Australian Centre for
HIV and Hepatitis Virology Research

THE AUSTRALIAN CENTRE FOR HIV AND HEPATITIS VIROLOGY RESEARCH

SCIENTIFIC CONFERENCE

Australian Centre for
HIV and Hepatitis Virology Research

ACH4 19th Scientific Conference

Wednesday 25th – Friday 27th June 2025

The Coogee Bay Hotel

253 Coogee Bay Road,
Coogee NSW 2034

Organising Committee:

HIV	HEPATITIS
Dr Lindi Masson	Dr Arunasingam (Arun) Abayasingam
Dr Gabriel Duette	A/Prof Branka Grubor

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https://www.ach4.org.au/

Australian Centre for
HIV and Hepatitis Virology Research

CONTENTS

SPEAKERS

INVITED SPEAKER

HIV Plenary – Associate Professor Marta Rodriguez Garcia

Department of Biochemistry, Microbiology and ImmunologyCS Mott Center
Wayne State University School of Medicine

Dr. Marta Rodriguez-Garcia obtained her MD from the University of Granada and PhD from the University of Barcelona (Spain). She completed her medical residency in Clinical Immunology and then moved to the USA for postdoctoral training, first at the Ragon Institute of MGH, MIT and Harvard, and then at Dartmouth to focus her research on reproductive immunology and HIV infection. She then became an Assistant Professor in the Department of Immunology at Tufts, and recently moved to Wayne State University as an Associate Professor in the Department of Biochemistry, Microbiology and Immunology and the C.S Mott Center for Human Research and Development.

She serves as a standing member for the HIV Comorbidities and Clinical Studies (HCCS) NIH Study Section, she is the treasurer for the American Society for Reproductive Immunology (ASRI) and she recently became a Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Diseases (PATH) awardee. Her research, funded by NIH and the Burroughs Welcome Fund, focuses on understanding the early events of mucosal HIV acquisition in the female genital tract and how innate immune protection changes with age.

Learn more here: *https://mott.med.wayne.edu/marta-rodriguez-garcia-lab-home/*
Email: *marta.rodriguez-garcia@wayne.edu*

INVITED SPEAKER

HEPATITIS Plenary – Professor Mansun Law

Department of Immunology and Microbiology
The Scripps Research Institute

Mansun Law is a Professor at The Scripps Research Institute, specializing in antiviral antibody and B cell responses. He graduated from the University of Hong Kong in 1995, and obtained his DPhil degree in 2001 from the University of Oxford, studying the roles of antibody and complement on poxvirus dissemination and pathogenesis.
He continued his investigation on antiviral antibodies in the Burton lab in 2004 at TSRI and started his independent lab in 2008. He became a tenured professor in 2023.

Dr. Law's research focuses primarily on understanding immune responses to viral infections, particularly chronic viral hepatitis B and C. His work has been instrumental in uncovering key mechanisms of HCV neutralization and the genetic biases in the generation of broadly neutralizing antiviral antibodies. His laboratory currently explores how B cell repertoires evolve during chronic infection and treatment.

Learn more here: *https://www.scripps.edu/faculty/law/*
Email: *mlaw@scripps.edu*

INVITED SPEAKER

HTLV-1 Plenary – Professor Yorifumi Satou

Professor of Genomics and Transcriptomics, Kumamoto University, Japan

During my PhD course I carried out studies on novel treatment of ATL (Leukemia 2004).
As a post doctorial study, I analyze antisense viral gene HBZ about expression and role in HTLV-1 pathogenesis by using clinical samples and transgenic mouse model (PNAS 2006, PLoS Pathogens 2011). I then performed molecular biology experiment to elucidate the mechanism underlying retroviral latency (PNAS 2016).

Since I obtained PI position in 2013, I have incorporated high through-put sequencing technology as a tool to understand genetic and epigenetic regulatory mechanism of retroviral latency regarding both HTLV-1 and HIV-1 (Cell Rep 2019, JCI 2021, Nature Commun 2022, Nature Microbiology accepted in principle)

Learn more here: *https://kumamoto-u-jrchri.jp/satou/en/*
Email: *y-satou@kumamoto-u.ac.jp*

GUEST SPEAKERS

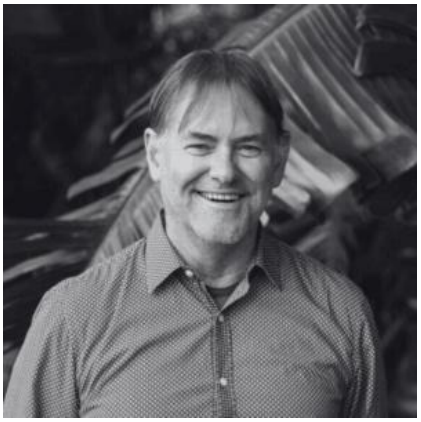
HIV Cure research – Dr Jillian Lau

Dr Jillian Lau is an Infectious Diseases physician in Melbourne and a postdoctoral research fellow at The Peter Doherty Institute for Infection and Immunity.

Her research focusing on clinical interventions in particular immunotherapy towards a cure for HIV. Jillian is also an NHMRC Emerging Leadership Fellow and is passionate about meaningfully engaging people living with HIV in cure-focused research

Learn more here: *https://www.doherty.edu.au/people/jillian-lau#panel2*
Email: *Jillian.lau@unimelb.edu.au*

HIV – National Association of People With HIV Australia (NAPWHA) – Dr John Rule



Dr John Rule, Director of Research, brings nearly 20 years' experience in the HIV sector and an extensive range of academic research achievements to NAPWHA.

As the Director of Research I drive NAPWHA's strategic engagement in HIV research. I have maintained and expanded research relationships with relevant partners and collaborators, and provide leadership and HIV-representative input into the development of relevant HIV research. My work is published in high quality academic journals. I work to translate research findings for the communities who have provided information and data and am also working within the organisation to link research information with policy development. I work in Australia and have a long track record of international project work, research and development activities

Learn more here: *https://napwha.org.au/*
Email: *john@napwha.org.au*

HIV – Positive Women Victoria – Dr Kristy Machon

Dr Kirsty Machon has more than twenty five years' experience in HIV journalism, policy, and advocacy. Kirsty has worked for national and state HIV organisations in Australia. She is currently the Executive Officer of Positive Women Victoria and is a former Chair of the Victorian AIDS Council.

Kirsty has co-authored recent guidance and papers on topics including infant feeding options for women living with HIV, quality of life, and barriers to the participation of women in HIV clinical and cure research. She was recently involved with the ACT NOW forum on global migration at the 12th IAS Conference on HIV Science in Brisbane, 2023.

Learn more here: *https://positivewomen.org.au/*
Email: *kirsty@positivewomen.org.au*

COVID – Dr Chan Phetsouphanh

Dr Chan Phetsouphanh completed his PhD in 2014 at UNSW. He then embarked on a postdoctoral position at the Peter Medawar Building for Pathogen Research at the University of Oxford (2015-2018), working on the role of MAIT cells and Cytotoxic CD4 T cells during HIV infection. He continued his postdoctoral training in Oxford at The Ludwig Institute for Cancer Research (2018-2020).

Since rejoining UNSW, he works as a Senior Lecturer within the Immuno-Virology and Pathogenesis Program (IVPP) at the Kirby Institute. His projects involve investigating T-cell responses following viral infections, evaluating the drivers of Long COVID, and harnessing immunopeptidomics for HIV eradication. He has keen interests in infectious diseases, T-cell biology, and immunotherapy

Learn more here: *https://research.unsw.edu.au/people/dr-chansavath-phetsouphanh*
Email: *cphetsouphanh@kirby.unsw.edu.au*

Human T-Lymphotropic Virus type 1 (HTLV-1) – Dr Lloyd Einsiedel

Associate Professor Lloyd Einsiedel is an infectious diseases physician who has provided a clinical service to central Australia for more than a decade. He has active research interests in Indigenous health with particular reference to interactions between the social determinants of health, health literacy and disease.

Learn more here: *https://findanexpert.unimelb.edu.au/profile/669687-lloyd-einsiedel*
Email: *lloyd.einsiedel@unimelb.edu.au*

HEPATITIS – Andrea Pizzie, General Manager, Hepatitis Australia

Andrea Pizzie is the General Manager of Hepatitis Australia, having previously led the Policy Team. Andrea has significant experience in health and social policy development, and specific experience in the development of national viral hepatitis policy and program design and costing. Andrea has led successful budget submissions and has played key leadership roles in the development and delivery of national policy platforms. Prior to working at Hepatitis Australia,

Andrea held positions in federal parliament and in public and private sector roles. Andrea is a values-based advocate and policy specialist who works strategically to achieve meaningful outcomes that centre the community.

Learn more here: *https://www.hepatitisaustralia.com/*
Email: *Andrea@hepatitisaustralia.com*

HEPATITIS – Emily Skillin, HepLink Program Manager, Hepatitis Australia

Emily Skillin is a HepLink Program Manager at Hepatitis Australia, supporting delivery of the HepLink program in partnership with the eight state and territory community hepatitis organisations to strengthen the national community response to viral hepatitis.

She brings broad experience in health policy and program development, with a background in pharmaceutical policy, economic evaluations of medicines, medical research strategy, grant funding, and clinical trials.

Learn more here: https://www.hepatitisaustralia.com/
Email: emily@hepatitisaustralia.com

CONFERENCE SPONSORS AND AWARDS

The Organising Committee for ACH4 2025 Conference acknowledges with gratitude the generous support received from the following organisations:

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> ARUN ABAYASINGAM

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> DISHEN COREY CHEN

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2025 PROGRAM AGENDA

Wednesday 25 June		
JOINT SESSION		
Coffee Cart > located in the Foyer Area, Level 1		
11:00am – 2:00pm	**Event Open –** **Registration & Lunch** **Registration Location: Seaview Room, Level 1**	
12:00pm – 1:00pm	**Lunch Location:** **Arden Lounge, Ground Floor – Cold Buffet**	
2:00pm – 2:30pm	**Welcome Address -** ACH4 & ACHV Professor Tony Cunningham & Professor Thomas Tu **Location: Seaview Room, Level 1**	
2:30pm – 3:30pm	**Keynote Presentation** **Hepatitis Plenary > Professor Mansun Law** **Talk Title: Hepatitis C Virus Immune Escape and Persistence** ***Chair: Professor Rowena Bull*** **Location: Seaview Room, Level 1**	
3:30pm – 3:45pm	**Brief Break**	
3:45pm – 5:00pm	**Abstract Session > Hepatitis C Session 1** **Chairs: Professor Rowena Bull & Dr Arun Abayasingam** **Location: Seaview Room, Level 1**	
3:45pm – 4:00pm	**Jordan Stoddart** Limitations of neutralising antibodies in an HCV reinfection case study	ABSTRACT #1
4:00pm – 4:15pm	**Eloise Kershler** Imprinting of broadly neutralising anti-HCV antibodies to inform vaccine design	ABSTRACT #2
4:15pm – 4:30pm	**Haiyi Ye** AlphaFold3-Enabled Discovery of Early Infection Antibody Targets in an HCV Super Clearer	ABSTRACT #3
4:45pm – 5:00pm	**Joey McGregor** Developing a mRNA-based HCV vaccine	ABSTRACT #4
5.00pm – 5:15pm	**Branka Grubor-Bauk** Induction of liver-resident memory T cell immunity against HCV by mRNA vaccination	ABSTRACT #5

5:15pm – 6:30pm	**FREE TIME**	
5:15pm – 6:15pm	**ACH4 Executive Meeting** **Location: Seaview Room, Level 1**	
6:30pm – 9:00pm	**Networking Event** Light mobile meal - Cocktail Style; Canapes & Beverages **Location: Arden Lounge, Ground Floor**	
Thursday 26 June		
Coffee Cart > located in the Foyer Area, Level 1		
Breakfast > Marra, Ground Floor, Opens at 7am		
8:00am – 8:50am	**ECR Breakfast Workshop Session** Consumer Engagement Facilitated by Sr Stephen Kent Breakfast banquet will be served in the room **Location: Seaview Room, Level 1**	
JOINT SESSION		
9:00am – 10:00am	**Keynote Presentation** **HIV Plenary > Associate Professor Marta Rodriguez Garcia** **Talk Title:** Innate immune control of HIV infection in the female genital tract ***Chair: Professor Andrew Harman*** **Location: Seaview Room, Level 1**	
10:00am – 10:15am	**MORNING TEA** **Location: Foyer Area, Level 1**	

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CONCURRENT SESSION		
10:15am – 12:00pm	**Breakout Session 1 > Hepatitis C (cont.) & Hepatitis B** **Chairs: A/Professor Chaturaka Rodrigo & Dr Melanie Walker** **Location: Seabreeze Room, Level 1**	
10:15am – 10:30am	**Inam Ulhaqazad** Landscape of epitopes targeted by T cells during Hepatitis C virus infection	ABSTRACT #6
10:30am – 10:45am	**Jean Pierre Ndabakuranye** Conductometric biosensing platform for early diagnosis of Hepatitis B and C	ABSTRACT #7
10:45am – 11:00am	**Bethany Horsburgh** Implementation of a novel tiling PCR for use in HCV diagnostics	ABSTRACT #8
11:00am – 11:15am	**Delgerbat Boldbaatar** A sensitive PCR assay to precisely quantify hepatitis B virus covalently closed circular (ccc)DNA	ABSTRACT #9
11:15am – 11:30am	**Dong Li** Investigating HBV DNA integration and its role in genomic instability through HBx expression	ABSTRACT #10
11:30am – 11:45am	**Bobby Boumelhem** A circulating biomarker of myofibroblasts is elevated in HBV infection but lacks a clear association with fibrosis	ABSTRACT #11
11:45am – 12:00pm	**Harout Ajoyan** High-throughput pipeline detecting viral integration reveals evidence for oncogenic insertional mutagenesis in domestic cat hepatitis B virus-associated hepatocellular carcinoma	ABSTRACT #12
10:15am – 12:00pm	**Breakout Session 2 > HIV – Transmission** **Chairs: Dr Kirstie Bertram & Dr Lindi Masson** **Location: Seaview Room, Level 1**	
10:15am – 10:30am	**Paula Ellenberg** Inhibitory activity of lactic acid against *L. iners* and BV-associated vaginal bacteria to prevent HIV transmission	ABSTRACT #13
10:30am – 10:45am	**Brianna Jesaveluk** Lactic acid, a key *Lactobacillus* metabolite, reduces HIV internalisation and migration through the cervicovaginal epithelial barrier	ABSTRACT #14
10:45am – 11:00am	**Freja Warner van Dijk** Understanding Inflammatory Mononuclear Phagocyte Heterogeneity in Human Anogenital Tissue	ABSTRACT #15
11:00am – 11:15am	**Lara Sarkawt** Epithelial dendritic cells: interactions with HIV in human penile mucosa	ABSTRACT #16

11:15am – 11:30am	**Daniel Buffa** Role of Epithelial Dendritic Cells in HIV Transmission and Their Potential as Vaccine Delivery Vehicles	ABSTRACT #17
11:30am – 11:45am	**Thomas O'Neil** An in situ quantitative map of mononuclear phagocyte interactions with HIV across anogenital mucosal tissues using high parameter spatial proteomic techniques	ABSTRACT #18
12:00pm – 1:00pm	**LUNCH** **Location: Marra, Ground Floor**	
12:00pm – 1:00pm	**ACHV AGM Working Lunch** **Location: Seaview Room, Level 1 (Banquet Lunch)**	
CONCURRENT SESSION		
1:15pm – 3:00pm	**Breakout 1 – Abstract Session > Hepatitis B** **Chairs: Professor Peter Revill & A/Professor Thomas Tu** **Location: Seabreeze Room, Level 1**	
1:15pm – 1:45pm	**National Guest Speaker > Hepatitis - Community** **Andrea Pizzie, General Manager & Emily Skillin, HepLink Program Manager, Hepatitis Australia** **Talk Title:** The Community response to viral hepatitis	
1:45pm – 2:00pm	**Sarah Bae** Developing personalized treatment pathways for Hepatitis B using novel assays and fine needle liver aspirates	ABSTRACT #19
2:00pm – 2:15pm	**Laura McCoullough** Developing CRISPR-Cas13b as a novel therapy for chronic hepatitis B virus infection	ABSTRACT #20
2:15pm – 2:30pm	**Zak Janetzki** Editing of HBV DNA and RNA in vitro and in vivo using A CRISPR/Cas9 Base Editor and CRISPR/Cas13 approach	ABSTRACT #21
2:30pm – 2:45pm	**Xiaonan Zhang (Presenting Author: Jack Bell)** Capsid-Antibody-Complexes (CACs): an early indicator of liver inflammation in chronic hepatitis B	ABSTRACT #22
2:45pm – 3:00pm	**Dishen Chen** Unleashing Immune Memory: Characterising HBV-Targeting Memory NK Cells for Next-Generation Cell Therapies	ABSTRACT #23
1:15pm – 3:00pm	**Breakout 2 – Abstract Session > HIV Therapy and Cure** **Chairs: Dr Paula Cevaal & Dr Rory Shepphard** **Location: Seaview Room, Level 1**	

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1:15pm – 1:45pm	**National Guest Speaker > HIV** **Dr Jillian Lau –** **Talk Title:** HIV cure in Australasia: Clinical Trials and Community Engagement	
1:45pm – 2:00pm	**Hannah King** CRISPR Cas13b-mediated knockdown of PD-1 mRNA: an alternative delivery strategy for immune checkpoint inhibitors	ABSTRACT #24
2:00pm – 2:15pm	**Le Wang** Applying Pro-apoptotic Agents to Combat Chronic HIV Infection in Vivo	ABSTRACT #25
2:15pm – 2:30pm	**Bruce Wines** The enhancement IgG hexamerisation through the H429F mutation enables potent complement killing of viral and cancer targets by otherwise inactive monoclonal antibodies	ABSTRACT #26
2:30pm – 2:45pm	**Kezia Singgih** Targeting Vδ2+ T cells to enhance immune mediated clearance of HIV-infected cells	ABSTRACT #27
2:45pm – 3:00pm	**Hafsa Rana** A novel strategy to eradicate HIV using CD8 CAR T cells	ABSTRACT #28
3:00pm – 3:30pm	**AFTERNOON TEA** **Location: Foyer Area, Level 1**	

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CONCURRENT SESSION		
3:00pm – 5:15pm	**Breakout 1 – Abstract Session > HTLV** **Chairs: Dr Nick Vandegraaff & Dr Chantelle Ahlenstiel** **Location: Seabreeze Room, Level 1**	
3:00pm – 3:30pm	**Keynote Presentation** **HTLV Plenary > Professor Yorifumi Satou** **Talk Title:** Mechanisms underlying HTLV-1 latency, persistence and pathogenesis	
3:30pm - 3:45pm	**Melissa John** Improving the Diagnosis and Monitoring of HTLV-1 in Australia	ABSTRACT #29
3:45pm - 4:00pm	**Andleeb Hanif** Promoter-Targeted siRNA as a Novel Therapeutic strategy for Oncogenic Viruses	ABSTRACT #30
4:00pm - 4:15pm	**Natasha Jansz** The HTLV-1c genomic landscape reveals host-virus interactions	ABSTRACT #31
4:15pm - 4:30pm	**Marcel Doerflinger** Combination antiretroviral therapy and MCL-1 inhibition mitigate HTLV-1 infection in vivo	ABSTRACT #32
4:30pm – 4:45pm	**Tiana Wang** Development of siRNA therapeutics targeting Human T-cell Leukaemia Virus Type-1 (HTLV-1) infection	ABSTRACT #33
4:45pm – 5:00pm	**Damian Purcell** Towards a universal mRNA-LNP vaccine to prevent HTLV-1 infection and disease	ABSTRACT #34
5:00pm – 5:15pm	**National Guest Speaker: HTLV-1 Update** **Associate Professor Lloyd Einsiedel** **Talk Title:** Report from the NACCHO HTLV research	
3:00pm – 5:00pm	**Breakout 2 – Abstract Session > COVID-19** **Chairs: Prof Gilda Tachedjian & A/Prof Branka Grubor-Bauk** **Location: Seaview Room, Level 1**	
3:00pm - 3:15pm	**Professor Tony Cunningham** **Director, ACH4** COVID Situation and Vaccine Update	
3:15pm – 3:30pm	**National Guest Speaker – COVID** **Dr Chan Phetsouphanh** **Talk Title:** Unravelling Long COVID- Immune Profiling and Insights into driver mechanisms	
3:30pm – 3:45pm	**Chantelle Ahlenstiel** Development of Intranasal Antiviral RNA Therapeutics targeting SARS-CoV-2 (DARTS)	ABSTRACT #35

3:45pm – 4:00pm	**Stephen Kent** COVID-19 booster vaccination in 2025 – when, how and why	ABSTRACT #36
4:00pm – 4:15pm	**Stephen Kent** Blood Distribution and degradation of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans	ABSTRACT #37
5:00pm – 6:30pm	**FREE TIME**	
6:30pm – 10:30pm	**Onsite: Gala Dinner Event** **Location: Seaview Room, Level 1** **Pre-dinner drinks, followed by a 3-course meal**	

Friday 27 June

Coffee Cart > located in the Foyer Area, Level 1

Breakfast > Marra, Ground Floor, Opens at 7am

JOINT SESSION

9:00am – 10:30am	**Abstract Session > HIV Latency and Pathogenesis** **Chairs: Dr Bruce Wines & Dr Gabriel Duette** **Location: Seaview Room, Level 1**	
9:00am – 9:30am	**National Guest Speaker – HIV Community Talk** **Dr John Rule, NAPWHA & Kirsty Machon, Positive Women Victoria** **Talk Title:** What are the research interests of people living with HIV?	
9:30am – 9:45am	**Paula Cevaal** Exploring CRISPR-based epigenetic tools for the reversal or promotion of HIV latency	ABSTRACT #38
9:45am – 10:00am	**Bridget Fisher** Improving the potency and safety of Tat-LNPs for HIV latency reversal	ABSTRACT #39
10:00am – 10:15am	**Rory Shepherd** Optimising long read sequencing of cell associated HIV RNA to understand latency reversal	ABSTRACT #40
10:15am – 10:30am	**Josefina Marin-Rojas** A novel IMmunoinformatics Analysis Pipeline (IMAP) identifies genetically-conserved and immunogenic peptides found in rebound HIV-1 during analytical treatment interruption	ABSTRACT #41
10:30am – 11:00am	**MORNING TEA** **Location: Foyer Area, Level 1**	

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JOINT SESSION		
11:00am – 12:00pm	**Abstract Session > CO-INFECTION** **Chairs: Dr Najla Nasr & Dr Bethany Horsburgh** **Location: Seaview Room, Level 1**	
11:00am – 11:15am	**Andrea Pereyra Casanova** Characterising the HIV reservoir in people living with HIV and HCV after treatment for HCV	ABSTRACT #42
11:15am – 11:30am	**Samantha Cronin** The Tuberculosis-Associated Microenvironment Promotes Viral Persistence in People Living with HIV and Tuberculosis	ABSTRACT #43
11:30am – 11:45am	**Jennifer Simpson** Utilising multi-omics analysis to characterise HIV persistence at the site of HIV and Tuberculosis coinfection	ABSTRACT #44
11:45am – 12:00pm	**Professor Tony Cunningham** **Director, ACH4** Global HIV Research impacted by US Funding cut	
12:00pm – 1:00pm	**LUNCH** – **Location: Marra, Ground Floor**	
1:00pm – 2:00pm	**Closing ceremony** Award presentation EVENT CLOSE	
2:15pm	**DEPART** **Buses Awaiting**	

THANK YOU FOR ATTENDING THE 2025 ACH4 CONFERENCE

ABSTRACTS

Abstract #1

Limitations of neutralising antibodies in an HCV reinfection case study

Jordan Stoddart[1,2], Alexander Underwood[1,2], Nicholas Brasher[1,2], Arunasingam Abayasingam[1,2], Money Gupta[1], Lisa Maher[2], Andrew Lloyd[2], Nicodemus Tedla[1], Rowena Bull[1,2]

[1]School of Biomedical Sciences, The University of New South Wales, Sydney, NSW, Australia

[2]The Kirby Institute, The University of New South Wales, Sydney, NSW, Australia.

Introduction: HCV vaccines are a key strategy to achieve elimination, though each approach presents its own considerations. One popular approach hinges on inducing broadly neutralising antibodies (BnAbs), targeting conserved portions of the virus. The Antigenic Region 3 (AR3) is particularly popular, broadly conserved and in the CD81-binding region thus facilitating viral entry. Furthermore, broadly neutralising antibodies towards it have been discovered in the public repertoire, further supporting its strength as a candidate. We investigate a case study of an individual who developed these antibodies and was able to clear one viral infection but failed to clear a second and try to determine immunological and virological features that could be responsible.

Methods: The individual was enrolled into the Hepatitis C Incidence and Transmission Study prison (HITS-p) cohort, a prospective cohort following people who injected drugs in NSW prisons. E2-specific B cells were isolated from the individual over the course of the study and were single cell sorted, allowing the B cell receptors (BCR) to be sequenced. The E2-specific BCRs were expressed as monoclonal antibodies, and their neutralisation and epitope mapping profile were determined. Recombinant E2 protein from the first and second infections were produced and used to investigate the change of antibody binding over time, through ELISAs and surface plasmon resonance.

Results: While numerous factors could contribute to the failure of this patient to clear their second infection, it seems that their failure to clear was driven by a difficult-to-neutralise virus. Serum analysis showed the initial infection resulted in broadly neutralising antibodies, which quickly returned upon reinfection. After screening isolated BCRs, BnAbs, including those with AR3-targeting capacity were isolated. Generally, antibody binding to the reinfecting virus showed low affinity. Conspicuously, the second viral E2 bound little to AR3-targetting antibodies and CD81, though this capacity seemed to be recovered later in the second infection.

Discussion: These results suggest that the individual may have been reinfected with a difficult-to-neutralise virus, with key mutations that prevented neutralising antibodies from binding with affinity. Fortunately, other broadly neutralising epitopes, such as Domain E, seem to have remained a viable target. Vaccine strategies involving neutralising antibodies should take this into account and focus on inducing antibodies to multiple epitopes to prevent escape.

Conclusions: Difficult-to-neutralise viruses may present a barrier to vaccine design and so must be considered in the process. Further research is needed into the frequency of these viruses and if there are any commonalities between them.

Abstract #2

Imprinting of broadly neutralising antiHCV antibodies to inform vaccine design

Eloise Kershler1,2, Arunasingam Abayasingam2, David Agapiou2, Ellise Roper1,3, Bethany Horsburgh2, Gail Mathews2, Gregory Dore2, Elise Tu2, Lisa Maher2, Nicodemus Tedla1, Jason Grebely2, Clara Young3, Deborah Burnett 1,3, Andrew Lloyd2, Rowena Bull1,2

1 School of Biomedical Sciences, Medicine & Health, University of New South Wales, Sydney, NSW, Australia
2 The Kirby Institute, Medicine & Health, University of New South Wales, Sydney, NSW, Australia
3 Garvan Institute of Medical Research, Sydney, NSW, Australia

Introduction: It is unclear why candidate HCV vaccines have been unable to induce broadly neutralising antibodies (bnAbs) across multiple recipients. A more comprehensive understanding of the impact of the infecting HCV variant on host antibody responses may offer new insights. Recent studies have shown that some HIV-1 and SARS-CoV-2 viral variants can induce similar bnAb responses across multiple individuals through a mechanism known as antibody imprinting. The overall aim of this project is to identify and characterise HCV variants that have the capacity to imprint antibody responses across individuals. If confirmed, this could inform the design and development of a broad and effective HCV vaccine.

Methods: Viral sequences were obtained using Sanger sequencing from people with current HCV infection from four prospective cohorts of acute and chronic HCV (HITS-p, HITS-c, SToP-C, and ATAHC). Sequences were aligned and a genetic distance matrix was generated to identify transmission clusters of individuals infected by highly similar viruses (>96.5% nucleotide identity). To determine the correlation of antibody responses between members of these transmission clusters, ELISAs were used to test antibody binding and epitope mapping, and pseudoparticle neutralisation assays were used to test antibody potency and breadth. Based on this data, recombinant E2 (rE2) proteins were generated from three HCV variants shown to imprint favourable or unfavourable antibody responses across members of the transmission cluster. To test if the responses observed in humans could be recapitulated in an in vivo setting, Ig-humanised Trianni mice were vaccinated four Mmes with these rE2 proteins. PBMCs and serum were collected for epitope mapping using flow cytometry and ELISAs, and to test antibody potency using pseudoparticle neutralisation assays.

Results: Among 1,023 viral sequences from people with current HCV infection, 38 transmission clusters were identified. Our findings show that transmission cluster members were more likely to have comparable antibody binding magnitude and epitope specificity for seven of the eight epitopes tested (Spearman $r = 0.3\text{-}0.43$, $P < 0.05$). Serum antibodies from these clusters also featured potent neutralisation against HCV pseudoparticles known to be neutralisation resistant. Similar results were also observed in the Trianni mouse model – the mice vaccinated with rE2s from viruses that imprinted favourable responses in humans had faster onset of antibodies targeting epitopes associated with neutralisaMon, and these antibodies had more potent neutralisation of HCV pseudoparticles than the antibodies from mice vaccinated with rE2s which imprinted unfavourable responses ($P = 0.001$).

Discussion: This study demonstrated people infected with the same HCV variant generate similar antibody responses and identified HCV antigens capable of bnAb induction in humans and Ig-humanised mouse models.
These findings suggest a novel strategy for optimal immunogen selection during vaccine development for HCV and other antigenically diverse viruses including influenza and SARS-CoV-2.

Abstract #3

AlphaFold3-Enabled Discovery of Early Infection Antibody Targets in an HCV Super Clearer

Haiyi Ye1, Syed Faraz Ahmed1,2, Sam Wilks1, Deborah L. Burnett4,5,6, Melanie R. Walker3,4, Arunasingam Abayasingam3,4, Rowena A. Bull3,4,*, Matthew R. McKay1,2,7,*, Ahmed Abdul Quadeer1,*

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Introduction: Hepatitis C virus (HCV) infection typically progresses to chronicity; however, a small subset of individuals achieves spontaneous viral clearance. Of particular interest are the "super clearers", who can rapidly resolve multiple successive HCV infections. Understanding the early-stage antibody (Ab) responses targeting the HCV envelope glycoprotein E2 in these super clearers can reveal mechanisms of spontaneous viral elimination and inform rational vaccine design. Traditional experimental approaches to comprehensive epitope characterization of these Abs are both labour and resource intensive. In this study, we validate and employ AlphaFold3 (AF3), a state-of-the-art computational model for antigen-Ab structure prediction, to define a comprehensive epitope map of early-stage E2-reactive Abs in an HCV super clearer.

Methods: We implemented AF3 on a local high-performance GPU server to facilitate extensive structural predictions of HCV E2-Ab complexes. The predictive accuracy and reliability of AF3 were initially evaluated through comprehensive benchmarking against all available E2-Ab crystal structures deposited post AF3 training data cutoff date in public repositories, including the Protein Data Bank. Complementary validation of AF3- predicted epitope was achieved through in-house E2-Ab binding assays with experimentally confirmed epitope domain information. E2-specific B-cell receptors (BCRs) and autologous E2 from the subject at initial re- infection point were identified and sequenced as previously described [1]. The immunoglobulin gene information and the translated sequences for BCRs were obtained using the IMGT/V-QUEST program. We then employed AF3 to predict complex structures formed between each BCR sequence and the autologous E2 consensus sequence. Epitopes were defined as E2 residues located within a 5Å proximity to the predicted BCR structure.

Results: AF3 successfully predicted most benchmarked HCV E2-Ab interfaces, with accuracy varying across E2 antigenic domains. Epitope prediction accuracy correlated positively with the confidence score provided by AF3, allowing us to define a threshold to assess prediction reliability. Remarkably, AF3 accurately identified novel epitope residues involving recently resolved E2-Ab complexes. At the analysed time point, VH1-69 encoded the majority of studied BCRs, followed by VH3-23 and VH1-18. Among all reliable AF3-predicted E2- BCR structures, most BCRs were predicted to target antigenic region 3 (AR3) and CD81-binding loop on E2.

Discussions: Our findings highlight the capability of AF3 in predicting HCV E2-Ab interactions, even extending to novel E2 variants and Abs. This suggests that AF3 could be broadly applicable for Ab epitope mapping in HCV, and potentially other viruses. Identification of AR3 and the CD81-binding loop as primary early BCR targets, along with diverse VH gene usage, facilitates further studies of early BCRs essential for effective viral clearance.

Conclusion: This study shows that AF3-based computational epitope mapping reliably identifies early-stage BCR epitopes targeting E2 in an HCV super clearer, providing novel insights into the initial immunological determinants associated with natural viral clearance.

References: [1] Underwood, A.P., Gupta, M., Wu, B.R., Eltahla, A.A., Boo, I., Wang, J.J., Agapiou, D., Abayasingam, A., Reynaldi, A., Keoshke rian, E. and Zhao, Y., 2024. B-cell characteristics define HCV reinfection outcome. Journal of Hepatology, 81(3), pp.415-428

Abstract #4

Developing a mRNA-based HCV vaccine

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Hepatitis C virus (HCV) poses a significant global health challenge, affecting an estimated 71 million people worldwide. It's diverse genotypes and multiple immune evasion mechanisms underscore the need for innovative vaccination strategies. The viral glycoprotein E2 harbors crucial neutralising antibody epitopes, yet immune evasion mechanisms hinder the generation of broadly neutralising antibodies (bNAbs). Our research has discovered a minimised form of E2 (E2Δ123), characterised by the removal of hypervariable regions known to act as immune decoys and induce the production of immunodominant non-neutralising antibodies.

E2Δ123 demonstrates enhanced presentation of bNAb epitopes and minimal production of non-neutralising antibodies. Additionally, E2Δ123A7, a modified variant, with 7 cysteine residues mutated to alanine, offers a consistent monomeric structure. This allows for the potential to serve as an immunogen composed of repetitive monomers, such as virus-like particles, effectively exposing a multitude of the required epitopes to elicit a robust immune response. Moreover, the homogeneous monomeric structure of E2Δ123A7 facilitates the development of vaccine formulations that can be easily standardised and manufactured at scale. Here we show synthetic mRNA production of E2Δ123 and E2Δ123A7 yields proteins with conformational similarities to their DNA-produced counterparts, effectively binding bNAbs and the entry receptor CD81.

Vaccination studies in small animals have demonstrated that mRNA-based E2Δ123 and E2Δ123A7 induce immune responses equivalent to purified protein vaccines, suggesting potential broad protection against HCV genotypes. Moreover, mRNA technology enables rapid, flexible, and cost-effective vaccine production, offering promise for combating HCV infection globally.

Abstract #5

Introduction of liver-resident memory T cell immunity against HCV by mRNA vaccination

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Introduction: Chronic hepatitis C virus (HCV) infection affects an estimated 58 million people worldwide and remains a leading driver of cirrhosis and hepatocellular carcinoma. Although direct-acting antivirals achieve >95 % virological cure, their cost, limited availability and the fact that ~79 % of infections are undiagnosed mean new cases still outpace cures. Modelling by the Burnet Institute indicates that even a partially effective vaccine could prevent >1 million new infections and save >US $11 billion in healthcare costs this decade. Increasing evidence points to liver-resident memory $CD8^+$ T (T_{RM}) cells as critical mediators of protection against hepatotropic pathogens. We therefore asked whether an mRNA platform could be engineered to establish robust intra-hepatic T_{RM} responses against HCV.

Methods: Nucleoside-modified mRNA encoding the non-structural polymerase NS5B from HCV genotype 3a was encapsulated in three formulations (i) ALC-0315 or ii) MC3 (Onpattro) lipid nanoparticles (LNPs) or (ii) electrostatically complexed with DOTAP/DOPE liposomes to form lipid-complexed RNA (LPX). To enhance immunogenicity, an invariant NKT-cell agonist, α- galactosyl-ceramide analogue (α-GC_B), was co-formulated with LPX. Six- to eight-week-old female BALB/c mice (n = 5–6 per group) received 5 µg mRNA per dose: ALC-0315-LNP intramuscularly or intradermally on days 0/14; MC3-LNP intravenously on days 0/14; LPX ± α-GCB intravenously on day 0 with or without a homologous boost on day 30. HCV-specific $CD8^+$ T cells were quantified by using MHC class I tetramer ($K^d NS5B_{451-459}$), IFN-γ ELISPOT and intracellular cytokine staining in both liver and spleen.

Results: All formulations induced NS5B-specific $CD8^+$ T cells systemically and in the liver. However, only LPX-mRNA induced liver-resident T_{RM} cells. Co-delivery with aGC_B adjuvant elicited a significantly stronger immune response and a higher frequency of T_{RM} cells compared to non-adjuvanted LPX, via iNKT cells. Furthermore, boosting with a second LPX dose 30 days after the prime vaccination led to a significant increase in the frequency of NS5B-specific T_{RM} cells in the liver.

Discussion and conclusion: LPX's hepatic tropism, coupled with the strong NKT cell signal provided by α-GC_B, creates a cytokine milieu favourable for T_{RM} imprinting and efficiently seeds high frequency polyfunctional liver resident T_{RM}. As a T cell–based vaccine against HCV should ideally generate localised immune responses within the liver, this study provides key insights toward achieving that objective. The findings highlight LPX-mRNA aGC_B, vaccines, as a promising strategy for developing HCV vaccines that target protective intrahepatic T cell responses. Because LPX is prepared by simple mixing of mRNA with pre-formed liposomes, the platform is readily adaptable to multi-genotypic HCV antigens.

Abstract #6

Landscape of epitopes targeted by T cells during Hepatitis C virus infection

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Introduction: A prophylactic vaccine is essential for eliminating Hepatitis C virus (HCV), but HCV vaccine development remains a challenge. A key factor is the virus's large genetic diversity and high mutation rate. T cell-mediated immunity has been demonstrated to be critical for protection from HCV, yet knowledge of the overall HCV epitope landscape remains limited. This knowledge gap hampers the identification of epitope targets that can elicit broadly protective immune responses across diverse HCV genotypes and human populations. To address this, we aim to define a comprehensive T cell epitope landscape for HCV by integrating immunological and viral sequence data.

Methods: We analyzed experimentally determined HCV T cell epitope data from the Immune Epitope Database (IEDB), comprising over 6,000 T cell assays. The dataset includes diverse genotypes, source proteins, assay methods, HLA associations, and other metadata features. Extensive curation resolved issues including missing metadata, duplicate entries, and absent source protein annotations. To map epitopes to their source proteins, we utilized reference sequences available at the HCV-GLUE database and a sliding window approach. For epitopes lacking exact matches, we applied a 70% sequence identity threshold to account for HCV's variability. Immunodominant epitopes were identified by calculating response frequency (RF) as the fraction of subjects testing positive for an epitope across studies. Epitope conservation, defined as the fraction of available protein sequences for a HCV genotype that contain the exact epitope sequence, was assessed using a string-matching approach. We averaged the per-genotype conservation values across the seven major HCV genotypes to obtain a mean cross-genotype conservation score for each epitope.R**esults:** We found 1,601 human T cell assays corresponding to 332 unique epitopes associated with 50 HLA alleles. These assays were derived from 1,033 HCV-infected subjects across various studies. The majority of assays (88%) were linked to HLA class I alleles. Source protein mapping revealed that most of the epitopes (77%) were derived from non-structural proteins, with 38% of epitopes originating from the NS3 protein. RF analysis identified 16 epitope-HLA pairs with consistently high RF (≥0.5) across multiple studies, suggesting their immunodominance and immunoprevalence. Moreover, 12 epitopes exhibited a high mean cross-genotype conservation (≥0.8), with five of them demonstrating exceptional conservation (≥0.9) per genotype for all genotypes, supporting their potential to serve as cross-genotypically-conserved vaccine targets.

Discussion: Our analysis presents the first comprehensive landscape of HCV T cell epitopes by combining immunological assay data with viral sequence information. The predominance of epitopes in non-structural proteins, particularly NS3, aligns with prior findings on their immunogenicity, making them promising candidates for T cell-based vaccine design[1]. The preponderance of HLA Class I restricted epitopes highlights the importance of cytotoxic T lymphocytes in HCV immunity. Conservation analysis shows that, despite HCV's high genetic diversity across genotypes, there are epitopes that remain highly conserved. Identifying immunodominant, immunoprevalent, and conserved epitopes across multiple HCV genotypes provides the foundation for intelligently selecting vaccine antigens capable of eliciting broad and durable immune responses. Further investigation into their HLA population coverage is warranted to evaluate their inclusion in vaccine formulations. **Conclusions:** By systematically integrating immunological assay data from IEDB with sequence conservation analysis from HCV-GLUE, we have defined a comprehensive landscape of HCV T cell epitopes characterized by multiple attributes including conservation within and across HCV genotypes, response frequency, and HLA association. This epitope landscape provides concrete targets for next-generation HCV vaccine development that can potentially overcome the challenges posed by HCV diversity. **References:** [1] J. R. Bailey, E. Barnes, and A. L. Cox, "Approaches, Progress, and Challenges to Hepatitis C Vaccine Development," Gastroenterology, vol. 156, no. 2, pp. 418–430, Jan. 2019.

Abstract #7

Conductometric biosensing platform for early diagnosis of Hepatitis B and C

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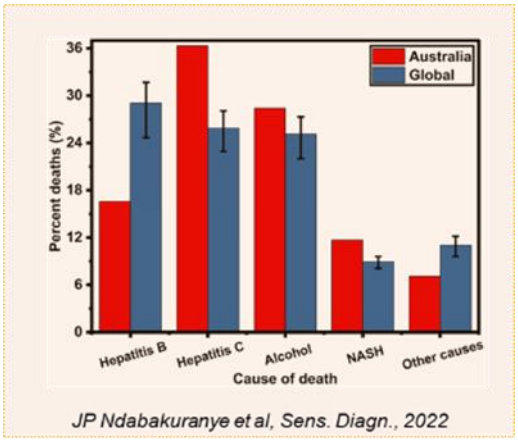
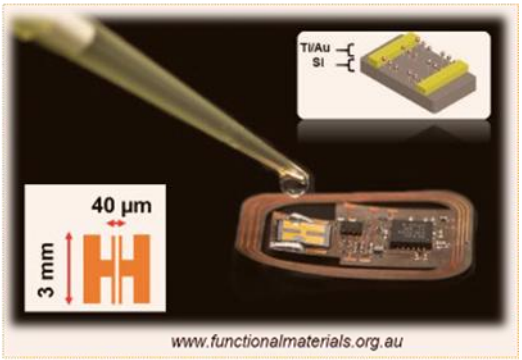
Introduction: Hepatitis B (HBV) and Hepatitis C (HCV) are major causes of liver-related morbidity, including hepatocellular carcinoma and cirrhosis, and constitute significant national (Australia) and global health burdens. Early diagnosis is critical for timely intervention but remains challenging due to the asymptomatic nature of early infection. Current diagnostic modalities rely heavily on centralised laboratory infrastructures, limiting accessibility, particularly in underserved communities. To bridge this diagnostic gap, we propose a saliva-based, conductometric biosensing platform designed for early point-of-care (PoC) detection and monitoring of HBV and HCV infections.
Methods: In this presentation, we propose a conductometric biosensing approach for early diagnosis of HVB and HVC. This platform leverages high-resistivity silicon as the active sensing substrate with optimised surface chemistry for enhanced biomarker bonding to detect a panel of strategically selected inflammatory biomarkers shared for HBV and HCV. Building upon previous work involving cardiac and COVID-19 biomarker detection, our biosensors can be integrated into a fully packaged, multiplexed device featuring Bluetooth data transmission to a mobile app interface. Minimal sample volumes (<10 µL) can be used to ensure usability ease and patient comfort.

Results: Preliminary findings from our biosensing platform have demonstrated high specificity and sensitivity across six orders of magnitude for cardiac and COVID-19 biomarkers, with performance metrics benchmarked against Enzyme-Linked Immunosorbent Assay (ELISA). A fully integrated prototype featuring wireless communication and user-centric design principles has been successfully fabricated. Early work suggests that the conductometric approach is robust, sensitive, and suitable for adaptation to a broad range of targets. Future experiments will focus on translating these successes to HBV/HCV biomarker detection.





Discussion: The ability to detect multiple biomarkers from a non-invasive saliva sample offers a transformative opportunity to shift HBV/HCV diagnostics from centralised laboratories to home-based environments. If validated, this platform could empower early detection, support timely clinical decision-making, and reduce the burden of disease progression. Furthermore, its portability and simplicity would enhance accessibility in remote communities, aligning with Australia's national hepatitis elimination goals for 2030 and supporting WHO strategies for global hepatitis control.

Conclusions: This proposed platform represents a promising avenue for advancing early HBV and HCV POC detection. By facilitating non-invasive, rapid, and decentralised testing, the technology could significantly contribute to reducing hepatitis-related morbidity and mortality. Further validation studies are essential to realize its full potential and position it for clinical and translational applications.

Abstract #8

Implementation of a novel tiling PCR for use in HCV diagnostics

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Introduction: Australia is aiming to end the transmission of hepatitis C virus (HCV) by 2030. The National Hepatitis C Strategy highlights the importance of timely high-quality data to improve public health responses. Such data could be obtained through a molecular epidemiology surveillance system for HCV transmissions, but for successful implementation a robust, time- and cost-effective method for HCV sequencing must first be developed. To fulfill this purpose, a novel tiling PCR assay was designed to rapidly sequence HCV genomes in under 24 hours. However, the utility of this method in a real-world (diagnostic) setting needs to be assessed.

Methods: Residual HCV-positive samples from a single site (Prince of Wales Hospital, Sydney) were identified, retained, and amplified using the novel PCR method in overlapping segments of ~1kb. After Oxford Nanopore sequencing, the resulting HCV genomic sequences underwent assessment for putative clusters of transmission using HIVTrace. A cluster was defined by a shared genetic identity of ≥98% between sequences generated from ≥2 samples. Deidentified sample metadata, including patient age range, gender and date of sampling were stored in a secured RedCap database and overlayed on identified clusters to assess for linkage traits.

Results: Amplification was attempted on 134 residual HCV+ samples using the HCV tiling assay, and 98% of these (n=131) generated usable genomic sequence, with an average sequence length of 7592 bp (maximum 9080 bp). All samples that were successfully sequenced could be genotyped; only 33% of samples had been genotyped prior to using the tiling PCR. From the 131 sequences, 16 putative transmission clusters were identified, with 53 samples (40% of the data set) forming part of a cluster. Clusters ranged in size from two to nine patient samples. Eleven clusters contained samples that were collected more than two months apart (median four months), likely indicative of ongoing transmission events. Samples were found to cluster by gender, and a trend was seen for samples to cluster by age range.

Discussion and Conclusions: A high success rate at generating sequence, coupled with the ability to use the sequence for genotyping as part of patient care, means that the novel tiling PCR has high utility in a diagnostic setting. Moreover, the resulting data can be used for transmission cluster analysis, with cluster characteristics at a single site similar to that which has been observed in other jurisdictions in Australia. Future work will determine if there are other epidemiological traits that link these putative clusters of transmission. The novel tiling PCR method will also be used in a pilot study in the NSW justice system, to determine the utility of using molecular epidemiology in near-real-time to curb HCV transmissions.

Abstract #9

A sensitive PCR assay to precisely quantify hepatitis B virus covalently closed circular (ccc)DNA

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Introduction: Covalently closed circular (ccc)DNA is a driver of persistence of hepatitis B virus (HBV) infection. The inability to quantify cccDNA sensitively and accurately has prevented research into a hepatitis B cure. Here, we describe a novel, sensitive method to quantify HBV cccDNA in patient tissues.

Methods: Assay specificity and sensitivity were determined for a pan-genotypic cccDNA inversion and quantification PCR (pan-cinqPCR) assay developed to simultaneously quantify cccDNA and cellular gene RNaseP. As negative controls, we isolated HBV virion DNA from mouse serum or cell culture supernatant. As positive controls, we used recombinant cccDNA (rcccDNA) and DNA extracts of infected primary human hepatocytes. Human tissue samples from two clinical cohorts were analysed: 1) resected liver tissue from 33 patients with hepatitis B: 18 with chronic infection (HBsAg- positive) and 15 who had cleared the virus (HBsAg-negative); and 2) fine needle aspirates (FNA) from 11 patients with hepatitis B.

Results: Pan-cinqPCR could readily distinguish between cccDNA and virion-derived HBV DNA at a ratio of ~1/1000 for both HBV genotype D and A. Sensitivity was ~1 copy of cccDNA per digital PCR (dPCR) reaction for both genotypes. We quantified intrahepatic cccDNA in tissue resected from patients infected with HBV genotype B or C. Compared to HBsAg- positive patients (n = 18), liver tissues of HBsAg-negative patients (n = 15) contained significantly lower levels of cccDNA (means = 0.0013 vs 0.0155 copies/per cell, p=0.001). The assay required a minimal input of <10 infected cells, allowing analysis of FNA samples.

Discussion: We show that lower (but not undetectable) cccDNA levels were significantly associated with HBsAg-loss. Pan- cinqPCR solves key issues in quantifying cccDNA: discriminating between low copy cccDNA forms (1-10 copies per cell) and the high levels of replicative intermediate DNA (100s per cell) including rcDNA, dslDNA and ssDNA; high precision through conservation of cellular DNA to normalise results; and use with clinical samples.

Conclusion: Measuring HBV cccDNA in hepatocytes from minimally-invasive FNA will provide a valuable monitoring tool for clinical trials of anti-HBV therapies and directly influence health decisions in clinical care of people with hepatitis B.

Abstract #10

Investigating HBV DNA integration and its role in genomic instability through HBx expression

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Introduction: Most Hepatitis B virus (HBV)-associated liver cancers contain viral integrations, suggesting that these phenomena are linked, but the mechanism remains unknown. HBV integration occurs at double stranded DNA breaks in the host genome in ~1/10,000 cells. Integrations can encode the HBV X protein (HBx), which has been reported to induce genomic instability by degrading the structural maintenance of chromosome 5/6 (SMC5/6) complex. However, integration-derived HBx often contains C-terminal truncations, which may affect its function. We hypothesise that integration-derived HBx enables increased DNA damage (like wild type HBx), inducing more integrations, fostering a feed-forward loop and HCC development.

Methods: We characterized integrated HBV DNA by generating 118 HepG2-NTCP-derived clones using a recombinant reporter HBV expressing a Zeocin-resistance gene (in place of HBs). Integrations were quantified and sequenced using inverse PCR coupled with digital droplet PCR or targeted sequencing. Expression of HBx transcripts was measured using quantitative 5' rapid amplification of cDNA ends (5'RACE). HBx function was analyzed using Western blot to quantify SMC5/6 and *γ*-H2AX (marker for DNA damage). The functional consequences of HBx were also determined by infecting the clones with wild-type HBV and quantifying integration rates using inverse PCR.

Results: The HepG2-NTCP clones each contained 1-2 copies of integrated HBV DNA and the motif for SMC5/6 degradation (amino acids 45 to 140) was complete in >85% of integrations. Quantitative 5'RACE revealed that HBx expression in the clones ranged from 3 - 518 copies per µg RNA, on average ~4-fold lower than HBV-infected cells expressing HBx from cccDNA. The integration-derived HBx appeared to be functional: compared to parental cells, SMC5/6 (DNA stabilizer complex) was reduced by 1.9-fold (p=0.024, two-tailed t-test) and γ-H2AX (DNA damage marker) was increased by 3.8-fold (p=0.024, two-tailed t-test) in cells with transcriptionally-active integrations. These changes were not as marked in clones with low HBx expression [no reduction in SMC5/6 and 2.4-fold increase in γ-H2AX (p=0.1, two-tailed t-test)] showing the dependence of DNA instability on HBx expression. This increased DNA damage had functional consequences: cells with transcriptionally-active integrations were 2-12-fold more susceptible to additional integrations after a new HBV infection.

Discussion: Our data indicate that cells with HBV integrations can express functional HBx and may be more susceptible to additional integrations, promoting a self-amplifying process. The resultant exponential increase in genomic instability then fuels accelerated acquisition of cancer driver mutations.
Conclusions: Integration-derived HBx could be key to driving liver cancer progression and may be a target for tumour prevention.

Abstract #11

A circulating biomarker of myofibroblasts is elevated in HBV infection but lacks a clear association with fibrosis.

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Metabolic dysfunction-associated fatty liver disease (MAFLD) drives chronic liver injury leading to fibrosis, which is the major correlate of adverse health outcomes in MAFLD. Hepatitis B virus also acts as a driver of chronic liver damage that can lead to cirrhosis. The greatest mortality in both MAFLD and HBV is during end- stage fibrosis. There is a clear and urgent medical need for more accurate non-invasive tests to detect advanced liver fibrosis. We previously showed that fibroblast activation protein (FAP) is strongly and specifically expressed by myofibroblasts and activated stellate cells in human cirrhosis of many etiologies. Here, we investigated the utility of circulating FAP (cFAP) to detect fibrosis in MAFLD and HBV.

The FAP molecule shed from cell surfaces as an active enzyme is stable in human serum and plasma. We applied our quantitative, specific, rapid, one-step FAP enzyme assay to sera from a bariatric cohort (training cohort P, n = 160, 20.3% F3-F4), a bariatric/MAFLD cross-validation cohort (n = 332, 11.4% F3-F4) and an HBV cohort (n=623, 2.6% F3-F4). Fibrosis was associated with cFAP in MAFLD cohorts ($p < 0.05$). We coined the term FAP Index for a multi-variate model that was developed, which combines cFAP with age, diabetes status and ALT to evaluate liver fibrosis risk, especially in individuals who had an indeterminate result in a FIB4 test. The training cohort AUROC was 0.875 with a negative prediction value of 92% and a positive prediction value of 95% for FAP Index, indicating high accuracy. The AUROC for the cross-validation cohort was 0.84, with 98.5% specificity and 55% sensitivity. By serial application of FIB4 (age, AST, platelet count and ALT) then the FAP Index, the proportion of indeterminate results more than halved, to < 15%. No direct association was found between cFAP and fibrosis in the HBV cohort. However, patients with active viral replication showed significantly increased cFAP compared to virus inactive patients ($p = 0.014$). Additionally, the HBV cohort had significantly elevated baseline cFAP activity compared to the MAFLD cohort, with or without severe fibrosis ($P < 0.0001$).

We present a novel, inexpensive diagnostic tool for assessment and triage of individuals with diabetes, steatosis or obesity. While cFAP does not provide clear insights into disease progression of HBV, it suggests a possible contribution from the immune system in cFAP activity. Additional investigations are needed because inclusion of our FAP index algorithm in clinical fibrosis assessments could greatly improve economies of time and expense and lower the need for obesity-specialised secondary testing.

Abstract #12

High-throughput pipeline detecting viral integration reveals evidence for oncogenic insertional mutagenesis in domestic cat hepatitis B virus-associated hepatocellular carcinoma

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Introduction: Hepadnavirus infections and viral DNA integration have been associated with hepatocellular carcinoma (HCC) in humans and other species. Understanding molecular mechanisms of integration has been challenging, given the numerous viral DNA forms in infected cells with identical sequences. We developed a targeted sequencing pipeline to characterise and quantify hepadnavirus integrations. We established this pipeline using *in vitro* cell lines containing clonal HBV DNA integrations from *de novo* infection. We then used this approach to investigate viral integrations in cats infected with a newly-discovered virus, domestic cat hepatitis B virus (DCHBV).

Methods: A replication-deficient reporter-HBV virus encoding zeocin-resistance was used to generate, select, and isolate HepG2-NTCP clones with integrations. Virus-cell junctions were detected by targeted sequencing and validated by inverse PCR. Our custom bioinformatics pipeline identified chimeric reads, assembled them into contigs, and annotated them using HOMER. Formalin-fixed paraffin-embedded liver tumour samples from 16 DCHBV-positive cases and 4 DCHBV-negative controls were analysed using our pipeline and confirmed with whole genome sequencing.

Results: HBV integrations were detected in 108 of 108 cell clones analysed. HBV DNA inversions (~20% of integrations) and chromosomal translocations (~8%) were detected, suggesting genomic rearrangements seen in patient tumours may occur directly after infection. Integrations were detected in 11 out of 16 HCCs from DCHBV-infected cats, but none of the 4 HCC from DCHBV-negative cats. Integration sites showed a 2.4-fold enrichment in host exons and introns, similar to HBV integration (1.9- fold). Significant enrichment (z-score = 3.14) of integrations was observed in chromosome E2 proximal to the *CCNE1* oncogene (6/45 unique integrations).

Discussion: We establish a high-throughput targeted sequencing pipeline to detect hepadnaviral integrations. Using this approach, we show that HBV DNA integrations can undergo genomic rearrangements directly after infection that may potentially promote HCC. Furthermore, we provide the first evidence not only for the existence of DCHBV DNA integrations but also their role in carcinogenesis via insertional mutagenesis within or near *CCNE1*.

Conclusions: This work highlights viral integration as an oncogenic mechanism in cats infected with DCHBV. Our pipeline enables cost-effective and high-throughput detection of hepadnaviral integration

Abstract #13

Inhibitory activity of lactic acid against *L. iners* and BV-associated vaginal bacteria to prevent HIV transmission.

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Introduction: In 2023, the global HIV burden remains high with women and girls accounting for 44% of the 1.3 million new HIV infections (UNAIDS). A common vaginal condition that increases a women's risk of acquiring HIV, is bacterial vaginosis (BV), characterised by a diverse, *Lactobacillus*-depleted vaginal microbiota and local inflammation. We have discovered that actic acid (LA), a metabolite produced by *Lactobacillus s*pecies, has immunobiological properties that may help protect women from acquiring HIV. This study aimed to investigate the bactericidal activity of LA against vaginal bacteria.

Methods: To determine bactericidal activity, cultures of ~10^6 colony forming units (CFU) of *Lactobacillus iners* (ATCC 55195), *Lactobacillus crispatus* (ATCC 33820), and *Gardnerella vaginalis* (ATCC 14018) were treated with 1% DL-LA pH 3.8 at 37°C for 1h under anaerobic conditions. Viable bacteria were quantified by CFU/ml. For competition assays, mixed cultures of G. *vaginalis, L. iners*, and *L. crispatus* were incubated at 37°C for 24h with 1% DL-LA in S-Broth at pH 4.5 under anaerobic conditions. After treatment, mixed cultures were subjected to taxon-specific PMA-qPCR to quantify viable bacteria. For proteomics, *L. crispatus* cultures were adjusted to 0.5 OD600 and incubated with 1% DL-LA in MRS broth for 6h. Washed bacterial pellets were subjected to LC-MS/MS and differential protein abundance was quantified. To determine morphological changes, *L. crispatus* and *L. iners* cultures were adjusted to 1 OD600 and treated with PBS pH 7, PBS pH 3.8, or 1% DL-LA pH 3.8 for 1h at 37°C. Bacterial pellets were washed, fixed, and processed for TEM.

Results: Treatment with 1% DL-LA pH 3.8 for 1h showed selective bactericidal activity against *G. vaginalis* (50,000-fold reduction, n=3, $p<0.0001$) and *L. iners* (128-fold reduction, n=6, p=0.003), whereas *L. crispatus* (n=3, $p>0.93$) viability was unaffected compared to untreated bacteria. LA's activity was more potent than media adjusted to pH 3.8 with HCl, ndicating an LA-specific effect. Treatment of mixed cultures for 24h at 37°C with 1% DL-LA pH 4.5 resulted in undetectable evels of *G. vaginalis* and *L. iners*, and increased *L. crispatus* copy numbers. Top differentially expressed proteins in *L. crispatus* after LA treatment included D-lactate dehydrogenase (LDHD), GroEL, and transcription factor GreA. Pilot experiments showed *L. iners* treated with 1% DL-LA for 1h had altered morphology compared to HCl-treated bacteria, while *L. crispatus* cell walls remained mostly unaffected.

Discussion: LA at a physiological concentration and pH shifts a mixed culture dominated by *L. iners* and *G. vaginalis* towards *L. crispatus* dominance. We identified a set of *L.crispatus* proteins differentially expressed after LA treatment, that include acid stress response proteins observed in non-vaginal *Lactobacillus* spp.

Conclusions: LA targets key BV-associated vaginal bacteria and the less stable *L. iners*, which persists after metronidazole treatment. These findings suggest a potential intervention to prevent BV recurrence and, consequently, HIV acquisition.

Abstract #14

Lactic acid, a key *Lactobacillus* metabolite, reduces HIV internalisation and migration through the cervicovaginal epithelial barrier

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Background: Young women and adolescent girls in sub-Saharan Africa are disproportionately affected by HIV. There are several factors that influence viral transmission in women, including effects of the vaginal microbiota and their acid metabolites, on epithelial barrier integrity. An optimal *Lactobacillus*-dominated cervicovaginal microbiome lowers the female reproductive tract (FRT) pH to below 4.5. *Lactobacillus* species such as *L. crispatus* reduce the risk of HIV acquisition by decreasing genital inflammation, which disrupts the FRT epithelial barrier and enables the virus to infect target cells in the submucosa. We have discovered that lactic acid (LA), a key metabolite of optimal *Lactobacillus* spp. strengthens the cervicovaginal epithelial barrier. However, LA's ability to inhibit passage of cell-free virus between epithelial cells (transmigration) or HIV uptake (internalisation) and transcellular migration through epithelial cells (transcytosis), as well as mechanisms underlying these interactions, are unknown.

Methods: Immortalised human ectocervical (Ect1) and vaginal (VK2) epithelial cell lines were cultured in a transwell system, treated apically for 1 h with 0.3% LA (pH 3.9), lactate (pH 7.0), or acidified media (pH 3.9, HCl adjusted). At 24 h post-treatment, cells were thoroughly washed, and HIV (HIVBa-L, 10 ng p24) or HIV infected mononuclear target cells derived from peripheral blood (PBMCs) were added apically for 24 h, after which p24 was quantified in basolateral supernatant and epithelial cell lysates. In addition, Ect1 and VK2 cells were treated simultaneously with LA and toll-like receptor (TLR) agonists poly I:C (PIC, TLR3), FSL-1 (TLR2/6), or PAM3CSK4 (TLR1/2). Surface expression of intercellular adhesion molecule-1 (ICAM-1) was quantified by flow cytometry.

Results: LA treatment (pH 3.9), but not HCl (pH 3.9) or lactate (pH 7.0) reduced HIV migration to the basolateral supernatant by 72 ± 5.8% in Ect1 (mean ± SEM) and 89 ± 6.8% in VK2 cells relative to untreated cells. LA reduced internalised virus in cell lysates by 49 ± 7.3% in Ect1 and 67 ± 10% in VK2 cells ($p < 0.05$, n= 5-11). LA additionally reduced HIV internalisation from infected PBMCs ($p < 0.05$). Stimulation of Ect cells with PIC significantly increased ICAM-1 expression by 1.6-fold (n= 4, $p < 0.05$). This increase was abrogated 1.4-fold by treatment with LA, but not HCl-acidified media or lactate. Similar findings were observed for Ect cells treated with FSL-1 and PAM3CSK4, as well as TLR-stimulated VK2 cells.

Discussion: LA treatment significantly reduced HIV transmigration and internalisation in FRT epithelial cells. LA treatment reduced ICAM-1 expression on FRT epithelial cells in the presence of TLR stimulation, indicating a potential protective mechanism for LA. Inhibition of ICAM-1 upregulation is specifically mediated by the uncharged form of LA, which is present in the FRT at an optimal, low pH.

Conclusions: This study is the first to demonstrate a direct effect of LA on HIV migration through epithelial cells and provides novel insights into its potential mechanism of action. These findings have implications for developing novel strategies to prevent HIV transmission in women utilising the protective properties of optimal FRT microbial metabolites. LA-containing intravaginal gels are undergoing preclinical studies to advance to a phase I study in women.

Abstract #15

Understanding Inflammatory Mononuclear Phagocyte Heterogeneity in Human Anogenital Tissue

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Introduction: Anogenital inflammation is a critical risk factor for the sexual transmission of HIV. Crucially, the primary HIV preventative treatment, pre-exposure prophylaxis (PrEP), can be ineffective in inflammation. However, the key inflammatory HIV target cells in anogenital tissues are poorly understood. Mononuclear phagocytes (MNP) are innate immune cells and known HIV targets, comprising of Dendritic cells (DC), macrophages and Langerhans cells (LC). DCs and LCs are professional antigen presenting cells and responsible for sampling and delivering invading pathogens to CD4 T cells, subsequently causing systemic HIV infection. This study aims to identify and characterise all known mononuclear phagocytes in inflamed human anogenital tissues using high-parameter flow cytometry and investigate their role in HIV transmission.

Method: Through extensive collaborations with surgeons across Western Sydney our lab has access to all human anogenital tissues including labia, vagina, cervix, foreskin, glans penis, fossa navicularis, anus and rectum. We process these tissues within 30 minutes of removal from the body utilising optimised tissue digestion protocols and perform flow cytometry with a 26- parameter panel or HIV uptake and infection assays.

Results: We have demonstrated that MNP populations are highly heterogenous across different anogenital tissues, both in cell composition and HIV binding capacity. Furthermore, inflammatory MNPs in human anogenital tissue have different phenotypical expressions and functions compared to healthy tissue. We have shown that the novel inflammatory Axl+Siglec-6+ DCs (ASDCs) are present in inflamed anogenital tissues, where HIV transmission occurs, and demonstrated blood-derived ASDCs are capable of both HIV uptake and infection. Furthermore, we confirmed that the recently identified DC3s, which are increasingly recognised as a central inflammatory DC, are enriched in inflamed anogenital mucosa. Yet, their interactions with HIV remain unexplored.

Conclusion: As anogenital inflammation in inextricably linked with sexual HIV transmission, a comprehensive exploration and profiling of inflammatory MNPs will ultimately allow for the development of targeted HIV therapeutic strategies and modified PrEP regimes.

Abstract #16

Epithelial dendritic cells: interactions with HIV in human penile mucosa

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Introduction: Antigen-presenting cells (APC) play a vital role in transmission of HIV by capturing the virus within the anogenital mucosa and then migrating to lymphoid tissues where they interact with and transfer the virus to CD4 T cells—the primary targets for HIV replication. Recently it has become apparent that HIV transmission is highly correlated with inflammation. The stratified squamous epithelium (SSE) forms the outermost layer of genital tissue, making it the first point of contact for sexually transmitted pathogens. Langerhans cells (LCs) were previously believed to be the only APCs present in the SSE, but we have shown that dendritic cells (epi-DCs) are also present. Notably, epi-DCs are more abundant than LCs in genital tissues and demonstrate greater efficiency in capturing and transmitting HIV to CD4 T cells. Here we use human genital tissues to examine if these cells migrate towards HIV and to determine relative abundance in steady-state and inflamed tissues.

Methods: HIV-1 was topically applied to penile genital mucosa (fossa navicularis) and their migration quantified *in situ* using immunofluorescence microscopy. We also utilised immunofluorescence microscopy to compare the proportions of epi-DCs, LCs and T cells in steady state and inflamed human foreskin derived from Papua New Guinea.

Results: In fossa navicularis, we utilised a novel migration marker (CD1a) to show that topical applications of HIV increased DC migration towards the HIV virus particles. We also saw a general trend of increased epi-DC density in the epithelium of these genital tissues. We found that epi-DCs were not enriched in inflamed foreskin however CD4 T cells were.

Discussion: Expanding on our earlier findings that epi-DCs are efficient at transmitting HIV to CD4 T cells, we now demonstrate that these cells are not only part of the initial immune response but also are migratory cells within the genital mucosa. This helps explain their preferential role in viral transmission. Therefore, interfering with epi-DC migration may represent a promising prophylactic strategy by preventing their movement toward the virus and thereby limiting transmission. Although epi-DCs were not enriched in inflamed tissues, T cells were raising the possibility that transfer of HIV from epi-DCs to T cells may occur with the SSE.

Conclusions: Epi-DCs migrate towards HIV in human penile tissue but are not enriched in inflamed tissue but T cells are. These characteristics suggest a potential for new PrEP regimes in patients who do not respond to current treatments, as it is currently known that current treatments are not effective across an inflamed mucosa. Identifying the pathways that lead to the migration towards HIV and then to CD4 T cells for transfer, may have significant implications in new strategies for PrEP as well as the possibility of targeting these cells for vaccine delivery.

Abstract #17

Role of Epithelial Dendritic Cells in HIV Transmission and Their Potential as Vaccine Delivery Vehicles.

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Introduction: In 2019, our lab identified dendritic cells (epi-DCs) within the stratified squamous epithelium of the skin and type II mucosa, the body's primary barrier against pathogens. This discovery challenged the long-standing belief that Langerhans Cells (LCs) were the sole antigen-presenting cells (APCs) responsible for initiating adaptive immunity in these tissues. Following their identification, we compared epi-DCs to LCs and found that epi-DCs were more abundant in genital mucosa and functionally superior in facilitating HIV transmission to CD4+ T cells. These findings suggest that epi-DCs may play a pivotal role in HIV infection and immune priming. In this study, we further explore the capacity of epi-DCs to prime adaptive immunity and investigate their role in HIV transmission by examining their localisation in response to topical HIV application.

Methods: To assess the ability of epi-DCs to drive T cell activation, we isolated epi-DCs and LCs from human tissues using fluorescence-activated cell sorting (FACS). These cells were then co-cultured with allogeneic naïve T cells, and T cell proliferation was measured using flow cytometry. Additionally, cytokine production and T helper cell polarisation were analysed using LEGENDplex assays. To investigate the effects of topical HIV application on epi-DC localisation within genital tissue, we applied HIVbal to penile genital mucosa and quantified migration *in situ* using immunofluorescence microscopy.

Results: Our results demonstrate that epi-DCs were significantly more efficient than LCs in promoting T-cell activation. Epi-DCs induced, on average, a 14-fold increase in CD4+ T cell proliferation (n=5) and a 7-fold increase in CD8+ T cell proliferation (n=3). Furthermore, epi-DCs effectively polarised CD4+ T cells into distinct helper subsets. To explore their localisation in response to HIV, we identified a novel migration marker for epi-DCs. Leveraging this migration marker, we found that topical HIV application not only increased DC migration towards the virus but also resulted in a net increase in DCs within the epithelium of genital tissue.

Discussion: These results highlight the exceptional potential of epi-DCs as vaccine delivery cells. Their accessibility within the epithelium and strong ability to prime adaptive immune responses make them ideal candidates for targeted vaccine strategies. Additionally, their migratory behaviour in response to HIV further positions them as key facilitators of transmission. Building on our previous work demonstrating their ability to infect CD4+ T cells, we now show that they are not only present in the body's first line of defence against HIV but also migrate towards the virus within genital mucosa. Importantly, these findings suggest that targeting the migration mechanisms of epi-DCs could provide a novel approach for prophylactic strategies, potentially blocking their migration towards HIV and reducing transmission. Further research into these mechanisms could offer valuable insights for enhancing vaccine delivery or developing novel antagonistic prophylactics.

Conclusions: Epi-DCs are potent in priming adaptive immunity and play a crucial role in mucosal immunity. Their innate migration towards the virus *in situ* further implicates these cells as key facilitators of HIV transmission. Moreover, their high efficiency in driving T cell proliferation and polarisation makes them strong candidates for use as vaccine delivery vehicles. Identifying the pathways that regulate their migration could lead to new strategies for prophylactics as well as enhancing vaccine efficacy and HIV immunity.

Abstract #18

An *in situ* quantitative map of mononuclear phagocyte interactions with HIV across anogenital mucosal tissues using high parameter spatial proteomic techniques

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Tissue resident mononuclear phagocytes (MNPs) play critical roles in pathogen recognition and antigen presentation to CD4 T cells. Despite this, most of our knowledge of MNPs at the initial host-pathogen interface is derived from studies of *ex vivo* isolated cells. Detection of most pathogens *in situ* can only be achieved after multiple rounds of replication long after initial exposure. As such, we do not have a comparative map of initial MNP-pathogen responses in intact human tissues, especially in the context of inflammation.

We have developed a spatial proteomic analysis workflow that enables *in situ* quantification of immune cells within human mucosal tissues. We have also developed a model for investigating initial responses to transmission by topically applying HIV to mucosal surfaces and integrating virus-specific probes into existing imaging workflows, to further characterise host-viral interactions in initial transmission events.

We have constructed a comprehensive proteomic spatial atlas of the colorectal immune cell landscape. We found that within just 2 hours of exposure, HIV was enriched in dendritic cells, which rapidly clustered with CD4 T cells and trafficked the virus to lymphoid follicles. The lymphoid follicles then become early sanctuaries of high viral titres.

The application of high parameter imaging platforms and the development of our analysis pipelines significantly advance our understanding of early host defences to pathogens by (i) detailing the immune cell composition of key tissue niches and (ii) demonstrating that lymphoid follicles provide a sanctuary for pathogens following rapid trafficking. Importantly, this work provides a framework for *in situ* studies of DC responses to pathogens in human mucosal tissues.

Developing personalised treatment pathways for Hepatitis B using novel assays and fine needle liver aspirates

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Introduction: Hepatitis B is incurable, requiring long-term suppressive antiviral therapy. Treatment cessation is possible under international guidelines, but it is not widely practiced as outcomes are difficult to predict. After stopping treatment in HBeAg negative patients, ~20% of patients achieve functional cure (loss of HBsAg), ~30% maintain virological control and 50–70% relapse with risk of disease progression. This is because current peripheral biomarkers do not reflect the persistent viral forms in the liver, i.e. covalently closed circular DNA (cccDNA) and integrated DNA (iDNA). We hypothesise that lower levels of cccDNA and iDNA predict viral clearance, but these are challenging to quantity as invasive tissue sampling is required, traditionally core liver biopsies due to the low sensitivity of existing assays. Fine needle aspirates (FNAs) offer a less invasive method to assess these viral forms, but due to the low tissue yield better assays are needed.

Methods: Our lab has developed novel, highly sensitive PCR-based assays to measure cccDNA and iDNA in liver FNAs. We are recruiting adults with HBeAg-negative hepatitis B who have been on long-term antiviral therapy, with HBV DNA < 20 IU/ml for ≥ 3 years and HBsAg < 1000 IU/ml. Prior to treatment cessation, liver FNAs and blood samples are collected. Different cell populations in FNAs are quantified by flow cytometry, using fluorescently-labelled myrcludex B to identify hepatocytes, anti-CD45-PE for white blood cells and DAPI for dead cells. Immune cell populations are further characterised using standard antibody panels. Blood samples collected over a 4-year follow-up period will be used to monitor biomarkers including HBV DNA, HBV RNA, HBsAg, HBcAg, HBeAg, ALT and peripheral blood mononuclear cells.

Results: 25 recruited patients (target 130) had HBV DNA <20 IU/ml prior to treatment cessation and HBsAg ranged from 0.05 – 938 IU/ml. To date, baseline FNAs have been obtained from 25 patients and the procedure was well tolerated, with only mild (45%) or moderate (55%) pain reported. Average recovery time was ~48 hours with minimal post-operative care. Flow cytometry analysis of FNAs yielded an average of 3800 live hepatocytes per pass and further analysis of immune cell populations is in progress. After treatment cessation, most patients have remained well with minimal flares (ALT < 2x ULN and HBV DNA < 2000 IU/ml). Four patients cleared HBsAg (one with anti-HBs), and three resumed therapy due to flares. Those who achieved functional cure had lower baseline HBsAg (0.05–51.1 IU/mL) and those who resumed therapy had higher levels (374–856 IU/mL). We are now quantifying cccDNA and iDNA from FNA-derived liver tissue, using our highly sensitive assays.

Conclusion: Our preliminary data are in line with current literature as they show that lower HBsAg levels at treatment cessation are associated with a higher chance of achieving functional cure. Similarly, those with higher HBsAg have a higher risk of HBV DNA relapse. We are currently quantifying cccDNA and iDNA from liver FNAs and will assess the performance of these markers to predict clinical outcomes. We aim to explore associations between intrahepatic markers, clinical outcomes and blood-based biomarkers to develop a predictive clinical algorithm for personalised treatment decisions. Ultimately, this approach could help identify patients who can safely stop therapy and maximise chances of achieving hepatitis B cure.

Abstract #20

Developing CRISPR-Cas13b as a novel therapy for chronic hepatitis B virus infection

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Introduction: There is global consensus that new therapies are urgently required to target multiple stages of the hepatitis B virus (HBV) replication cycle to improve rates of functional cure. Although a DNA virus, RNA plays a key role in the HBV replication cycle, and represents a novel target for new therapies, which has been highlighted by previous RNA interference and antisense oligonucleotide studies. CRISPR-Cas13b is a bacterial endonuclease that can be re-purposed to target RNA in mammalian cells, including viral RNA. Cas13b has high specificity because of a 30 nucleotide CRISPR RNA (crRNA) that is designed to be complementary to the target RNA of interest. We have previously shown that CRISPR-Cas13b can be re- purposed to target the HBV RNAs to strongly reduce HBV replication and protein expression *in vitro* and can reduce sera HBsAg by 50% in a pilot *in vivo* study. Here, we expand on these studies and test Cas13b in HBV-infected primary human hepatocytes and in mice with persistent HBV replication using Cas13b mRNA as the delivery system.

Methods: Primary human hepatocytes were infected with HBV and then treated with Cas13b mRNA and crRNA five days post- infection. Secreted HBeAg and HBsAg were measured as markers of viral replication. In a pilot *in vivo* study, mice were hydrodynamically injected (HDI) with HBV plasmid, and then intravenously injected (IV) with lipid nanoparticle (LNP)- encapsulated Cas13b mRNA and crRNA eight weeks post-HDI. Sera HBeAg, HBsAg and viral loads were measured at several time points. To confirm LNP delivery of Cas13b to the liver, mice were intravenously injected with lipid nanoparticles encapsulating Cas13b mRNA tagged to nanoluciferase. Livers were harvested at different time points and imaged using the IVIS (Revvity).

Results: Cas13b strongly reduced secreted HBeAg and HBsAg from primary human hepatocytes compared to the non-targeting crRNA control. Cas13b-nanoluciferase expression was confirmed in the livers of mice up to four days post-IV. In an exciting finding, one out of three mice with persistent HBV replication cleared HBV infection one week post-IV of LNP/Cas13b in the pilot *in vivo* study.

Discussion: This is one of the first studies to use CRISPR-Cas13b *in vivo*, using mRNA and LNPs to deliver Cas13b. Our pilot *in vivo* study showed promise that Cas13b can suppress HBV replication after one dose of LNP/mRNA. Further *in vivo* work is underway. Multiple dosing of LNP/Cas13b is also being explored which may improve suppression of HBV infection after treatment.

Conclusions: As current HBV therapies rarely achieve HBV functional cure, new therapies are desperately needed. These studies expand on our previous findings that CRISPR-Cas13b can target the HBV RNAs to strongly reduce HBV replication and antigen expression. This further demonstrates the potential of using CRISPR-Cas13b as a novel treatment option for chronic HBV infection.

Abstract #21

Editing of HBV DNA and RNA *in vitro* and *in vivo* using a CRISPR/Cas9 Base Editor and CRISPR/Cas13 approach

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Introduction: Current hepatitis B virus (HBV) treatments do not target the HBV covalently closed circular DNA (cccDNA) minichromosome reservoir, HBV integrated DNA or HBV RNA. There is a desperate need to develop novel therapeutics that target cccDNA, integrated DNA and HBV RNA; to improve HBV cure rates.

CRISPR/Cas9 base editors (BEs) are a promising approach as they utilise the CRISPR/Cas9 guiding system to introduce specific C:G to T:A edits into target DNA without introducing insertions or deletions that hamper traditional Cas9 approaches. Edits can prevent protein expression with reduced likelihood of genome instability when targeting integrated HBV DNA. CRISPR/Cas13 works in a similar manner as CRISPR/Cas9 but instead targets RNA for degradation. The aim of this project is to analyse Cas9 BE single guide RNA (sgRNAs) targeting all HBV open reading frames (ORFs) and to test the efficacy of BEs and Cas13b in combination to simultaneously target HBV DNA and RNA, reducing HBV replication and protein expression *in vitro* and *in vivo*.

Methods: sgRNAs were designed to introduce premature stop codons to reduce HBV protein expression and replication. Screening for the best sgRNA per ORF was performed via plasmid transfection of HBV DNA, Cas9 BE and sgRNA into HepG2 cells. Secreted HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) were measured using quantitative serology whilst HBV x protein expression was investigated via western blotting. Southern blotting was also utilised to investigate intracellular core-associated HBV DNA and qPCR was used to measure secreted HBV DNA. After determining the best sgRNA for each ORF, an additional screening step was undertaken to determine the best Cas9 BE. The best acting Cas9 BE and sgRNAs from each ORF were also transfected as RNA in HepG2.NTCP cells. A combination of sgRNAs as well as a combination of one sgRNA with Cas13b were also investigated. HBV replication markers and protein expression were measured and compared to a non-targeting control sgRNA.

Results: sgRNAs achieved knockdown of HBV proteins and intracellular core-associated HBV DNA with varying efficacy when delivered as DNA. Preliminary studies showed that transient RNA transfection of Cas9 BE and sgRNAs decreased secreted HBV protein expression. Further analysis of the impact on HBV RNA, DNA and protein expression *in vitro* will be performed. The efficacy of a combination of Cas9 BEs and Cas13b is currently being explored. BEs will also be delivered as mRNA packaged in lipid nanoparticles *in vivo* with pilot studies confirming the presence of BEs in the liver and spleen by detecting nanoluciferase (conjugated to Cas9 BE).

Conclusion: These studies will determine the utility of Cas9 BEs for introducing specific base changes into the HBV cccDNA and integrated DNA as an important first step towards developing this approach as a novel HBV therapeutic. Additionally, these studies will determine the utility of Cas9 BEs in combination with Cas13, to simultaneously target HBV DNA and RNA aspects of the viral 'life cycle', a combination never tested before.

Abstract #22

Capsid-Antibody-Complexes (CACs): an early indicator of liver inflammation in chronic hepatitis B

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Introduction: We previously reported the existence of Capsid-Antibody Complexes (CACs) in serum of CHB patients and demonstrated its close association with histological evidence of intrahepatic inflammation. Here we aim to investigate the dynamics of CACs during the antiviral therapy, the spatial relationship between complement deposition and viral antigen expression in liver biopsies, and the effect of co-existing metabolic fatty liver disease on the CACs level.

Methods: We developed a simple microplate-based assay (ELISA) informing their relative quantity. The levels of CACs in CHB patients were measured in multiple cohorts and their association with key virological, serological and histological parameters (H&E staining, C4d, HBsAg immunohistochemistry) was investigated.

Results: In a longitudinal cohort consisting of 35 treatment-naïve CHB patients who were treated with nucleos(t)ide analogues (entecavir, tenofovir), 19 of whom underwent liver biopsy before and 96 weeks after initiation of therapy, we observed the normalization of CACs during the first 48 weeks of treatment, a trend consistent with the normalization of serum ALT. Moreover, the reduced level of CACs is accompanied by the lowered Scheuer Grades in these patients. To further confirm the causative role of viral activity in the intrahepatic complement deposition, we performed immunohistochemistry of C4d and HBsAg in serial sections of liver biopsies. In specimens with a patchy parenchymal C4d staining pattern, a spatially correlated HBsAg IHC signal was observed in adjacent sections suggesting that complement deposition is due to the viral activity. Further co-staining experiments are underway to confirm this observation. Lastly, in a pilot cohort of HBeAg negative CHB patients with or without coexisting MAFLD, we found a significant lower level of CACs in CHB/MAFLD group compared with simple CHB. This suggests that metabolic fatty liver disease modifies the level of CACs in circulation. Further study is needed to extend the observation on both e negative and e positive individuals. In summary, these observations further substantiate CACs as a leading indicator for liver inflammation in CHB. The roles of immune complex-mediated immunopathy in the development chronic liver disease should be further explored. The effects of various co-morbidities on the CACs level and on the trajectory of liver disease in general should be further examined.

Abstract #23

Unleashing Immune Memory: Characterising HBV-Targeting Memory NK Cells for Next-Generation Cell Therapies

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Introduction: Chronic Hepatitis B (CHB) affects over 300 million people globally, causing approximately 800,000 deaths annually. Despite available antiviral therapies, achieving a functional cure, defined as the clearance of hepatitis B virus (HBV) surface antigen (HBsAg), remains a significant challenge. Natural Killer (NK) cells are traditionally considered part of the innate immune system but have recently been shown to mount antigen-specific memory responses against viral infections, including HBV. This study aims to characterise memory NK (mNK) cell maturation and identity to better understand mNK of antigen specificity. Findings from this work will lay the foundation for novel cell-based therapies targeting HBV-infected hepatocytes.

Methods: A total of 20 vaccinated individuals and 76 CHB patients were recruited. We developed a 28-color flow cytometry panel to characterise mNKs using the CYTEK Aurora spectral flow cytometer. Antigen-specific mNKs were identified using tetramers, enabling precise isolation of mNKs based on prior antigen exposure. Ongoing studies include single-cell RNA sequencing and surface protein profiling using the BD AbSeq and Rhapsody platforms to gain deeper insights into mNK identity and function. NK cell expansion was performed using feeder K562 cells expressing membrane-bound IL-15, IL-21, CD86, and 4-1BBL, which promote robust mNK cell expansion and antigen-specific functional responses.

Results: Initial findings confirmed that mNK cells from vaccinated individuals primarily recognised HBsAg, while those from CHB patients responded to both HBsAg and HBV polymerase, reflecting broader antigen exposure. Preliminary expansion studies have demonstrated up to 5,000-fold increase in total NK cells, a 200-fold increase in HBsAg tetramer binding, and a five-fold enhancement in antigen-specific target cell killing following feeder cell co-culture.

Discussion: These results underscore the potential of mNK cells as targeted immunotherapies for CHB, capable of selectively recognising and killing HBV-infected hepatocytes. Using tetramers to identify antigen-specific mNK cells is a significant advancement, allowing for the precise isolation of highly specific mNK populations.

This approach not only provides critical insights into mNK cell biology but also offers a platform for future therapeutic development.

Conclusions: Our novel approach to mNK identification using tetramers provides a powerful tool for isolating pure mNK populations, revealing critical insights into their identity, antigen specificity, and therapeutic potential. These findings may pave the way for the development of novel cell-based therapies capable of specifically targeting and eliminating HBV-infected hepatocytes, addressing a major unmet need in CHB management.

Abstract #24

CRISPR Cas13b-mediated knockdown of PD-1 mRNA: an alternative delivery strategy for immune checkpoint inhibitors

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Introduction: Chronic HIV antigen exposure in people living with HIV leads to exhaustion of HIV-specific T cells, reducing their function and limiting the ability to achieve immune-mediated control of HIV. Expression of the immune checkpoint programmed-death 1 (PD-1) on CD8 T cells, which is upregulated following HIV infection, is a major driver of this exhausted phenotype. Blockade of PD-1 to reverse immune exhaustion is emerging as an exciting potential component of HIV cure strategies, able to both restore immunological function and drive HIV latency reversal. However, a major barrier to this treatment is use of monoclonal antibodies for PD-1 blockade, which can cause serious immune related adverse events. Here we are investigating an alternative strategy for PD-1 blockade.

Methods: We used the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas13b ortholog and guide RNAs to the PD1 locus to transiently reduce expression of PD1. Cas13b is a programmable RNA nuclease that can knock down expression of specific mRNA inside a cell. We are delivering CRISPR-Cas13b mRNA via a novel lipid nanoparticle formulation that efficiently delivers mRNA to resting T-cells. We used computational design to create a panel of guide RNAs predicted to pair with the PD-1 mRNA sequence. To quantify the silencing potency of these guides we used a PD-1 fluorescence reporter system in 293T cells and assessed downregulation of endogenous PD1 in the Jurkat T cell line using flow cytometry.

Results: Our computational design identified two guide RNAs able to potently reduce PD-1 expression as measured by reporter fluorescence and cell surface protein expression (~95% and ~65% reduction, respectively) in 293T cells. In the Jurkat T cell line, cell surface expression of PD-1 was reduced by ~50% compared to a non-targeting control guide RNA.

Discussion: This data indicates PD-1 expression can be knocked down using CRISPR-Cas13b targeting of mRNA. Experiments to increase the degree of PD-1 knockdown, and to assess the kinetics of PD1 expression and impact on T cell functionality are ongoing.

Conclusion: Transient inhibition of PD1 expression using Cas13b represents a highly promising alternative to monoclonal antibodies, both for potential scalability and reduction in side effects due to the transient nature of this knockdown. The highly programmable nature of Cas13b also leaves open the possibility for concurrent targeting of additional immune checkpoints, in combination with PD-1.

Abstract #25

Applying Pro-apoptotic Agents to Combat Chronic HIV Infection in Vivo

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Introduction: HIV reservoir is responsible for viral rebound upon ART interruption, making HIV infection a chronic, lifelong disease. Recent *ex vivo* studies suggest that resistance to cell death is an important feature of the HIV reservoir, which serves as a therapeutic target. In this project, we used pro-apoptotic agents such as SMAC Mimetics and BH3 Mimetics to target the pro-survival proteins (cIAPs, BCL2) in HIV infected cells *in vivo*.

Methods: We used a Humanized Immune System (HIS) mouse model to study HIV infection *in vivo*. Newborn NOD/SCID/IL2rγnull (NSG) mice were irradiated and engrafted with human CD34+ cord blood cells. After a reconstitution period of 16 weeks, they were infected with HIV-JRCSF (week 0). 3 weeks after infection, mice began ART treatment until week 16. During the ART treatment, HIV-suppressed mice were given either vehicle or different pro-apoptotic treatments to reduce HIV reservoir. After treatment, we assess the efficacy of our treatment using either a 4-weeks Analytical Treatment Interruption (ATI) or the Intact Proviral DNA Assay (IPDA).

Results: HIS mice recapitulate key features of human HIV infection. 6 weeks of Xevinapant treatment induced a significant delay in HIV rebound comparing with vehicle group. A combination treatment of Xevinapant and Venetoclax also showed a trend to selectively reduce the intact proviral load, without affecting the defective proviruses, as determined by IPDA.

Discussion: To our knowledge, this is one of the first *in vivo* studies to characterize SMAC Mimetics alone and in combination with other pro-apoptotic drugs to reduce the HIV reservoir in a latent model. Our 6-week Xevinapant treatment showed a significant delay in HIV rebound, which suggests a potential reduction of HIV reservoir size. We are currently conducting more ATI and IPDA experiments to strengthen our findings. We are also studying the impact of these treatments on HIV latency, HIV- specific immune response, and cytokine responses.

Conclusions: SMAC Mimetics are able to partially purge the HIV reservoir *in vivo*, and the combination of SMAC Mimetics and BH3 Mimetic also showed promising efficacy. These findings suggest that exploiting cell death pathways offers a potential new direction towards an HIV cure. Combination of pro-apoptotic treatment with other strategies such as latency reversing agents may help better clear the HIV reservoir

Abstract #26

The enhancement IgG hexamerisation through the H429F mutation enables potent complement killing of viral and cancer targets by otherwise inactive monoclonal antibodies.

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Immune effector functions are key elements of protective immunity mediated by IgG antibodies, including anti-HIV mAbs. Across a range of indications, many therapeutic monoclonal antibodies (mAbs) are ineffective in utilising a key immune function -complement dependent cytotoxicity (CDC). IgG antibodies optimally activate the complement pathway by the arrangement of their Fc portions into hexamers, a pattern that forms an optimal binding and activation platform for the hexameric complement protein C1.

Our Stellabody® H429F mutation in the mAb heavy chain produces monomeric IgG but enhances the Fc-Fc hexamer interface so that antibody hexamers form efficiently on target surfaces. When the hexamerising H429F mutation is engineered into SARS-CoV-2 broadly neutralising antibodies it can confer CDC killing of spike expressing target cells. The activity of FcγRIIa, an important receptor in phagocytosis and antigen presentation is also enhanced.

This hexamerising mutation was also transformative of the activity of an ACE2-Fc decoy protein which neutralised SARS-CoV-2. The decoy with an unmodified Fc did not activate complement, while a H429F modification conferred complement killing of spike expressing targets.

The Fc-hexamerising mutation also improved the killing potency of standard of care CD20 mAbs against chronic lymphocytic leukemia (CLL) cells, in particular conferring efficient complement-mediated killing to CLL with low CD20 expression that are otherwise CDC resistant. Similarly, the CDC ineffective CD38 mAb isatuximab with H429F mutation becomes potent against multiple myeloma patient cells.

Given the potency of hexamerising mutations for COVID and cancer mAbs, this modification is predicted to enhance the therapeutic potential of HIV BNAbs. Natural immunity involves polyclonal responses to pathogens and individual antibody clones can interact together via conserved Fc portions to form hexamer effector activating platforms. Thus Stellabody® is highly effective with mixtures of mAbs whereby individual mAbs contribute Fcs to co-operatively activate killing function. This allows us to test the efficacy of mixes of HIV mAbs.

This work focuses on HIV mAbs specific to the V1V2 glycan site, the V3 glycan site, and the CD4 binding site currently used in clinical trials. These will be engineered by Stellabody® modification to optimize mAb potency and analysed individually and as mixes for the complement dependent inactivation of virus and the lysis of virus infected cells and the activation of FcγRIIa.

Abstract #27

Targeting Vδ2+ T cells to enhance immune mediated clearance of HIV-infected cells

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Introduction: HIV can persist on antiretroviral therapy (ART) as latently infected cells. One strategy to eliminate the HIV latent reservoir is to enhance HIV-specific immunity, thereby promoting elimination of infected cells and maintenance of long-term viral control off ART. We hypothesised that gamma-delta (γδ) T cells could play an important role in eliminating HIV-infected cells that persist. In peripheral blood, most γδ T cells utilise the Vδ2 T cell receptor (TCR) chain to recognise small non-peptide antigens – phosphoantigens (pAg) – presented by butyrophilin (BTN) complexes. Here, we investigated the role of γδTCR and BTNs in facilitating recognition and clearance of HIV-infected cells.

Methods: Expanded Vδ2+ T cells from HIV-negative (HIV-) individuals were cultured with a latently infected cell line, ACH2 which contains a full length integrated provirus with a mutation in the Tat binding region (HIV+) and the parental cell line A3.01 (HIV-) overnight, and the relative number of ACH2 to A3.01 was used to quantify HIV-specific killing. Separately, primary CD4+ T cells from HIV- individuals were infected *in vitro* with a green fluorescent protein (GFP)-reporter HIV and cultured with allogeneic expanded Vδ2+ T cells. All cultures were performed in the presence of raltegravir and T20 to limit infection of bystander cells. These assays were performed with the antagonistic anti-BTN3A antibody (clone 103.2), or isotype control, prior to culture with Vδ2+ T cells. To assess the impact of pAg on Vδ2+ T cell-mediated clearance of productive HIV-infection (GFP+), HIV-infected CD4+ T cells were treated with the amino-bisphosphonate zoledronate prior to culture with allogeneic expanded Vδ2+ T cells. Changes in the Vδ2+ T cells activation profile were quantified using flow cytometry.

Results: Vδ2+ T cell-mediated clearance was preferentially targeted towards ACH2 cells (27.9%) in the presence of A3.01 cells (10.7%, p = 0.001), highlighting specificity of killing towards HIV+ cells. Separately, Vδ2+ T cells can recognise HIV- infected CD4+ T cells, as determined by elevated expression of activation markers, but this did not result in clearance of GFP+ HIV-infected CD4+ T cells. Zoledronate enhanced Vδ2+ T cell-mediated recognition of HIV-infected CD4+ T cells, as indicated by a 64.9% (p = 0.016) increase of cells expressing activation markers, which led to clearance of GFP+ HIV-infected CD4+ T cells. The addition of the antagonistic anti-BTN3A antibody (clone 103.2) abrogated clearance of ACH2 and GFP+ HIV- infected CD4+ T cells, indicating a crucial involvement of γδTCR and BTN3A in γδ T cell-mediated anti-HIV immunity.

Discussion: These data demonstrated Vδ2+ T cell-mediated specific clearance of HIV-infected cell in a γδTCR and BTN- dependent manner. Clearance of HIV-infected CD4+ T cells was only observed in the presence of zoledronate, suggesting a need for pAg augmentation to effectively eliminate HIV-infected CD4+ T cells. Therefore, use of zoledronate as an enhancer of pAg production might be required to prime Vδ2+ T cell responses *in vivo* to support anti-HIV immunity.

Conclusions: Our findings elucidate a novel role for γδTCR and BTNs in facilitating Vδ2+ T cell-mediated clearance of HIV- infected cells. This will inform strategies to repurpose zoledronate as an immune-enhancing agent to increase Vδ2+ T cell- mediated anti-HIV immunity.

Abstract #28

A novel strategy to eradicate HIV using CD8 CAR T cells

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The latent HIV reservoir remains a barrier for an HIV cure as it is not recognised by CD8+ T cells. Latency Reversing Agents (LRAs) used to reactivate latent HIV are not optimal as the HIV specific CD8 T cells are dysfunctional in chronic HIV infection. Broadly neutralising antibodies (bNabs) can significantly control HIV replication by binding to highly conserved regions of gp120. Thus, we engineered CD8+ T cells with chimeric antigen receptor (CAR)s expressing short chain variable fragment (scfv)'s of two bNabs in tandem to investigate whether they can reduce/eliminate the HIV reservoir.

The *piggyBac* transposon system was used to generate CD8+ CAR T cells via electroporation expressing the scfv of PGT121 (targeting CCR5-binding site of gp120), and the scfv of a novel bovine cross-reactive bNab, MEL-1872 (targeting CD4-binding site of gp120) developed in the Purcell lab. Single CAR T cells as well as two iterations of Tandem CAR (TanCAR) T cells were generated, targeting different regions of HIV gp120 to avoid viral escape by mutation, and lead to specific targeting of infected cells. These cells were expanded over 14 days and their cytotoxic function against HIV-infected autologous CD4 T cells was measured. CAR19 (used in anti-tumour therapy) cells were used as a negative control.

All single and tandem bNab-based CAR T cells (PGT121 and MEL-1872), both showed high expression of the CAR after 14 days of culture as determined by GFP expression detected using flow cytometry. Both TanCAR T cell subtypes induced targeted killing of HIV-infected autologous CD4 T cells with similar efficiency in coculture with TanCARs and single CAR T cells. All CAR T cells also showed little to no expression of exhaustion markers and most cells differentiated into Central or Effector Memory T cells. After determination of the best iteration of TanCAR, we will continue these studies with cells extracted from people living with HIV, to generate CAR T cells and determine their functionality against autologous reactivated CD4 T cells.

We thus show that bNabs are feasible antigen recognition domains for the development of HIV specific CAR T cells to target HIV infected cells without off-target toxicity. Our lab has previously shown that IFNa can reactivate latent HIV in in vitro infected CD4 T cells. with IFNa-reactivation and dual targeting of gp120 by CD8+ CAR T cells, we hypothesise a novel and unique strategy complimentary to 'shock and kill' silent cells which could contribute to the elimination/reduction of the latent HIV reservoir.

Abstract #29

Improving the Diagnosis and Monitoring of HTLV-1 in Australia

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Introduction: Human T-cell lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2) are retroviruses that share approximately 70% nucleotide identity. Of the two, HTLV-1 is associated with diseases such as adult T-cell leukemia (ATL) and HTLV-1- associated myelopathy/tropical spastic paraparesis (HAM/TSP), while HTLV-2 has not been definitively linked to clinical disease to date. HTLV-1 comprises seven known subtypes (A–G), with the HTLV-1c subtype endemic at high prevalence rates (>15%) among Aboriginal communities in Central Australia. Diagnosis of HTLV in Australia typically involves confirmatory testing at the NRL following a reactive antibody screen conducted in state-based laboratories. This process uses a combination of high- throughput chemiluminescent immunoassays (CLIA) and Western blot assays. However, in approximately 10% of cases, the Western blot yields an indeterminate (IND) result, leaving a significant proportion of patients with an unconfirmed HTLV status. To address this, the NRL also offers a qPCR-based HTLV-1 proviral load test which, if used following an IND result, can significantly reduce the number of unresolved cases. However, the current HTLV-1 proviral load assay does not differentiate between HTLV types or subtypes and is only approved for use as a monitoring tool. As a result, its utility for diagnosis and surveillance in Australia remains limited.

Methods: Primers and probes (Merck) were designed to target the highly conserved *tax* region. Clonal in-house cell lines for HTLV-1c (JX891478) and HTLV-2 (NC_001488) were developed as reference material by stably transfecting pcDNA3.1+N-eGFP plasmid carrying Px region sequences (GenScript) into Jurkat T cells using Lipofectamine 3000. MT-2 cells were used as a reference for HTLV-1(non-c). Total DNA preparations from cells were obtained using the QiaAMP DNA Blood kits (Qiagen) and qPCRs were performed using the CFX OPUS instrumentation (Bio-Rad).

Results: PCR performed on total DNA extracted from clonal cells was used to verify the identity and clonal nature of in-house reference clones. qPCRs for the multiplex assay were optimised with respect to cycling conditions, with annealing temperature of 60°C giving complete discrimination between all reference sequences without compromising PCR efficiency. Amplification for all targets was highly linear over 6 logs, with the preliminary Limits of Detection (LOD95) for HTLV-1c, HTLV-1(non-c) and HTLV-2 all <5 copies. HTLV proviral DNA was confirmed in all cases for a panel of 18 confirmed HTLV-1 positive residual clinical samples obtained through MTA with VIDRL, with 4 being identified as HTLV-1(non-c) using the new assay. Moreover, the proviral loads reported by the new assay for those that were identified as HTLV-1c were in close agreement with that obtained using the current NRL in-house HTLV-1 PVL Assay, as well as with results from VIDRL. Cell-free DNA from HTLV-1–positive serum and plasma has also been characterised for both HTLV-1 non-c and HTLV-1c subtypes.

Discussion and Conclusion: When incorporated into the confirmatory algorithm for HTLV infection, this new molecular test is expected to improve confirmed diagnosis rates by resolving a significant proportion of samples that yield indeterminate results on the Western blot (WB). Further validation of the test is ongoing, and the NRL is currently collaborating with the Molecular Diagnostic Unit at Imperial College London to obtain a selection of HTLV (non-c) samples for this purpose. With the recent release of updated HTLV testing guidelines by ASHM and the National Aboriginal Community Controlled Health Organisation (NACCHO), both awareness and demand for accurate HTLV diagnosis in Australia are expected to increase.

Abstract #30

Promoter-Targeted siRNA as a Novel Therapeutic strategy for Oncogenic Viruses

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Background: Oncogenic viruses, such as Human T-cell Leukemia Virus Type 1 (HTLV-1) and Human Papillomavirus Type 18 (HPV-18), contribute to the development of aggressive malignancies, including adult T- cell leukemia/lymphoma and cervical cancer. These viruses establish chronic infections characterized by persistent transcriptional activity of viral oncogenes, which leads to disease progression and immune evasion. Current therapies fail to address the underlying causes of oncogenic viral expression that drive malignancy due to limited symptomatic or cytotoxic interventions. We present a novel onco-therapeutic approach utilizing promoter-targeted short interfering (si)RNAs to induce transcriptional gene silencing (TGS) of viral oncogenes via epigenetic remodeling of the viral promoter. This mechanism recruits repressive chromatin marks, effectively blocking transcription and locking the viral genome in a silent state, to provide a potential 'block and lock' functional cure.

Methods: To investigate the potential of promoter targeted siRNAs to silence viral oncogenes, we used two well- established models: HTLV-1-infected MJ and MT-2 T-cell lines, and HPV-18-infected HeLa T4 cervical epithelial cells. These cells were Lipofectamine transfected with siRNAs specifically designed to target conserved sequences within the viral promoter regions. Following transfection, viral gene expression was quantified at transcript and protein levels via real-time RT-qPCR and immunoblotting, respectively. To explore the underlying epigenetic mechanisms, ChIP assay was performed, targeting Ago1, the core RITS component, as well as key histone marks associated with transcriptional activity and repression: H3K9me3, H3K27me3 (repressive), and H3K9Ac, H3K27Ac (activating), and HDAC1. Additionally, to visualize intracellular and nuclear co-localization of GFP labelled Ago1 and Cy3-labeled siRNAs, fixed-cell imaging was conducted using high-resolution 3D confocal microscopy (Leica Stellaris 5). **Results:** In HTLV-1-infected MT-2 and MJ cells, transfection with promoter-targeted siRNAs (particularly siR2 and siR17) led to a marked reduction in viral gene expression. *TAX* and *HBZ* transcripts were suppressed by 68% ($p<0.01$) and 74% ($p<0.01$), respectively. In HPV-18-infected HeLa cells, promoter-targeted siRNAs showed significant repression of viral oncogenes, E6 and E7, transcript levels by 83% ($p<0.001$) and 86% ($p<0.001$). Transcriptional silencing was confirmed at the protein level, with TAX and E7 protein expression dropping by up to 90% ($p<0.0001$). In HPV-18 cells, repression remained stable over a 21-days, whereas HTLV-1 exhibited a more dynamic silencing pattern, with partial rebound at later time points. ChIP assay revealed significant recruitment of Ago1 to the viral promoter regions, accompanied by an 8-9-fold enrichment of H3K9me3 and H3K27me3 in

HTLV-1 MT-2 ($p <0.0001$) and 1.5-3.4-fold increase in HPV-18 HeLa ($p <0.001$), respectively. At the same time, activating histone marks (H3K9Ac and H3K27Ac) were comparatively depleted, along with significant recruitment of histone deacetylase marker, HDAC1 ($p<0.001$), indicating the formation of repressive chromatin. 3D confocal imaging further supported these findings with significant nuclear co-localization of Ago1-GFP with Cy3-labeled promoter-targeted siRNAs (Pearson's correlation coefficients (PCC) $p<0.0001$), consistent with targeted chromatin engagement compared to minimal nuclear events in scrambled controls both in HTLV-1 MT- 2 and HPV-18 HeLa cells, reinforcing the promoter-specific nuclear localization of the RNA-induced silencing complex. **Conclusion:** This study provides the first demonstration of siRNA-induced promoter-targeted transcriptional silencing of two oncogenic viruses through epigenetic remodeling via recruitment of Ago1 and repressive epigenetic markers to viral promoters. This mechanism locks viral genomes in a silent state, offering a sustained and programmable antiviral approach. These findings establish a foundation for siRNA-guided 'block and lock' functional cures for incurable oncogenic viruses like HTLV-1, HPV-18, and potentially other latent, cancer- associated viruses. *Keywords: Small interfering RNA (siRNA), HTLV-1, HPV-18, Argonaut-1 (Ago1), Oncogenic viruses, epigenetic silencing, functional cure*

Abstract #31

The HTLV-1c genomic landscape reveals host-virus interactions

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Introduction: Human T-cell leukemia virus 1 (HTLV-1) is endemic in Central Australia with a prevalence of ~ 50% reported in some remote First Nations Communities. HTLV-1c is the divergent molecular variant of HTLV-1 found in Australia. Upon infection, HTLV-1 integrates its reverse transcribed 9 kb proviral DNA genome into the host cell nuclear DNA. The integrated provirus and host genome become enmeshed and have an ongoing reciprocal influence on one another, impacting both host function and viral fitness. The integrated provirus thus has the potential to archive host-virus interactions.

Aim: To characterise the proviral and epigenetic diversity of HTLV-1c infection associated with pulmonary disease

Methods: We have used the Oxford Nanopore Technologies long-read sequencing platform to perform an in-depth, nucleotide resolution characterisation of near-full length HTLV-1c genomes in a patient cohort with HTLV-1 Associated Pulmonary Disease (HAPD) from Central Australia, alongside analysis in a humanised mouse (hu-mouse) model of HTLV-1c infection. We additionally characterised integration site selection and DNA methylation landscape in the HTLV-1c+ hu-mice.

Results: We uncovered extensive structural diversity in natural HTLV-1c infection, which included host-viral chimeric genomes and large indels that are predicted to perturb the transcriptional landscape of the provirus. We found that structural variants retaining the coding sequence of the *hbz* virulence factor stratify by HAPD status. We have identified, to our knowledge, the first HTLV-1c–human chimeric proviral genomes, which are predicted to encode novel protein products, render the proviral genome defective, and impact both host and viral gene expression. These findings are recapitulated in HTLV-1c+ hu-mice. In the mouse model, we observed a relationship between 5mC distribution along the provirus and defective genome breakpoint junctions.

Conclusions: The HTLV-1c proviral genome acquires extensive structural defects in natural infection, and genetic aberrations in the provirus that retain the *hbz* gene associate with pulmonary disease, presenting *hbz* as a candidate for therapeutic development. Genomic defects correlate with the 5mC landscape in HTLV-1c+ hu-mice.

Abstract #32

Combination antiretroviral therapy and MCL-1 inhibition mitigate HTLV-1 infection *in vivo*

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Satisfactory preventative or therapeutic drugs are lacking for HTLV-1, a retrovirus closely related to HIV. We established and characterised a humanized mouse model of HTLV-1c infection and identify that HTLV-1c disease appears slightly more aggressive than the prevalent HTLV-1a subtype, which may underpin increased risk for infection associated pulmonary complications in HTLV-1c. Combination antiretroviral therapy with Tenofovir and Dolutegravir at clinically relevant doses significantly reduced HTLV-1c transmission and disease progression *in vivo*. Single cell RNAseq and intracellular flow cytometry identified that HTLV-1c infection leads to dysregulated intrinsic apoptosis in infected cells *in vivo*. Pharmacological inhibition using BH3 mimetic compounds against MCL-1, but not BCL-2, BCL-xL or BCL-w, killed HTLV-1c-infected cells *in vitro* and *in vivo*, and significantly delayed disease progression when combined with tenofovir and dolutegravir in mice. Our data suggests combination antiretroviral therapy with MCL-1 antagonism may represent an effective, clinically relevant, potentially curative strategy against HTLV-1c. We are now expanding our efforts towards next-generation therapeutics with decreased treatment toxicity and increased cell specificity by utilizing precision targeting of T cells by LNP- based delivery of RNA therapeutics. These include siRNA against host apoptosis regulators including MCL-1, as well as the generation of nanobodies that can be delivered as mRNA to target viral virulence factors.

Abstract #33

Development of siRNA therapeutics targeting Human T-cell Leukaemia Virus Type-1 (HTLV-1) infection

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Introduction: No cure, vaccine, or treatment is currently available for the ~10 million people worldwide living with the lifelong infection caused by Human T-cell Leukaemia Virus Type-1 (HTLV-1). RNA therapeutics offer sequence specific targeting of HTLV-1. In this study, we designed and screened custom siRNA targeting the HTLV-1 5`LTR, 3`LTR, tax and hbz regions. We identified novel siRNAs that silence these regions in custom HTLV-1 GFP reporter cell lines and in infected T cell lines MJ and MT-2.

Methods: Preliminary screens were performed in HTLV-1 293T-GFP transduced reporter cell lines and GFP suppression was measured at day four using flow cytometry. Subsequent validation was performed in MJ or MT-2 T-cell lymphoma cell line. LTR, tax and hbz siRNA targets were validated on the mRNA level using RT-qPCR. All siRNA were assessed for off target interferon stimulated gene (ISG) response induction in HeLA T4 or MT-2 cells, and dose response was determined. Lipofectamine RNAiMax was used to transfected all siRNA.

Results: Significant GFP suppression was observed in four 5'LTR, seven 3'LTR, seven tax, and 13 hbz targeted novel siRNAs ($p<0.05$, $n = 6$) in the GFP reporter system at 96 hours post-transfection. RT-qPCR confirmation on the mRNA level showed that 13 hbz, four 3'LTR, six tax and six 5'LTR siRNA targets demonstrated significant hbz or tax mRNA knockdown in leukaemic T cells ($p<0.05$, $n = 3$). Of these hits, three 3'LTR and hbz targeted siRNAs demonstrated silencing potency down to 0.05 nM, and two 5'LTR targeted siRNAs were potent at a 0.005 nM dose ($p<0.05$, $n = 3$).

Discussion and Conclusions: This study demonstrates siRNAs can provide direct acting antiviral treatment by suppressing the activity of promoters and oncogenes in HTLV-1 GFP reporter cells and infected T cell lines. Confirmation on the protein level using Western Blots will provide further validation. Future development of siRNA using lipid nanoparticles may provide the opportunity for clinical translation.

Abstract #34

Towards a universal mRNA-LNP vaccine to prevent HTLV-1 infection and disease

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Introduction: Human T-cell leukemia virus type 1 (HTLV-1) is endemic to numerous regions worldwide, including First Nations communities of Central Australia that has adult infection rates around 40%. The lack of an approved vaccine preventing transmission or pathogenesis highlights HTLV-1 as a neglected public health issue. We have demonstrated that potent neutralising antibodies (nAbs) targeting the HTLV-1 Envelope (Env) protein are induced during natural infection with HTLV-1 subtype C that cross-neutralise other subtypes of HTLV-1. Here, we have developed env mRNA-lipid nanoparticles (LNP) vaccines that incorporate mutations to stabilise of the Env trimer prefusion conformation and reduce toxicities, while stimulating the robust neutralising responses observed during natural infection.

Methods: A panel of mutant Env expression constructs was generated and used to transfect ExpiCHO cells. Soluble Env expression and stabilisation was assessed by SDS-PAGE, BN-PAGE and ELISA. Key mutants were selected, and mRNA was in vitro transcribed (IVT) using N1-methyl pseudouridine and CleanCap. Protein expression was confirmed by transfection of HEK293 cells. Purified mRNA was encapsulated for delivery into lipid nanoparticles (LNPs) formulated using GenVoy ILM and used to vaccinate groups of C57Bl/6 mice (n=5). Membrane-tethered vs soluble forms of Env with stabilising mutations were compared at two different doses. Serum was collected 2 weeks post-boost and Env binding of serum IgG assessed by ELISA and virus-neutralisation assayed using reporter-pseudovirus.

Results: Characterisation of a comprehensive panel of HTLV-1 Env mutants has identified Pro substitutions that enhance expression of stable native-like trimer. Vaccination with soluble Env constructs elicited robust nAb in mice after the third vaccination. The mRNA vaccines expressing membrane-tethered Env yielded significantly lower nAb in vaccinated mice compared to soluble Env mutants.

Conclusions: We have shown that HTLV-1 Env expressed during natural infection can generate a robust protective serological response. We have recapitulated similar responses with multiple doses of mRNA-LNP expressing prefusion- stabilised Env mutants.

Development of Intranasal Antiviral RNA Therapeutics targeting SARS-CoV-2 (DARTS)

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Introduction: RNA therapeutics are an exciting treatment modality. Antiviral short interfering (si)RNA therapeutics that are highly conserved have potential to treat a range of diverse viral infections. The current antiviral treatments of COVID are limited and have many side effects with drug-drug interactions due to intravenous or oral administration. We have developed broad spectrum antiviral siRNA targeting the SARS-CoV-2 Nsp1 region. Here we investigated their antiviral efficacy in vitro and in vivo following encapsulated with an optimised lipid nanoparticle (LNP) formulation and delivery using an intranasal spray device (MedSpray) for delivery directly to the respiratory tract.

Methods: siRNA-LNP complexes were generated using the Nanoassembr Ignite platform. The proprietary optimised LNP was based on the FDA-approved Alnylam (Onpattro) formulation. LNP QC included measuring encapsulation efficiency (EE%), nanosize, polydispersity and zeta potential. Off-target effects of four interferon stimulated genes (ISGs) were measured by RT-qPCR of mRNA levels. Antiviral efficacy was assessed using cell survival assays and viral mRNA levels by RT-qPCR in VeroE6 and Calu-3 cells. Aerosol performance was assessed via industry standard human respiratory models (Alberta idealised nasal inlet-AINI, and Next Generator Impactor-NGI) and novel nasal expansion chamber models.
Preclinical safety and efficacy was assessed ex vivo using the air-liquid interface (ALI)-primary human bronchial epithelial cell (BEC) cultures model and in vivo the K18-ACE2 transgenic mouse model of SARS-Co-2 infection.

Results: siRNA-LNP formulations were <100 nm, with EE% of >80%, Polydispersity of <0.3, and a neutral charge zeta potential. No significant off-target effects were induced by the siRNA-LNP formulation. Virus suppression induced by siRNA-LNP in infected VeroE6 and Calu-3 cells was reported ranging between 1.94 and 3.03 log, compared to controls. Biodistribution studies reported aerosol deposition of siRNA-LNP (84.6%) in the nasopharynx region of the AINI-NGI respiratory model. Intranasal instillation of siRNA-LNP in the transgenic mouse model demonstrated the optimised LNP formulation was safe and non-inflammatory in the lung and resulted in reduced the clinical outcome with decreased viral RNA levels in the lung by ~1/2 log, compared to control groups. This is comparable to current antivirals.

Discussion and Conclusions: This study demonstrates broad-spectrum siRNA encapsulated in an optimised LNP have potential to treat COVID infection without off-target effects in vitro and in vivo. Nasopharynx biodistribution post-device treatment offers a targeted therapeutic approach for siRNA-LNP in the upper respiratory tract to suppress SARS-CoV-2 infection. This platform technology is also applicable to all respiratory virus infections.

Abstract #36

COVID-19 booster vaccination in 2025 – when, how and why.

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BACKGROUND. There is uncertainty about delivering SARS-CoV-2 booster vaccinations in highly immune adults now. What should be the interval of vaccination? Should they be given together with influenza vaccination in autumn – and if so, should they be given in the same or opposite arms? Are the immune correlates of protection still the same?

METHODS. We conducted 2 randomised controlled trials. First in 52 fully vaccinated adults randomised to an immediate or a 3-month delayed mRNA booster vaccine. Second, in 56 adults given both an mRNA booster and an inactivated influenza vaccine on the same day in either the same or opposite arms. We studied antibody and T cell responses in serial blood and mucosal samples and followed participants for breakthrough COVID-19.

RESULTS. We found: (i) delaying vaccination did not improve immune responses, (ii) SARS-CoV-2 and influenza vaccination can safely and effectively be given at the same time in either in the same or opposite arms although some antibody responses had stronger rises in the opposite arm group, and (iii) neutralising antibody responses (but not T cell responses) correlated with prevention of symptomatic and asymptomatic breakthrough infection during follow up.

CONCLUSIONS. Our trials showed no benefit in delaying COVID-19 mRNA booster vaccination. We found that giving COVID-19 and Flu vaccines simultaneously was a safe and effective strategy that could improve vaccine uptake. We also confirmed neutralising antibodies as a strong correlate of protection from SARS-CoV-2

Abstract #37

Blood Distribution and degradation of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans

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Lipid nanoparticle mRNA vaccines for COVID-19 and other diseases are an exciting but emerging technology used in humans. There is limited understanding of the factors that influence their biodistribution and immunogenicity. We developed methods to quantify both the vaccine mRNA and ionizable lipid in frequent serial blood samples from over 40 subjects receiving Moderna SPIKEVAX or Pfizer BNT162b2 mRNA booster immunization. Both the vaccine mRNA and ionizable lipid peaked in blood 1–2 days post vaccination (median peak level 0.19 and 3.22 ng/ml, respectively).

The vaccine mRNA was detectable and quantifiable up to 14–15 days postvaccination in at least 37% of subjects. We measured the proportion of vaccine mRNA that was relatively intact in blood over time and found that the decay kinetics of the intact mRNA and ionizable lipid were identical, suggesting the intact lipid nanoparticle recirculates in blood. Decay rates of mRNA and ionizable lipids did not correlate with baseline levels of Spike- or PEG-specific antibodies although the magnitude of mRNA and ionizable lipid detected in blood did correlate with the boost in the level of PEG antibodies.

Decay kinetics of the Pfizer vaccine were slower than that of the Moderna vaccine, consistent with lower levels of anti-PEG antibodies induced by the Pfizer vaccine. Furthermore, the ability of a subject's monocytes to phagocytose lipid nanoparticles was inversely related to the rise in PEG antibodies. This suggests that the circulation of mRNA lipid nanoparticles into the blood and their clearance by phagocytes influence immunogenicity aspects of mRNA vaccines. Overall, this work defines the pharmacokinetics of lipid nanoparticle mRNA vaccine components in human blood after intramuscular injection and the factors that influence these processes. These insights should be valuable in improving the future safety and efficacy of lipid nanoparticle mRNA vaccines and therapeutics.

Exploring CRISPR-based epigenetic tools for the reversal or promotion of HIV latency

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Introduction: The use of conventional CRISPR-Cas9 has been explored for excision of the HIV provirus. However, the induction of double-stranded DNA can induce genotoxicity. Alternative CRISPR tools have been developed that use a catalytically inactive (dead) Cas9 (dCas9) fused to transcriptional activators or transcriptional repressors to specifically activate or repress a gene of interest. We aimed to exploit these advances by assessing the potency of dCas9 to activate HIV transcription (CRISPR activation (CRISPRa)) or permanently silence HIV transcription (CRISPRoff), as part of 'shock and kill' and 'block and lock' strategies towards an HIV cure, respectively.
Methods: mRNAs encoding the dCas9-effector protein combined with HIV LTR-targeting guide (g)RNAs were encapsulated into lipid nanoparticles using an optimized lipid formulation (LNP X) that can efficiently deliver mRNA to resting T-cells. CRISPRa machinery consists of an mRNA encoding dCas9-VP64, a second mRNA encoding p65 and heat-shock-factor 1 (HSF1), and previously described guide (g)RNAs (termed L and O), with VP64, p65 and HSF1 acting as transcriptional activators. CRISPRa-LNP potency was assessed in latently infected cell line JLat A2, which contains an integrated construct of green fluorescent protein (GFP) under the control of the HIV LTR, and in CD4+ T-cells from people living with HIV on suppressive antiretroviral therapy (n=8). Digital PCR was used to quantify transcription initiation, elongation and splicing of HIV transcripts. CRISPRoff mRNA included an mRNA encoding dCas9 fused to DNA methyltransferase 3 (DNMT3) and Krüppel-associated box (KRAB) combined with gRNA-L. CRISPRoff-LNP potency was assessed in the TZMbl cell line, which contains a luciferase reporter under the control of the HIV LTR and has a constitutive low levels of luciferase expression. Viability was assessed using an MTS assay.
Results: CRISPRa-LNPs induced potent HIV transcription in Jlat A2 cells, with highest potency observed using gRNA-L (mean±SEM %GFP+ 76.4±3.6). Multiplexing of gRNA-L and gRNA-O, which bind non-overlapping targets in the U3 LTR region, demonstrated synergistic activity (Bliss-independence score 0.1±0.02). CD4+ T-cells treated ex vivo with CRISPRa-LNPs (gRNAs L+O) demonstrated median 2.0-fold increase in cell-associated unspliced HIV transcripts compared to untreated cells, but no increase in multiply-spliced transcripts. In preliminary experiments, CRISPRoff-LNP induced a mean 45% reduction in luciferase expression after 24 hr, with minimal reduction in viability (73%) compared to untreated cells.
Discussion: CRISPRa can be delivered to a resting T-cells using mRNA-LNP technology and activate HIV transcription, however the effects was modest. Further optimisation will be needed to enable a higher proportion of cells to express dCas9. CRISPRoff appears to suppress HIV transcription in a basic model of HIV latency but, importantly, the durability of this suppression needs to be carefully studied in order to identify CRISPRoff as a novel 'block and lock' compound.
Conclusions: LNP technology enables novel HIV cure strategies that leverage mRNA-based therapeutics, including variants of the CRISPR-Cas9 toolbox.

Abstract #39

Improving the potency and safety of Tat-LNPs for HIV latency reversal

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Introduction: One strategy to eliminate the HIV reservoir, which persists on effective antiretroviral therapy (ART), is to activate
proviral transcription and protein production using latency reversing agents (LRAs), to trigger virus-mediated cytotoxicity or immune clearance of infected cells. We recently demonstrated that lipid nanoparticles (LNPs) delivering mRNA encoding the HIV Tat protein (Tat-LNPs) potently reversed latency in CD4 T cells from people with HIV (PWH) on suppressive ART. However, this strategy has potential toxicities pertaining to Tat secretion and uptake, and the reliance of Tat on the expression of the HIV Transactivating Response element – a marker of transcription initiation. We sought to determine: (1) Can Tat secretion and uptake be reduced through point mutations? (2) Can Tat-LNP potency be enhanced by combining with other LRAs that boost transcription initiation?

Methods: To limit Tat secretion and uptake, we engineered mRNA encoding the first coding exon of Tat with specific substitutions: W11A (to block secretion), R57S (to block uptake), or both. To assess potency enhancement, Tat-LNPs were combined with the following LRAs: 1μM JQ1 (bromodomain inhibitor), 10nM Bryostatin (Protein Kinase C agonist) or 1μM BV6 (second mitochondrial-derived activator of caspases mimetic). Latency reversal was evaluated in the latently infected cells lines JLAT A2 (Tat-IRES-green fluorescent protein [GFP]), JLAT 10.6 (full-length GFP-reporter virus) or JLAT 6.3 (full-length GFPreporter virus) cell lines. Reactivation and viability were assessed by flow cytometry. Synergy was calculated using Bliss Independence Model.

Results: The percentage of GFP positive cells in JLAT A2 cells was comparable following expression of wildtype Tat (mean±SEM %GFP+ cells 67.5%±2.1%) and R57S variants (66.2%±3.1%). However, a reduction in the percentage of GFP cells was observed following expression of variants containing the W11A mutation (W11A mean±SEM %GFP+ cells 57.2%±3.4%; W11A+R57S 53.8%±3.5%), resulting in a 5.34-fold increase in EC50 compared to wildtype Tat. A similar pattern was observed in JLAT 10.6 cells following expression of the variant W11A (25.5%±1.9%), R57S (33.6%±1.8%), W11A+R57S (22.1%±0.2%), and wildtype Tat (30.7%±4.5%). Strong synergy was observed when Tat-LNPs were combined with BV6 (Bliss Independence score 0.45), Bryostatin (1.4), and JQ1 (0.1) in JLAT 6.3 cells. There was minimal effect on cell viability throughout.

Discussion: Point mutations in Tat that reduce secretion and uptake do not change the potency of latency reversal activity in cell lines. Combining Tat-LNPs with other LRAs significantly enhances efficacy, likely through increased transcription initiation. BV6 and Bryostatin are the lead candidates for combination therapy due to their strong synergy with minimal effects on cell viability and will be tested ex vivo in CD4 T cells from PWH on ART.
Conclusions: Tat-LNPs can be optimized for safety without compromising latency reversal activity and show increased potency when paired with additional LRAs. Future studies will focus on the use of ELISA and intracellular staining to quantify Tat protein secretion and uptake, respectively, following treatment with Tat variants. Ongoing studies are evaluating the performance of Tat-LNPs plus BV6 or Bryostatin in CD4 T cells from PWH on ART ex vivo.

Abstract #40

Optimising long read sequencing of cell associated HIV RNA to understand latency reversal

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Introduction: Latency reversal in HIV is often quantified using PCR with primer sets targeting specific viral RNA transcripts; however, not all of these RNAs reliably correlate with protein production, and a more complete characterisation of viral transcripts is needed. Given HIV's complex splicing patterns and the production of incomplete transcripts following latency reversal, we hypothesised that long-read RNA sequencing would allow for an improved understanding of the viral transcriptome. However, a major challenge is the relative low abundance of HIV transcripts compared to host background.

Methods: We assessed HEK293T cells transfected with proviral NL4.3 plasmid, the ACH2 cell line which contains an integrated copy of HIV1 LAI with key mutations that limit virus replication, and CD4+ T cells from people living with HIV (PLWH) on antiretroviral therapy (subtype B, n=2). Cells were stimulated with phorbol myristate acetate (PMA) and Ionomycin. RNA was purified from ACH2 cells and converted to cDNA with oligo-dT primers, prepared as a library (SQK-DCS109, EXP-NBD104, ONT) before sequencing on a FLO-MIN106 Flow cell (ONT). We assessed enrichment using short-read sequencing technologies. Here, RNA was reverse transcribed using random hexamers and libraries prepared (Twist Bioscience). For enrichment, a custom HIV panel comprising >75,000 HIV biotinylated oligos was used (Twist Bioscience), and enriched libraries sequenced on an Illumina iSeq100 platform.

Results: Long-read sequencing of ACH2 cells revealed that unstimulated cells contained 0.12% HIV transcripts, while stimulated cells had 2.04% HIV transcripts of total detectable reads, reflecting increased transcription following reactivation. In HEK293T cells, HIV transcripts represented 0.30% of detectable reads without enrichment and 73.2% of detectable reads following enrichment. In unstimulated ACH2 cells, HIV transcripts represented 0.018% of detectable reads, increasing to 58.4% of detectable reads following enrichment. In stimulated ACH2, HIV transcripts of detectable reads increased from 0.81% to 94.4% after enrichment. Preliminary results from CD4+ T cells isolated from PLWH found HIV transcripts undetectable without enrichment, but after enrichment represented 0.002% of reads for unstimulated cells and 0.006% for stimulated cells.

Discussion: These results demonstrate the effectiveness of using an HIV-specific enrichment strategy to improve capture of viral transcripts in cell line models of HIV latency and reactivation. Integrating the enrichment strategy with long-read sequencing will further allow us to comprehensively profile HIV reactivation and support efforts to identify signatures and targets of effective latency reversal. Further work will be needed to optimise long read sequencing in cells from PLWH.

Conclusions: We present an enrichment-based method for the characterisation of HIV transcriptomes *in vitro* and *ex vivo* samples during latency reversal which will be readily combined with long-read sequencing.

Abstract #41

A novel IMmunoinformatics Analysis Pipeline (IMAP) identifies genetically-conserved and immunogenic peptides found in rebound HIV-1 during analytical treatment interruption.

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Introduction: HIV-1-specific CD8 T-cell responses directed against genetically-conserved HIV-1 protein regions have been associated with viral control. Therefore, we applied a novel IMmunoinformatics Analysis Pipeline (IMAP) to identify 182 peptides (IMAP-peptides) from 5 HIV-1 protein regions (Gag, Pol, Vif, Vpr, and Env), which were genetically conserved across global HIV-1 variants while avoiding known immune escape mutations. We assessed whether the selected IMAP-peptides were found within rebound virus derived from participants who experienced analytic treatment interruption (ATI). Furthermore, we characterized the corresponding CD8 T-cell response.

Methods: During the PULSE clinical trial, 68 men who have sex with men living with HIV-1 in Australia underwent three consecutive ATIs. Remarkably, seven participants transiently controlled HIV rebound during the 3rd ATI. We obtained plasma and/or blood-derived mononuclear cells from four non-controllers (NCs) who experienced rapid viral rebound during all ATIs, and five transientcontrollers (TCs) who exhibited control during the 3rd ATI. We assessed whether the IMAPpeptides were present within HIV-1 RNA sequences from rebound virus across the ATIs in NCs and TCs. In addition, the effector response to these IMAP-peptides and NIH-provided control peptide pool (without IMAP-peptides) were determined by IFN-γ/TNF-α production and degranulation (CD107a/b).

Results: Near full-length HIV-1 RNA sequencing of rebound virus from 3 NCs and 2 TCs revealed the Gag, Pol, Vif, Vpr, and Env IMAP-peptides were found in 52-100% of the viral sequences obtained from these five participants across three ATI time points. Moreover, the CD8 T-cells from 3 TCs had a 15-53-fold higher effector response to the IMAP-peptides compared to the CD8 T-cells from 2 NCs. Notably, in NCs, the relative response to the IMAP-peptides was 20-fold lower compared to the control peptides, whereas in TCs, the IMAP-peptide response was similar to the control response (1-1.58-fold change).

Discussion and Conclusions: HIV-1 cure strategies are complicated by the rapid mutation rate of the virus and its high genetic diversity. To address this, our novel IMAP successfully identified 182 potentially immunogenic and mutationally constrained peptides within the HIV-1 proteome. The IMAP-peptides were found within the HIV-1 RNA sequences during viral rebound in transient-controllers and noncontrollers. This indicates these peptides are expressed during HIV-1infection and can be potentially presented to HIV-1-specific CD8 T-cells. The higher potential of CD8 T-cells from transient-controllers to recognize and respond to mutational-constrained HIV-1 epitopes, may contribute to their virological control. These results highlight the potential of IMAP for identifying novel immunogens for therapeutic strategies to enhance CD8 T-cell response.

Abstract #42

Characterising the HIV reservoir in people living with HIV and HCV after treatment for HCV

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Introduction Previous studies have shown that early HCV treatment, consisting of interferon-alpha (IFN-α) and the antiviral ribavirin (RBV) in people living with HIV-1 and HCV (PLWH/HCV) could reduce total HIV-1 DNA within CD4 T cells, suggesting a potential impact on the HIV-1 reservoir. With the introduction of new direct-acting antivirals (DAAs) for HCV treatment, recent findings have shown that total HIV-1 DNA remains stable after HCV clearance DAA therapy. However, it is unclear whether different HCV clearance strategies may modulate the size and/or genetic landscape of the replication-competent HIV-1 reservoir. To address this question, we have genetically characterised the HIV-1 reservoir during coinfection in ten participants receiving HCV therapy.

Methods To study the impact of HCV treatment on the HIV-1 reservoir, peripheral blood mononuclear cells (PBMCs) were collected from ten PLWH/HCV from Argentina. All ten participants were on suppressive antiretroviral therapy (ART): four were treated with interferon-based therapy for HCV (ART-IFN/RBV group), and six achieved HCV cure via DAA (ART-DAA group). We employed full-length individual proviral sequencing (FLIPS), developed in our lab, to characterise the HIV-1 proviral landscape in both groups. Moreover, we quantified the infection frequency of cells infected with genetically-intact and defective proviral HIV-1 at baseline (pre-treatment) and post-HCV treatment timepoints. Results Our sequence analysis revealed that the majority of the HIV-1 proviruses are genetically defective across all cohorts (70%-100 %). We observed no significant difference in the frequency of genetically-intact HIV-1 proviruses at either baseline or post-treatment timepoints in both the ART-DAA and ART-IFN/RBV groups (ART-DAA p=0.8; ART-IFN/RBV p=0.8). Similarly, no significant changes in total infection frequency were observed over time within either treatment group (ART-DAA p=0.1; ART-IFN/RBV p=0.8).

Comparison of intact infection frequencies between treatment groups at matched timepoints revealed no significant difference (p>0.9). These findings indicate that neither DAA- nor IFN-based HCV therapies significantly impacted the frequency of intact or total HIV-1 proviruses in PLWH/HCV. Discussion and Conclusion While previous studies have observed reductions in total HIV-1 DNA after IFN-α therapy, our results showed that HCV IFN-α/RBV treatment does not significantly alter the size or genetic composition of the HIV-1 reservoir in PLWH/HCV. This discrepancy between our findings and previous studies is likely attributed to our near full-length genetic characterisation of HIV-1, which allows for the quantification

Abstract #43

The Tuberculosis-Associated Microenvironment Promotes Viral Persistence in People Living with HIV and Tuberculosis.

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Introduction: The primary barrier to achieving an HIV cure is the persistence of HIV-infected cells containing genetically-intact & replication-competent virus. *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), is the most common coinfection for people living with HIV (PLWH), with an estimated 14 million people experiencing concurrent HIV and *Mtb* infection. Despite the frequency of this coinfection, we do not fully understand the impact of concurrent TB on the immune response to HIV and the persistent HIV reservoir.
Therapeutically aspirated TB-associated pleural effusion (TB-PE) represents a physiologically relevant model to investigate local immune dysfunction and viral persistence at the site of coinfection. In this study, we have investigated the persistence of cells infected with genetically-intact HIV at the site of the coinfection and in the blood of people living with HIV/TB (PLWH/TB). In addition, we have also assessed the effect of the TB-associated immune microenvironment on the functionality of HIV-specific CD8+ T cells.
Methods: We performed full-length individual proviral sequencing (FLIPS) to quantify the frequency of genetically-intact HIV proviruses in peripheral blood mononuclear cells (PBMCs) from five PLWH and five PLWH/TB from Argentina. We have also applied FLIPS to PBMC and pleural effusion mononuclear cells (PEMC) samples from three PLWH/TB.
To assess the effect of the TB-associated microenvironment on HIV-specific CD8+ T cells, cells from five PLWH were stimulated with HIV peptides and expanded for two weeks. Expanded CD8+ T cells were re-stimulated with HIV peptides and the expression of effector cytokines (IFN-γ and TNF-α) and degranulation (CD107a/b) was measured by flow cytometry in the presence or absence of TB-PE. In addition, CD8+ T cell cytotoxicity was assessed by coculturing CD8+ T cells with autologous HIV-infected CD4+ T cells in the presence or absence of TB-PE.
Results: Our analyses revealed a five-fold increase in the frequency of cells infected with genetically-intact HIV in PLWH/TB when compared to participants with HIV only (54.86±55.70 vs 10.96±14.24; $p=0.095$). In three PLWH/TB, PEMCs from the site of the coinfection had a significantly higher frequency of cells infected with genetically-intact HIV when compared to PBMCs (144±34.05 vs 3.623±3.909; $p<0.05$). Interestingly, HIV-specific CD8+ T cells treated with TB-PE exhibited diminished production of TNF-α ($p<0.001$), IFN-γ ($p<0.01$), and CD107a/b ($p<0.001$). Furthermore, these cells exhibited a reduced capacity to eliminate HIV-infected CD4+ T cells *in vitro* ($p=0.0021$).
Discussion and Conclusions: Our results suggest that the TB-associated microenvironment impairs CD8+ T cell-mediated control of HIV infection, potentially contributing to the higher frequency of cells infected with genetically-intact HIV observed in PLWH/TB. In part, this could explain the worsened clinical outcomes seen in PLWH/TB. Understanding the immunological consequences of HIV/*Mtb* coinfection is also important for the field of HIV cure, as some novel cure approaches rely on immune-mediated clearance of the viral reservoir. Furthermore, our study highlights the significant challenge that concurrent TB poses to HIV curative strategies and underscores the critical need for targeted interventions for individuals living with HIV/TB.

Abstract #44

Utilising multi-omics analysis to characterise HIV persistence at the site of HIV and Tuberculosis coinfection

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Introduction: HIV and *Mycobacterium tuberculosis* (Mtb) coinfection is a considerable burden on global mortality, with tuberculosis being the leading cause of death among people living with HIV (PLWH). Active Mtb infection is associated with pleural effusion (PE), an excess of fluid recovered from the pleural space. In PLWH, the PE exhibits higher HIV viral titres than observed in the plasma, suggesting PE represents an important compartment for interactions between the virus and the host immune system. To investigate HIV persistence during HIV/Mtb coinfection, we used single-cell multi-omics to characterise the virus-specific CD8 T-cells and the HIV infected CD4 T-cells in the PE from a participant with HIV/Mtb coinfection.
Methods: PE mononuclear cells (PEMCs) were obtained from a participant living with HIV/Mtb coinfection. Single-cell suspensions of the PEMCs were prepared for the BD Rhapsody single cell sequencing pipeline. 12,000 PEMCs were processed using the BD Rhapsody system and RNA and T-cell receptor (TCR) libraries were generated for each cell. Libraries were sequenced and data analysis included the BD Seven Bridges pipeline and the R packages Seurat, Escape, Enrichr and scRepertoire. HIV-infected cells were identified by using the HIV consensus sequence previously obtained from this participant as a reference to identify HIV transcripts. TCR sequences for cells specific for HIV, cytomegalovirus, Epstein-Barr virus, Influenza and SARS-Cov2 were obtained from the VDJdb database. Single-cell RNAseq data from PEMCs from participants with Mtb mono-infection was obtained utilising publicly available datasets (accession numbers HRA000910 and HRA00036) and used for comparative analysis.
Results: PEMCs from a HIV/Mtb coinfected individual exhibited a variety of different cell subsets, with a pronounced T-cell dominance. Analysis of antigen specificity identified CD8 T-cells specific for multiple viruses. Comparisons of these cells identified HIV-specific CD8 T-cells overexpress *TIGIT* and *CTLA4* leading to a more exhausted phenotype when compared with CD8 T-cells with other viral specificities. When comparing CD8 T-cells from HIV/Mtb coinfection PEMCs to cells from Mtb mono-infected PEMCs, CD8 T-cells from the coinfection showed a trend for higher expression of exhausted genes. HIV sequences were identified in PEMC-derived RNA transcripts from the participant living with HIV/Mtb coinfection. Examination of HIV-infected CD4 T-cells identified an enrichment of effector memory CD4 T-cells, with fewer HIV transcripts detected in central memory CD4 T-cells. Further analysis identified T-helper (Th)1/17 cells exhibited the highest number of HIV transcripts followed by follicular helper T-cells (TFH) and Th2 cells.
Discussion and conclusions: Upregulation of exhausted genes and pathways in HIV-specific CD8 T-cells suggest T-cell exhaustion and dysfunction occurs within the PE during HIV/Mtb coinfection. This dysfunction implies the HIV antiviral response is compromised in this compartment, possibly explaining the higher viral loads routinely observed in the PE compared to the plasma. Analysis of the HIV-infected cells revealed enrichment of Th1/Th17 and TFH cells, two cell subsets known to be expanded in the lung during Mtb infection. Together, these data suggest HIV/Mtb coinfection promotes a microenvironment in the pleural cavity that reduces CD8 T-cell functionality and possibly increases HIV target cells, leading to higher HIV viral load. Future analyses will provide potential therapeutic targets to ameliorate HIV/Mtb coinfection-associated morbidity.