviral testing.

Methods: Human liver organoids were generated from liver resections or core biopsies and LGR5+ stem cells were selectively expanded in 3D culture. Hepatocyte maturation was assessed using qRT-PCR, RNA-Seq, confocal and electron microscopy throughout differentiation to assess for metabolic markers (albumin, CYP2C9, CYP3A4), HBV receptor (NTCP) expression and morphologic changes. HBV infection was performed using HepAD38 cell or human plasma-derived HBV with/without antiviral treatment followed by immunofluorescence staining of HBcAg/HBsAg and qRT-PCR for HBV RNA at 12 dpi.

Results: Following differentiation, organoids demonstrated an increase in albumin, CYP2C9, CYP3A4 and reduction in LGR5 mRNA expression (qRT-PCR, confocal microscopy). Electron microscopy confirmed presence of tight junctions and bile canaliculi while functional NTCP expression was only detected following differentiation. Individual differences in the expression of metabolic markers and NTCP were evident by RNA-Seq. HBV infection in these differentiated organoids was demonstrated using confocal microscopy and an increase in HBV RNA. Treatment of liver organoids with Myrcludex resulted in reduction in infection whereas the opposite was seen with JAK-1/2 inhibitor treatment. We also assed the innate immune competency of these organoids and revealed they respond to both IFN-□ and poly I:C indicating their usefulness in assessing the innate response to viral infection.

Conclusions: We have demonstrated that LGR5+ liver organoids can express mature hepatocyte function, express functional NTCP expression following differentiation and respond to IFN-a and the viral RNA mimic poly I:C. They are permissive to HBV infection from both cell culture and human plasma derived HBV, thus showing their potential utility as a personalised antiviral testing platform.

[30]

Donor-derived liver organoids as a model of hepatitis B virus infection.

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Introduction: Physiologically relevant human models of hepatitis B infection are required to test novel antiviral candidates. Our aim is to establish 3D human liver organoids with host/viral markers of infection, as a potential model of chronic hepatitis B (CHB) that can be used to test novel antiviral candidates.

Methods: Patients with CHB-hepatocellular carcinoma (HCC) undergoing liver resection have been recruited to the study. Distal liver (>2 cm from the tumour margin) were collected and cells were enriched in vitro to establish liver organoids. Immunofluorescence was used to detect hepatitis B surface antigen (HBsAg). Quantitative (q)PCR assays were used to measure HBV DNA, covalently closed circular (ccc)DNA and envelope/core transcripts as a measure of HBV integration into the host DNA.

Results: Non-tumour liver specimens were collected from 7 donors with CHB-HCC. One donor was co-infected with hepatitis C virus (HCV). The liver organoids were derived from all 7 patients, with a success rate of 100%. Primary liver tissue was positive for markers of infection including HBsAg (n=5), expression of HBV DNA and cccDNA (n=2). In contrast, staining of CHB-liver organoids for HBsAg was inconclusive (n=2), the liver organoids were negative for HBV DNA and cccDNA expression (n=2). Liver organoids derived from the liver tissue of one donor with CHB-HCC, did not express HBV integration markers.

Discussion: These preliminary data suggest that liver organoids can be successfully generated from CHB-infected donor liver specimens. Further work is required to show organoids express HBsAg or enable HBV replication.

Conclusions: The generation of CHB-liver organoids requires further refinement of the enrichment and culture protocols.

Validation of functional assays to measure the activity of immunotherapeutic antivirals.

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Introduction: Natural killer (NK) cells may have both a protective (anti-viral) and damaging (hepatic injury) role in the liver of chronic hepatitis B (CHB) patients. The aim of this study is to validate cytotoxicity assays using model cell lines that can then be applied to CHB liver organoids to determine their utility in the discovery of novel antivirals including immunotherapies.

Methods: Target (T) cell lines (K562, HepG2) or human CHB liver organoids were co-cultured with NK-92 effector (E) cells at different E:T ratios, in a 96-well format. After 24 h, the co-culture supernatant was harvested and tested for the release of tumour necrosis factor (TNF) using ELISA. The co-cultures were treated with cell viability reagents and measured using chemiluminescence or fluorescence.

Results: In a co-culture with an E:T ratio of 10:1, NK-92 cells reduced the viability of K562 cells by ~60%. In contrast, HepG2 cultures were resistant to NK-92 induced cytotoxicity, at E:T ratios of 3:1, 10:1, 30:1 and 100:1.

Discussion: The resistance of HepG2 cultures to NK-92-induced cytotoxicity has been reported previously in the literature. The HepG2 cell line may serve as a good model to test immunomodulatory drugs that restore NK-92 activity against immunosuppressive target cells. Additional studies are required to validate the cytotoxicity of NK-92 towards target cell lines in the presence of immunomodulatory therapies such as checkpoint inhibitors.

Conclusions: Validation of assays that measure the function of cytotoxic immune cells towards target cell lines is required before they can be applied to CHB organoids to allow for the discovery of individualised therapies.

[32]

Refinement of culture conditions to improve the growth of donor-derived hepatocellular carcinoma tumouroids.

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Introduction: Chronic viral infection of the liver increases the risk of hepatocellular carcinoma (HCC). HCC has an increasing incidence, is associated with a poor prognosis and is one of the five most common malignancies worldwide. Immunotherapies are being trialed in HCC to reverse immune tolerance towards tumour cells and stimulate the innate and adaptive immune response to destroy the tumour, leading to a decrease in tumour growth. Our aim is to establish 3D human liver tumouroids, as a potential model of HCC that can be used to test novel immunotherapies in vitro.

Methods: Patients with viral/non-viral-HCC undergoing liver resection have been recruited to the study. Distal liver (>2 cm from the tumour margin) and HCC biopsies were collected and cells were enriched in vitro to establish liver/tumour organoids.

Results: Tumour and surrounding non-tumour liver were collected from 23 patients with HCC. The underlying causes of HCC included CHB (n=7), one of which was also co-infected with hepatitis C virus (HCV), HCV-HCC including cured HCV cases (n=10) and non-viral HCC (n=6). Two HCC patients received transarterial chemoemobilisation 3 months prior to resection and no tumouroids could be grown from the necrotic tissue. Of the 21 remaining patients, tumouroids could be generated from 10 specimens. This gives a success rate of ~47%. In contrast, "non-tumour" liver organoids were derived from all 23 patients, with a success rate of 100%.

Discussion: The success rate for generating HCC-donor tumouroids has been reported to be ~26%. Our higher tumouroid growth rate (47%) may result from setting up the cultures in 3 media, including classical-isolation, tumourisolation and the newly described human hepatocyte medium. In addition, we have moved to culturing liver organoids and tumouroids in a hypoxic chamber with 5% O_2 instead of 19% O_2, which may better mimic the liver tumour environment, where <1% O_2 has been observed.

Conclusions: These preliminary data suggest that both liver and tumour organoids can be successfully generated from HCC donor tissue. The generation of tumouroids requires further refinement of the enrichment and culture protocols and validation using functional assays.

Characterisation of hepatitis B virus spliced variants in the sera of individuals across different genotypes in three phases of chronic infection.

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Introduction: The hepatitis B virus (HBV) exists as several genotypes. The most commonly studied genotypes A to D, have distinct geographical prevalence and influence patients' progression through chronic infection. The natural history of chronic hepatitis B (CHB) infection can be broadly classified into five phases: (i) HBeAg- positive chronic infection (Phase I), (ii) HBeAg- positive chronic hepatitis B (Phase II), (iii) HBeAg- negative chronic infection (Phase III), (iv) HBeAg-negative chronic hepatitis B (Phase IV) and (v) HBsAg- negative phase (Phase V).

HBV is a DNA virus that replicates via an RNA intermediate. This pregenomic RNA transcript can be alternatively spliced to generate a range of different splice variants. Although not necessary for viral replication, splice variants are frequently found in the serum of CHB patients. An increased proportion of splice variants has been associated with severe liver disease and increased risk of developing hepatocellular carcinoma. However, the role of splice variants in chronic infection remains largely unclear. The aim of this project was to analyse the changes in the types and frequency of splice variants throughout Phases I, II and IV of CHB infection, with a focus on variations across genotypes A to D.

Method: Whole genome amplification was carried out on serum samples collected from patients in Phase I, II and IV of CHB infection. PCR products were pooled for high throughput sequencing using the Illumina MiSeq platform. Novel bioinformatics methods using an in-house NGS processing pipeline were employed to identify known splice variants in each NGS dataset. The number of reads per patient for each characterised splice variant was determined for each phase of natural history across multiple HBV genotypes.

Results: Five previously characterised splice variants were identified, Sp1, Sp3, Sp5, Sp9 and Sp13, with Sp1 the most common, followed by Sp13. Diversity was greatest in the Asian genotypes B and C, with all 5 splice variants identified, and lowest in genotype D with only 2 splice variants identified. Overall, splice variants were most frequently detected in Phase II, the immune active phase, and were present at lower levels in phases I and IV. The difference between phases I and II was most profound for variants Sp1 and Sp13 for Asian genotypes B and/or C.

Discussion: Our findings indicate the types and frequency of splice variants changes across CHB natural history, and that patterns of splicing are unique to each genotype. The diversity and levels of splice variants is greatest amongst patients in Phase II, where immune activation is induced. Low levels of splice variants were observed in phase I, the so-called immune tolerant phase, despite high HBV viral loads. In particular, the major splice variant Sp1 was more abundant in patients in Phase II than Phase I, highlighting a potential role of Sp1 in the onset of severe infection. Increased levels of splicing were observed in genotype C, which is associated with more serious pathology than other HBV genotypes. Taken together, our findings suggest a previously unidentified association between HBV splicing and immune responses, perhaps facilitating viral persistence, that warrants further investigation. Difference in the level of splice variants across genotypes suggests genotype- dependent regulation of splicing, utilising an as yet unidentified mechanism

Conclusions: The results of our study suggest an association between splice variants, genotypes and CHB natural history, which may provide insights into the relevance of splice variants in the progression of CHB infection.

HBV STOP Study – Large HBV Flares off NA Therapy cause Large Innate Immune Response

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Introduction*:* Current guidelines recommend indefinite Nucleot(s)ide Analogue (NA) therapy for patients with HBeAgnegative CHB. The HBV-STOP study is a prospective multi-centre study of NA discontinuation in patients who have achieved long-term virological suppression on treatment. We aim to detail the immunological response post treatment cessation, especially that of the innate immune system.

Methods*:* (1) *Stimulation Study:* To gauge the potential innate activation of the peripheral blood cells by the flare we stimulated PBMC samples of HBV-STOP study patients by TLR-specific ligands (TLR-2, TLR-3, TLR-4, TLR-7/8 & TLR-9) ex vivo. These patients either had a large biochemical flare (ALT > 10x ULN) or did not have a biochemical flare (ALT < ULN). PBMCs were tested at baseline and peak ALT time points and matched non-flare samples were used as controls. Cytokine levels (IL-6, IL-8, IL-10, TNF, CCL-2 & CXCL-10) were measured after PBMCs stimulation. (2) *Flow Cytometry Study:* Additionally, we performed flow cytometry on PBMC samples of HBV-STOP study patients who either had a large biochemical flare (ALT > 10x ULN) or did not have a biochemical flare. We did these studies at baseline & peak ALT time points. Non-flare samples were matched to the closest respective week. Flow cytometry was used to isolate specific NK cell & Monocyte populations using CD 56, CD 3, CD 16 & CD 14 cell surface markers. Expression of various immune markers were assessed in each cell population, including NKP46, NKG2D, TLR2, TLR4 & TREM1.

Results*:* The stimulation study cohort consisted of 13 flare patients and 12 non-flare patients. 8 of these flare patients and 6 of these non-flare patients were also used in the flow cytometry study.
(1) *Stimulation Study:* Comparisons between ratios of change from baseline to peak ALT samples with baseline to baseline samples were made for "Large Flare" & "non-Flare" subjects. Large flare values were superior to all non-flare values, except for CXCL-10 (TLR9). Large Flare ratios were all > 1, except for CXCL-10 (TLR9), CXCL-10 (TLR3) & unstimulated samples. Comparisons between Large Flare ratios had p-values < 0.05, except for IL-8 (TLR4), IL-10 (TLR4), TNF (TLR3), CXCL-10 (TLR3, TLR 7/8 & TLR9) & unstimulated samples. A p-value < 0.05 was seen in comparison between non-flare ratios only for CXCL-10 (TLR9), for which the baseline-flare ratio was superior to that of the Large flare ratio.
(2) *Flow Cytometry Study:* Comparisons between ratios of change from baseline to peak ALT samples with baseline to baseline samples were made for "Large Flare" & "non-Flare" subjects. Large flare values were superior to all non-flare values, except for classical monocytes (TLR2) & non-classical monocytes (TLR2). However, Large Flare ratios were all < 1 for Classical and intermediate monocytes (TLR2, TLR4 & TREM1), non-classical monocytes (TLR4), NK Bright (NKG2D) & NK Dim (NKG2D). Comparisons between Large Flare ratios had significant p-values < 0.05 for all NK & monocyte populations.

Discussions & Conclusions*:* In this analysis, we have shown that Large HBV Flares off NA therapy are associated with a significant inflammatory response from the innate immune system, including multiple pro-inflammatory cytokines which has not been shown previously in a cohort of patients experiencing HBV flares after NA Cessation. It is also associated with activation of the TLR receptors on NK cells and monocytes. Our study demonstrates that TLRs appear to play a key role in this innate immune response to HBV flares, which highlights their ongoing importance as potential immunotherapeutic options for CHB management.

Functional cure of chronic hepatitis B is associated with co-occurrence of HBsAg/anti-HBs immune complex peaks with ALT flares, and seroconversion to potently neutralising anti-HBs

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Introduction: HBsAg specific antibody (anti-HBs) responses during chronic hepatitis B infection are poorly defined due to the excessive amount of sub viral particles produced during infection and lack of standardised detection methodologies. The aim of this study was to investigate the nature of the anti-HBs responses in chronically infected HBV (CHB) patients during the process of functional cure (FC).

Methods: Longitudinal samples from 25 genotype A CHB patients undergoing nucleos(t)ide analogue (NA) treatment were examined, 14 of these patients achieved FC while 11 patients remained infected. HBsAg/anti-HBs Immune complexes (HBs-IC) were quantified using an in-house modification of a commercial diagnostic assay. Epitope-specific anti-HBs responses were detected using a 19-plex assay and neutralization efficacy of anti-HBs was measured using an *in vitro* HBV infection model.

Results: HBs-IC was detected in all patient's serum samples at fluctuating levels. In the 14 patients who achieved functional cure, 10 had an ALT flare immediately prior to HBsAg loss, and of these 10 patients, 9 had co-occurring HBs-IC peak responses. This co-occurrence was not observed in non-functionally cured patients (non-FC). Non-FC patients had lower anti-HBs responses at week 12 of NA treatment compared to FC patients. Anti-HBs derived from FC patients after seroconversion was more potent in neutralizing HBV infection *in vitro*, and recognised more anti-HBs epitopes compared to anti-HBs from vaccinees.

Discussion: Using several analytic assays, here we revealed previous unrecognized antibody responses during CHB infection. We demonstrated that peak responses of HBs-IC are closely associated with ALT flares in NA treatment, and this associate may play vital role in the virus clearance, further dissection of the HBs-IC function during ALT flare may provide insight in the cause of flare. The observation that early antibody responses during NA treatment is critical in defining patient outcomes is aligned with previous studies, indicating drug treatment may break immune tolerance for a short time frame, and patients who mount effect immune responses may achieve FC. CHB patients normally experience long term virus expose and their immune system may continuously evolve during the infection, this may explain the anti-HBs derived from FC patients are more efficient in neutralizing HBV infection compared to anti-HBs from vaccinees.

Conclusions: Anti-HBs responses are present and fluctuate during chronic hepatitis B infection. Functional cure in the analysed cohort was associated with co-occurring HBs-IC peak and ALT flare prior to clearance, and was followed by seroconversion to anti-HBs with broad HBsAg epitope recognition, which potently neutralize HBV infection. Failure of virus clearance was associated with lower anti-HBs responses in the early phase of NA treatment. These results demonstrate the presence and importance of broadly-reactive anti-HBs responses in clearing HBV infection.

Identifying CTL Epitopes for Immunotherapy Candidates Against Replication-competent and defective HIV

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Introduction: During effective antiretroviral therapy (ART), human immunodeficiency virus (HIV) persists in memory T-cells and virus from this cellular reservoir rebounds when patients stop therapy. To prevent this viral rebound, cells containing replication-competent proviruses would have to be eliminated by an immune response of HIV-specific cytotoxic T-cells (CTLs) to prevent viral spread. However, a majority of HIV-infected T-cells harbor defective proviruses, which can produce viral proteins that act as decoys for CTL response. To address these issues, we performed a systematic investigation to define immunogenic CTL epitopes within replication-competent and genetically defective proviruses which are conserved across HIV-infected individuals with different human leukocyte antigen class I (HLA-I) alleles.

Methods: By employing the full-length individual proviral sequencing assay (FLIPS), we characterized a total of 350 near full-length HIV genomes derived from six participants with known HLA-I alleles. From these proviral sequences we extracted the *gag, pol, vif, nef, vpr* and *env* genomic regions and generated a repertoire of 8-14 mer peptides. Subsequently, we employed the Protein BLAST (BLASTp) and NetMHCpan-4.0 algorithms to determine HIV-specific peptides with strong and weak binding affinities to HLA-I molecules. Next, we applied protein network analysis to determine which of these peptides contain highly networked amino acid residues that are crucial for structural and functional maintenance of the *gag, pol, vif, nef, vpr* and *env* HIV proteins. The peptide sequences representing immunodominant and highly networked epitopes were tested *in-silico* to determine their capacity to undergo HLA-I mediated antigen processing and presentation by using the immune epitope database (IEDB). We also performed an interaction network analysis to delineate the T-cell epitope derived peptides that can form a stable complex with both HLA-I molecules and T-cell receptor alpha and beta chains (TCRαβ).

Results: We obtained a repertoire of 17.6 million 8-14 mer peptides derived from *gag, pol, vif, nef, vpr* and *env* genomic regions extracted from proviral sequencing analysis involving six participants on prolonged therapy. However, only a fraction of these peptides (0.03%) were binders to participant specific HLA-I alleles despite that all six HIV genomic regions contained T-cell epitopes. Among the six HIV genomic regions, *vpr* contained the highest number of HIV-specific peptides that were strong (n=358) and weak (n=1306) binders to participant specific HLA-I alleles. Of these, our interaction network analysis revealed only three Vpr-derived peptides can form a stable complex with an HLA molecule and TCRαβ. In addition, these Vpr peptides are predicted to bind to multiple HLA-I alleles/supertypes with a global population coverage of 74%. Importantly, these peptides have a potential to target HIV-infected cells containing genetically intact (i.e. replication-competent) and defective proviruses.

Conclusions: By using our immunoinformatics analysis pipeline, we defined rare HIV-specific CTL epitope derived peptides within topologically important regions of the Vpr protein. These peptides have the potential to enhance CD8+ T-cell response to HIV--infected cells containing genetically intact and/or defective proviruses, especially when used with other latency-reversing HIV curative strategies during ART. Our approach that defines immunodominant regions is applicable to other viruses such as influenza and Severe Acute Respiratory Virus type 2.

Proof-of-concept that LDH Nanoparticle facilitates delivery and effectiveness of antiretroviral drugs to combat HIV-1 replication in the brain and central nervous system

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Introduction: Combination antiretroviral therapy (cART) has reduced the morbidity and mortality associated with HIV-1 (HIV) infection. However, cART does not cure HIV and individuals on cART develop pathologies, including the central nervous system (CNS) dysfunction. Due to the unique structure of the blood-brain-barrier (BBB), some anti-HIV drugs have restricted entry or penetrance into the CNS that contributes to low levels of HIV replication in CNS of people living with HIV, even though no viral RNA may be measurable in the blood or cerebral spinal fluid. In this project, we aim to adapt an advanced layered double hydroxide nanoparticle (LDH NP) platform for delivery of anti-HIV drugs to the CNS in order to improve inhibition of HIV replication in the brain.

Methods: Cellular binding and cytotoxicity of LDH NP loaded with tenofovir (LDH-TFV NP) in human peripheral blood mononuclear cell (hPBMC) were tested by flow cytometry analysis and MTT assay, respectively. Moreover, antiviral activity of LDH-TFV NP in hPMBC was examined *in vitro* by CAp24 enzyme-linked immunosorbent assay (ELISA). To determine whether BBB-targeted LDH NPs can facilitate anti-HIV drugs uptake in the CNS to inhibit HIV replication *in vivo*, we engrafted HIV-infected hPBMC into NSG mouse, making HIV-infected hPBMC (HIV-hPMBC) NSG mice. HIV-hPMBC NSG mice were then treated with BBB-targeted LDH-TFV NP or non-targeted LDH-TFV NP. After antiviral drug treatment, the relative levels of HIV RNA in mouse spleen and brain tissues were measured by RT-qPCR normalised to human cells in the sample.

Results: We found that hPMBC incubated with FITC-conjugated LDH (FITC-LDH) NP resulted in more than 90% FITC-LDH positive cells. The cell viability of hPBMC was above 95% following 24 h- and 48 h-LDH-TFV NP treatments at the TFV concentration ranging from 0.05µM to 5µM. There were more than 7-fold lower levels of HIV CAp24 in culture supernatants when HIV-hPMBCs were treated with LDH-TFV NP compared to non-treated control supernatants. After 14-days engraftment of HIV-hPMBCs, HIV-infected human T cells could be detected in brain and spleen tissues in engrafted mice.

Conclusions: Our data showed that LDH NP could efficiently deliver anti-HIV drugs to hPBMC and did not induce acute cytotoxicity. We also confirmed that LDH-TFV NP had antiviral activity against HIV replication in hPBMC. Evaluation of whether BBB-targeted LDH-TFV NP can inhibit HIV replication in the brain of mice is currently in progress.

[39]

Harnessing the glycan reactivity of PGT121-like bNAbs for potent FcγRIII mediated killing of Env expressing target cells.

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Introduction: FcγRIIIa is expressed on NK cells and CD16+ monocytes and is the principal receptor mediating NK antibody-dependent killing of virus infected target cells. FcγRIIIa has a highly glycosylated 20 kDa ectodomain containing six N-linked glycans, a density consistent with steric restraints on glycan processing. The HIV envelope trimer is also heavily glycosylated with up to 90 N-linked glycans/trimer (30 per 120 kDa) that shield the underlying protein from 'conventional' antibody recognition. This glycan shield is an unusual mix of high mannose, partially and fully processed glycans and, over years of infection, rare broadly neutralizing antibodies such as PGT121 can arise that recognize these structures as important components of their epitopes (Shivatare et al., 2018).
However, such glycans are produced by the host glycosylation machinery and so are displayed on a subset of highly glycosylated host proteins on some cell types. We recently made the exciting discovery that PGT121 binds to human leukocytes with the strongest binding to CD56+ natural killer (NK) cells. Blazkova et al. similarly found PGT121 binds to specific glycans of uninfected NK cells and activated lymphocytes. We have found the Fab of PGT121 interacts with FcγRIIIa glycans. Comparing WT and FcγR binding null LALA mutant forms of PGT121 revealed both Fc binding to FcγRIIIa and Fab:glycan interactions contributed to the PGT121 binding reaction with NK cells.
Since FcγRIIIa (CD16a) is the key receptor mediating NK antibody dependent cellular cytotoxicity (ADCC) we are exploring if the unusual reactivity of PGT121 can be exploited to engineer bispecific mAbs with improved ADCC activity.

Methods: We will use the "duobody" method based on IgG4 arm exchange to make bispecific mAbs comprising one Fab arm being a glycan self-reactive PGT121-Fab and the other Fab arm comprising a non-self-reactive bNAb PG9 (also VRC01). The PGT121 component of the bispecific will bind and pre-arm killer cells while the second Fab's specificity for HIV Env will target the primed killers to the HIV infected cells. Bispecific antibodies will be characterised biochemically and initially functionally using a unique luciferase reporter assay for FcγRIIIa activation. We will test the activation of human NK cells by the expression of CD107a and IFNγ and lastly for the capacity to mediate killing of HIV-infected CEM.NKr-CCR5 target cells using an established infected cell elimination assay with GFP reporter HIV-1AD8.

Results: We have generated "duobody" CH3 domain mutated variants of PGT121 and PG9. Reduction and reassociation reactions are being optimised for yield of the PGT121/PG9 bispecific antibody. The novel high through put FcγRIIIa activation luciferase assay has been fully optimised for the assessment of the bispecific anti-HIV reagents.

Discussion: We propose host glycan binding by PGT121, including to FcγRIIIa glycans, adversely affects its capacity to mediate ADCC. Progress on the production and characterisation of ADCC-enabled bispecific mAbs based on PGT121 will be presented.

References – 1: Shivatare et al. Unprecedented Role of Hybrid N-Glycans as Ligands for HIV-1 BnAbs. J Am Chem Soc. 2018, 140:5202-5210. 2: Blazkova et al., Glycan-dependent HIV-specific nAbs bind to cells of uninfected individuals. J. Clin Invest. 2019, 129:4832-4837.

[40]

Hepatitis C resistance-associated substitutions and antiviral salvage therapy outcomes across Australia

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Introduction: Hepatitis C virus (HCV) infection can now be cured with potent, well tolerated direct acting antiviral (DAA) therapy. However, a potential barrier to HCV elimination is the emergence of resistance-associated substitutions (RASs) in the virus genome, which reduce the efficacy of antiviral drugs. Patients who fail first-line DAA therapy select for RASs so are more likely to fail again, but studies assessing the clinical impact of RASs in the real world are limited. A recent advance in HCV salvage therapy is sofosbuvir/velpatasvir/voxilaprevir (Vosevi®), which combines pan-genotype DAAs from all three drug classes and was listed on the Australian PBS in April 2019. Here, we present real-world data comparing re-treatment outcomes for different salvage regimens, from patients across Australia, as well as an analysis of the impact of HCV RASs on DAA salvage outcomes.

Methods: We performed HCV resistance testing on blood referred from patients from across Australia who failed DAA therapy. Key regions of the HCV genome targeted by DAAs were amplified using published and in-house PCR protocols. Sanger sequencing was performed and clinically relevant RASs were identified using validated online databases (Geno2Pheno and RECALL). We have a growing clinical database on these patients including age, sex, fibrosis stage, HCV genotype, NS3/NS5A/NS5B RASs, details of failed first-line regimen and subsequent salvage regimens. We are continuing rolling data collection of retreatment outcomes, with follow up data currently available on 139 patients who have finished salvage therapy.

Results: Our preliminary data show that, of the 139 patients who have been retreated, 56 (40%) achieved sustained virologic response (SVR) and 16 (12%) failed retreatment. Other patients are either still undergoing treatment, awaiting SVR results, developed hepatocellular carcinoma, deceased, or were lost to follow up. Of the 34 patients that were retreated with Vosevi, 28 (82%) achieved SVR and 6 (18%) relapsed. Of the 56 patients who achieved SVR, 35 (66%) had NS5A RASs, the most common being Y93H (19). No patients had NS5B RASs and only six had NS3 RASs. Of the 16 patients who failed retreatment, nine (56%) has NS5A RASs but none had NS5B or NS3 RASs.

Discussion: The introduction of Vosevi onto the PBS has greatly improved retreatment of people in Australian who fail DAA therapy. In the Phase 3 POLARIS retreatment trials Vosevi produced SVR rates of >95% across all HCV genotypes. This was not the case in our initial cohort of real-world patients, which had an SVR rate of 82%. The presence of NS5A, NS5B, or NS3 RASs did not appear to influence retreatment outcomes, including high impact RASs such as Y93H. However the potential impact of HCV RASs should become clear as our cohort of patients with retreatment outcome data expands.

Conclusions: Hepatitis C treatment has greatly improved with DAA therapy, but viral resistance remains a potential hurdle to HCV elimination. In this study we present real-world data on HCV salvage treatment and outcomes in Australian, including the potential impact of HCV resistance.

Identification and analysis of a public antibody repertoire among Hepatitis C Virus infected patients

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Introduction: Neutralising antibodies (nAbs) play a key role in spontaneous clearance of hepatitis C virus (HCV) infection, and therefore have been a focus of research looking for an effective, long-term protective HCV vaccine. The ideal goal for such a vaccine is the ability of a common antigen to induce a protective broadly neutralising antibody (BnAb) response across subjects. Certain conserved epitopes have been associated with BnAb activity, but individuals induce varying responses to these BnAb epitopes. The aim of this study was to identify if specific V_H and V_L genes influence epitope specificity and if 'public' repertoires exist against specific epitopes; that is whether specific V_H and V_L genes are commonly induced between multiple individuals.

Methods: In this study, approximately 50 HCV-specific B cells each from ≥2 time points of 30 subjects (~3000 cells), who have either cleared or developed persistent HCV infections, were sequenced. These sequences were analysed for public BCR repertoires, finding approximately 200 public Abs among those sequenced. Abs cloned from those B cells are being mapped to identify specificity of binding to multi-epitopes on the HCV envelope protein E2. This data will be compared to sequence data to examine V gene usage biases for specific epitopes.

Results: To date, sequencing data has been generated for 21 subjects, with several timepoints for some subjects. From this, we have identified 50 shared clonotypes, with the three largest public clonotypes shared across 6 subjects. Initial comparison of V gene usage and epitopes targeted indicates a clear bias.

Conclusions: The findings from this study will inform on the hosts contribution to induce immune responses to protective epitopes and provide insight into how to target germline B cells for protective vaccine responses.

Functional characterisation of antigen-specific CD8 T_{SCM} during hepatitis C virus reinfection and clearance

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Introduction: Natural clearance of hepatitis C virus (HCV) occurs in approximately 25% of cases after primary infection, but generally does not result in protective immunity against subsequent infections. $CD8_+$ memory stem T cells (T_{SCM}) play an important role in long-lived protective immunity after natural infection or immunisation. This project hypothesized that HCV-specific T_{SCM} confer protection in a rare subset of HCV 'super-clearer' individuals who repeatedly clear viraemia via enhanced proliferative, multi-potency, and self-renewal ability. HCV-specific T_{SCM} were characterised in individuals who spontaneously resolved at least two infection episodes (super-clearers) and those who resolved the primary infection but subsequently developed chronic infection (clearer-chronics).

Methods: Longitudinally collected samples of HCV infections with these two outcomes were available from the Hepatitis C incidence and Transmission Study in prisons and community cohorts (HITS). Using our antigen-specific T_{SCM} assay systems previously developed for CMV-specific T_{SCM}, six subjects with good frequencies of HCV-specific $CD8_+$ T cells were selected, comprising three super-clearers, and three clearer-chronics. $CD8_+$ T_{SCM} ($CCR7_+CD45RA_+CD95_+$) were bulk sorted from peripheral blood monocular cells (PBMCs) followed by cell trace violet (CTV) labelling, and co-culture with cognate peptide in media supplemented with IL-2/IL-15 along with autologous PBMCs as antigen-presenting cells for five days. The function of the HCV-specific T_{SCM} identified in this assay system were assessed by quantifying the proliferation index, multi-potency index, and stemness index. Additionally, CTV_+ HCV-specific T_{SCM} were single cell index sorted for single cell RNA-seq to identify the molecular signature.

Results: HCV-specific CD8+ T cells were readily expanded *in vitro* with cognate peptide and cytokine co-stimulation. The frequencies increased 7.45 ± 2.32-fold (mean ± SEM, range 0.95 - 17.81) in super-clearers, compared with 4.10 ± 1.96-fold (range 0.55 – 14.87) in clearer-chronics. The HCV-T_{SCM} frequencies across timepoints of super-clearers was significantly higher than that of clearer-chronics (33.43 ± 12.22 vs. 6.18 ± 2.21). By contrast, the proliferative ability of HCV-T_{SCM} isolated from super-clearers was lower than that of clearer-chronics. Importantly, clearer-chronics had 'stemness' indices of zero prior to the reinfection (i.e no ability to generate T_{SCM} as progeny) whereas super-clearers consistently retained this key functional property. The transcriptomic analysis will describe the unique molecular signature of HCV-specific T_{SCM} comparing super-clearers and clearer-chronics to better understand the functional differences.

Discussion: These findings suggest that the ability to establish functional and long-lasting T_{SCM} memory T cell responses is important in the super-clearers outcome, suggesting a pivotal role of HCV-T_{SCM} in establishing protective immunity against subsequent infection. In particular, the protective role of HCV-T_{SCM} was associated with self-renewal ability (calculated as a stemness index). Future studies on the gene signature of HCV-T_{SCM} in super-clearers and clearer-chronics will help to identify the transcriptomic differences and to identify the genes responsible for protective $CD8_+$ T_{SCM} responses. The study is limited by the available sample size of subjects with these very rare clinical outcomes.

Conclusions: The ability to generate long-lived T_{SCM} with good stemness is likely to be a key element of both naturally-occurring and vaccine-conferred immunity against HCV.

Immunosilencing of the non-neutralizing face of HCV E2 glycoprotein for improved vaccine responses

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Introduction: Modelling suggests the elimination of HCV will require an effective preventative vaccine and direct acting antiviral drugs. Passive antibody transfer experiments in animals and studies of natural infection in humans suggest that broad-acting neutralizing antibodies (bNAb) against the HCV E2 glycoprotein will be a critical component of an effective vaccine. E2 has a neutralizing face consisting of structures that mediate binding to the cellular receptor CD81, and a non-neutralizing face that generates antibodies that cannot block infection (non-Nab) and in some cases may enhance infection. We aimed to suppress the immunogenicity of the non-neutralizing face by mutating key residues to alanine. We hypothesized that this would re-direct the immune system to generate antibodies that block receptor engagement and neutralize virus, thus creating immunogens with greater utility as vaccine candidates.

Methods: In the parental E2 molecule chosen (D3A7) the three hypervariable regions have been deleted, as these domains generally raise antibodies with narrow specificity. In addition, D3A7 has seven cysteine to alanine mutations that result in the expression of a homogeneous monomeric species, facilitating immunogen production. Guided by published crystal-derived structures, alanine scanning mutagenesis of D3A7 was performed across the nonneutralizing face of E2. We targeted surface exposed amino acids potentially forming part of the epitopes of known on-NAb and avoided amino acids with extensive contacts with buried residues, as mutation of these residues is likely to affect the global E2 conformation. In addition, specific regions were deleted or truncated in an attempt to ablate non-NAb epitopes. Mutant genes were synthesized, sub-cloned into expression vectors and used to transfect mammalian cells. The expression, antigenicity and ability of mutant molecules to bind CD81 was assessed by ELISA and biolayer interferometry and compared to parental E2.

Results: Mutations that were well-tolerated and yielded near wild-type levels of expression were selected. Our initial panel consisted of 14 mutants with single amino acid substitutions localised in three clusters and one mutant with an 11-amino acid deletion. The panel was screened to identify mutants that displayed reduction of binding to non-Nab and/or maintenance or enhancement of binding to bNAb. The binding of a linear bNAb targeting the first CD81 binding cluster and a conformational bNAb targeting the second CD81 binding cluster generally showed little variance in binding between the parental and mutant molecules. In contrast, a bNAb with a more complex conformational epitope showed variable binding to mutant D3A7 ranging from marked reduction to enhancement compared to parental D3A7. Several mutations in the E2 back layer markedly perturbed non-NAb binding but has less effect on Nab binding. Guided by these data and focusing on the back layer, we made a further series of 7 single amino acid substitutions, 3 deletion/truncation mutants and 2 double mutations. The deletion mutant and one truncation mutant disrupted the global E2 conformation, whereas a second truncation mutant preserved structure but removed the Cterminus of E2 and a non-NAb epitope. Similarly, the single amino acid substitutions identified additional residues that ablate non-NAb binding while leaving NAb binding intact.

Discussion and conclusion:
Our data indicate that selectively immunosilencing the non-neutralizing face of E2 is feasible. In future studies, we will combine favorable mutations in multiple mutants and re-test antigenicity. The most favorable mutant combinations will be placed in an immunization trial in small animals where we will assess the neutralization potency and breadth of the sera generated compared to parental forms of the immunogen.

Establishment of a VLP based Hepatitis C virus vaccine

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Despite the development of safe and highly effective direct-acting antiviral, hepatitis C virus (HCV) remains a major public health problem with over 71 million people infected globally causing 400,000 deaths each year. Furthermore, 1.7 million people are newly infected each year and this could be prevented by a vaccine. Viral clearance is associated with the rapid induction of neutralising antibodies. Cross-genotype neutralising monoclonal antibodies that recognise the E2 envelope glycoprotein of HCV protect against heterologous viral infection and suggest that a HCV vaccine may be achievable.

We have previously shown that a soluble recombinant form of glycoprotein E2, that lacks three variable regions (D123) is able to elicit a higher titre of broadly neutralizing antibodies in comparison to the parental wild type form (RBD).

In this study we engineered a virus-like particle (VLP) vaccine candidate that displays HCV glycoprotein E2 on a Duck hepatitis B virus S (DHBVS) antigen scaffold. Four variants of E2-DHBVS VLP's were constructed; D123 and RBD, and D123A7 and RBDA7 in which 7 cysteines were replaced with alanine. While all four E2-DHBVS VLP's display E2 as a surface antigen, the D123A7 and RBDA7 secreted VLPs most efficiently from transfected mammalian cells. Electron microscopy showed the formation of ~60nm particles all homogeneous in morphology, displaying epitopes recognized by cross-genotype neutralizing monoclonal antibodies. Both D123A7-DHBVS and RBDA7-DHBVS VLPs were immunogenic in guinea pigs generating high titre antibodies reactive to native E2, and able to prevent the interaction between E2 and the cellular receptor CD81. However, only animals immunized with D123A7-DHBVS elicited cross-genotype neutralizing antibodies to 7 genotypes of HCV. Immune serum generated by animals with neutralizing antibodies mapped to a major neutralization epitope located at residues 412-420 (epitope I) and antigenic region 3. VLP's that display E2 glycoproteins represent a promising vaccine platform for HCV.

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Characterization of a monoclonal antibody towards the N-terminal hypervariable region 1 (HVR1) and epitope I of Hepatitis C Virus Glycoprotein E2

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A Hepatitis C virus vaccine is urgently needed to achieve global elimination. Hepatitis C is one of the most antigenically variable human pathogens and an effective vaccine must generate immunity in the majority of the human population.

Glycoprotein E2 is present on the virion surface and is a major target of neutralizing antibodies which can prevent infection. All neutralizing domains identified to date work by blocking interaction between E2 and cell surface receptor CD81. The N-terminal hypervariable region 1, HVR1 (384-408) is an immunodominant region within E2 and elicits neutralizing antibodies that are usually type specific. HVR1 is known to play an essential role in binding of infectious serum derived HCV particles to scavenger receptor class B type 1 and glycosaminoglycans on the cell surface, essential for HCV entry. Cross neutralizing antibodies that include amino acids within HVR1 have not been characterized.

We have identified a novel rodent monoclonal antibody, MAb33, that binds to an unusual epitope bridging HVR1 and the adjacent target of broadly neutralizing antibodies referred to as epitope I (408-423). MAb33 potently neutralizes G1a viruses, and also has the ability to cross-neutralize 3 different HCV genotypes, however, it only weakly blocks the interaction between E2 andCD81 suggesting its mechanism of neutralization is distinct from previously defined bNAbs.

We have defined the epitope of MAb33 and for the first time resolved its structure in complex with its epitope at 2Å resolution. The structure of the epitope is a helix, and is in a different conformation to what is observed in unliganded structures that include this region of E2. These results suggest that this region could be flexible, raising the question whether there is a preferred conformation detected by neutralizing antibodies.

These studies will provide novel insight into the structure of HCV E2 and properties of antibodies directed towards HVR1 and help inform HCV vaccine development.

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Investigation of the optimal strategy to deliver a B cell immunogen for a HCV vaccine.

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Approximately 71 million people are infected with hepatitis C virus causing substantial morbidity and mortality worldwide. Efforts to eliminate HCV have been improved with the development of safe and highly effective directacting antivirals, but for elimination to be achieved, a vaccine must be developed to reduce the number of new infections and prevent reinfection. To date, no pathogen has been eliminated without the development of a prophylactic vaccine. Chimpanzee adenovirus (ChAd) is a leading vaccine platform for COVID-19 aimed at generating neutralizing antibodies to the SARS-CoV-2 spike protein. It is a viral vector platform developed at Oxford University from a genetically modified attenuated rare serotype (ChAdOx1), therefore there is low seroprevalence in the human population which will circumvent pre-existing immunity.

We investigated the use of this platform with a leading HCV vaccine candidate aimed at generating humoral immunity. We have previously reported that by removing the hypervariable regions from glycoprotein E2 a soluble recombinant protein (D123) can be produced and was shown to elicit cross neutralising antibodies in guinea pigs when administered as an adjuvanted soluble protein. In this study, we delivered the D123 vaccine candidate in ChAdOx1 and investigated the optimal primeboost strategy. Animals were administered three doses, three weeks apart of either ChAdOx1D123, ChAdOx1D123 and boosted with soluble adjuvanted D123, or three doses of soluble adjuvanted D123 protein. All strategies elicited E2 specific antibodies in mice, however, titres were significantly higher in groups that received D123 protein, either as a boost with ChAdOx1D123 prime or three doses of D123. Enhanced antibody mediated E2-CD81 inhibition was observed to be statistically highest using ChAdOx1D123 prime/D123 protein boost, with this heterologous combination also eliciting both type specific and cross neutralising antibodies. Type specific neutralising antibodies were targeted to 3 major neutralisation epitopes located at residues 412-428, 429-448 and 523-549 which includes the CD81 binding loop. The results of this study show that addition of a protein boost after a ChAdOx1 prime using D123 is the optimal strategy for delivering a B cell immunogen for an HCV vaccine and suggests that this strategy could be explored for other pathogens including COVID-19.
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