Victorian Infection & Immunity Network

Young Investigator Symposium

Thursday 9 November 2023
Monash Institute of Pharmaceutical Sciences

www.viin.org.au
#viinyis, #YoungVIIN2023

Welcome to the 2023 VIIN Young Investigator Symposium

Dear Colleagues and Friends,

On behalf of the members of the Victorian Infection and Immunity Network's Executive Committee, we welcome you to the 2023 Young Investigator Symposium.

Special welcome to our keynote speakers: Prof Ben Cowie of The Peter Doherty Institute for Infection and Immunity and Dr Kim O'Sullivan of Monash University.

This is the 16th year that the VIIN has convened a symposium for young investigators. We are delighted to be seeing you all in-person again and remind you to be vigilant about remaining COVID-safe.

We are indebted to many who have been vital to bringing the 2023 meeting about, namely:

- The Symposium Organising Committee, who have worked tirelessly to review abstracts and organise the many logistical elements of the day:

Catarina Almeida, University of Melbourne
Joshua Bourne, Monash University
Aaron Brice, Australian Centre for Disease Preparedness, CSIRO
Ruby Dawson, Hudson Institute of Medical Research
Ella Johnston, La Trobe University
Gabriela Khoury, Monash University
Kevin Lee, University of Melbourne
Stella Liong, RMIT University
Rhea Longley, Walter and Eliza Hall Institute
Christophe Macri, University of Melbourne
Joyanta Modak, Deakin University
Melanie Neeland, Murdoch Children's Research Institute
Linda Reiling, Burnet Institute
Stephany Sanchez, WHO Collaborating Centre for Reference and Research on Influenza
Stephen Scally, Walter and Eliza Hall Institute
Ghizal Siddiqui, Monash Institute of Pharmaceutical Sciences
Rebecca Smith, Victorian Infection & Immunity Network
Caroline Soliman, University of Melbourne
Praveena Thirunavukkarasu, Monash University
Ryan Toh, Murdoch Children's Research Institute
Jinxin Zhao, Monash University

- The 50+ Session chairs and judges for oral presentations, Science Bites and posters, which is a reflection of the ongoing popularity of this event. Thanks to each for your time and expertise.
- The sponsors and advertisers for this symposium. Your support is more and more important to the success of this event.
- The 15 Academic Institutions and government agencies that support VIIN annually through financial contributions. Without your support, our activities would cease.

Finally, thank you to everyone who is here as a delegate or to present a talk or poster. This meeting is for you. We always appreciate your participation and look forward to hearing your talks, Science Bites and posters.

Sincerely,

Prof Richard Ferrero and Prof Gilda Tachedjian, Co-Convenors of VIIN

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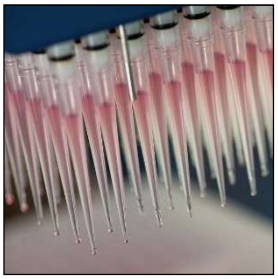
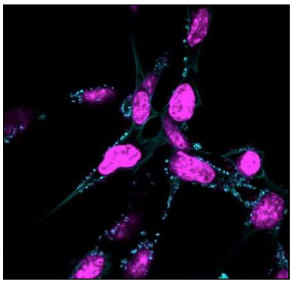
Victorian Infection & Immunity Network

Connecting Researchers, Students, Health Care and Industry to solve problems in infectious diseases and immunology

The Victorian Infection and Immunity Network (VIIN) brings together researchers from diverse disciplines across various nodes within Victoria who have an interest in infection and immunity. Through events such as the Lorne Infection and Immunity Conference and annual research symposia, VIIN connects established and emerging infection and immunity researchers with the goal of seeding new collaborations and strengthening research performance and capabilities.


The website at www.viin.org.au contains member profiles, contact details and fields of research, information on relevant news, events, platform technologies, career and funding opportunities.

VIIN Contributors include:

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A joint venture between the University of Melbourne and Melbourne Health

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For enquiries contact: info@viin.org.au

The Hartland Oration at Lorne

Professor Elizabeth Hartland was VIIN Co-Convenor between 2009 and 2017. Professor Hartland had key roles in co-convening the Lorne Infection and Immunity Conferences (2012-2017), the VIIN Industry Alliance (2012-2014) and the VIIN Young Investigator Symposia (2009 – 2016). Together with former Co-Convenor Professor Paul Hertzog, Liz oversaw the roll-out of VIIN's new website, its increasing presence on social media, implementation of the VIIN's annual careers evenings (2013-2017) and numerous other initiatives.

In honour of Liz's contribution to VIIN, the network is delighted to have established the Hartland Oration. This oration will be delivered at the Lorne Infection and Immunity Conference from 2018 onwards. The Hartland Orator will be selected at this VIIN Young Investigator Symposium and will be the post-doctoral researcher giving the best 10 minute oral presentation. This outstanding young researcher will receive free registration to the 2023 Lorne Infection and Immunity Conference and a cash contribution toward their accommodation, meals and transport.

Additional prizes at the VIIN Young Investigator Symposium

As in years past, prizes will also be awarded for:

- Poster prizes
- Science Bites prizes (3 minute oral presentations)
- Best VIIN Young Investigator Symposium PhD talk
- Best question prizes
- People's Choice Awards – stay tuned!

Would you like to join the VIIN Young Investigator Committee in 2024?

We are seeking expressions of interest from young investigators * who would like to join the VIIN Young Investigator Committee for 2024 for a 2-year term of commitment **.

Membership of the Young Investigator Committee is a prestigious opportunity for EMCRs to take leading roles in the infection and immunity community. By becoming part of the Young Investigator Committee, you are building your curriculum vitae in the areas of committee work, peer review, session chairing and skills relating to management, communication and influencing.

In this role, you will be required to:

- Attend Young Investigator Committee and Sub-Committee meetings (approximately 4 per year and held in either face-to-face or virtual modes).
- Participate in peer review for the annual Young Investigator Symposium and contribute to practical organisation of this event (including speaker management, sponsor recruitment, chairing sessions and other tasks).
- Contribute to a sub-committee responsible for the organisation of other workshops or networking activities throughout the year.
- After 12 months of service, a Young Investigator Committee member may be eligible for self-nomination as the ECR Representative to the VIIN Executive Committee.

To apply, please click here: https://forms.gle/8gFwJoSjNpgFgMhFA.

The deadline to apply is Thursday 16 November 2023, 11.59pm. Please note that these are voluntary roles.

* EMCRs within 10 years of graduation of their PhDs (career disruptions considered) are eligible to self-nominate.

** 2-year commitment is preferred, but flexible depending on professional and personal circumstances. For more information, please email info@viin.org.au and see here: https://www.viin.org.au/news/expressions-of-interest-to-join-2024-viin-young-investigator-committee or scan the QR code:

Program-at-a-Glance 2023

THURSDAY 9 NOVEMBER 2023	
08:15 – 08:45	**Registration, Sisson's Foyer in Cossar Hall**
08:45 – 08:55	**Welcome and Acknowledgement of Country, Lecture Theatre**
08:55 – 09:25	**Session 1, Lecture Theatre** **Oral Presentations 1: Research Assistants**
09:25 – 10:00	**Session 2, Lecture Theatre** **Science Bites 1**
10:00 – 10:45	**Session 3, Lecture Theatre** **Keynote Speaker 1: Prof Ben Cowie, The Peter Doherty Institute for Infection and Immunity**
10:45 – 11:25	**Morning tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
11:25 – 12:05	**Session 4, Lecture Theatre** **Oral Presentations 2: Honours students**
12:05 – 12:45	**Session 5, Lecture Theatre** **Oral Presentations 3: Early-career researchers**
12:45 – 14:00	**Lunch and Poster Session 1, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
14:05 – 15:10	**Session 6, Lecture Theatre** **Oral Presentations 4: PhD students**
15:10 – 15:50	**Afternoon Tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
15:50 – 16:20	**Session 7, Lecture Theatre** **Keynote Speaker 2: Dr Kim O'Sullivan, Monash University**
16:20 – 16:55	**Session 8, Lecture Theatre** **Science Bites 2**
17:00 – 18:00	**Evening networking and Poster Session 2, Cossar Hall**
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

Notes

Social media: TWITTER / X: We encourage you to join the conversation on Twitter / X with #YoungVIIN2023 and #viinyis. Please follow us @The_VIIN where we will be live tweeting symposium highlights. Please remember:
(1) Follow the VIIN's Social Media Policy https://www.viin.org.au/docs/viin-policy-on-social-media-draft-230713.pdf or via the QR code →
(2) **Have permission! Please only Tweet about presentations where speakers or poster presenters have explicitly given permission**, either verbally at the time of the presentation or by the use of a symbol on their slides or poster that conveys their wishes. (See the Social Media Policy above for examples of these symbols.)
(3) **Don't capture or transmit any data:** Please do not record or take photographs of any data slide in an oral presentation or Science Bite, or any data on a poster.

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Please visit the Registration Desk or ask our friendly catering team for information about where to collect your special meal.

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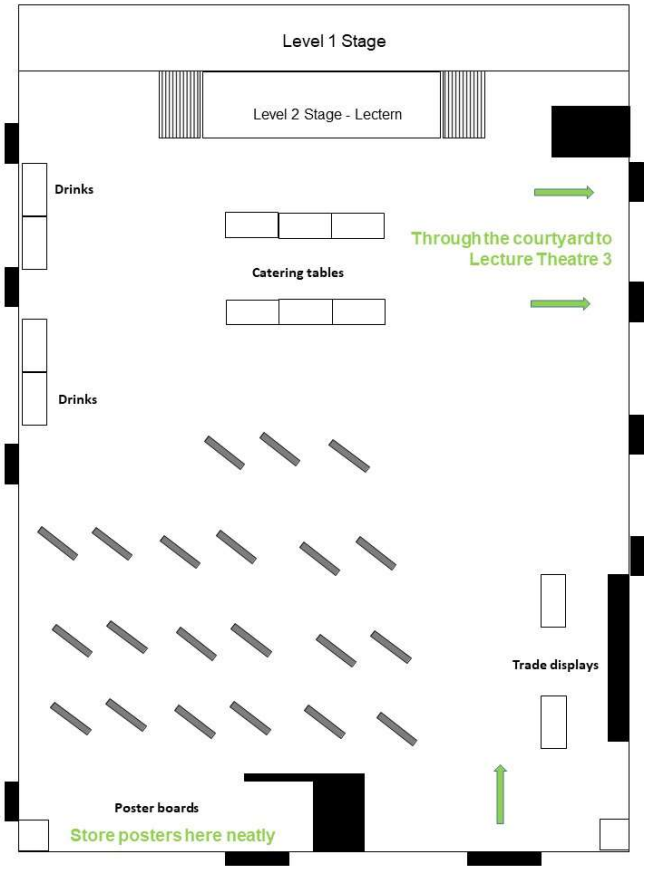
Please store your poster at the back of Cossar Hall, ensuring it is not a trip hazard.



Post event survey

Please feedback on the YIS in 2023 here:

Thursday 9 November

Thursday 9 November: 8.15am – 6.15pm

08:15 – 08:45	**Registration, Sisson's Foyer**
08:45 – 08:55	**Welcome and Acknowledgement of Country, Lecture Theatre** **Chairs: Kevin Lee, University of Melbourne and Christophe Macri, University of Melbourne**
08:55 – 9:25	**Session 1, Lecture Theatre** **Oral Presentations 1: Research Assistants** **Chairs: Kevin Lee, University of Melbourne and Christophe Macri, University of Melbourne**
08:55	**Identification of monoclonal antibodies against *Plasmodium vivax* Apical Membrane Antigen 1 that promote functional responses** Chiara Drago, Burnet Institute
09:05	**The Omicron BA.1 bivalent COVID-19 booster vaccine enhances the capacity of SARS-CoV-2-specific memory B cells to recognise Omicron BA.5 and BQ.1.1** Holly Fryer, Monash University
09:15	**Inflammatory profiles of vaginal *Gardnerella vaginalis* isolates from South African women with and without bacterial vaginosis** Sarah Amir Hamzah, Burnet Institute
09:25 – 10:00	**Session 2, Lecture Theatre** **Science Bites 1** **Chairs: Kevin Lee, University of Melbourne and Christophe Macri, University of Melbourne**
09:25	**Long-lived anti-cytokine autoantibodies associated with post-COVID-19 sequalae** Catherine Chen, Walter and Eliza Hall Institute of Medical Research
09:29	**Modelling & Targeting Cytokine Storm in Dengue virus-infected Mice** William Clow, Walter and Eliza Hall Institute of Medical Research
09:33	**The role of an innate-like T cell subset during *Plasmodium* sporozoite infection** Phoebe Dewar, University of Melbourne
09:37	**The *Helicobacter pylori* virulence factor, Tipα, is carried by bacterial extracellular vesicles to the nuclear compartment of host cells** Jack Emery, Hudson Institute of Medical Research
09:41	**Influenza vaccine responses to A(H1N1)pdm09 antigens in 2020 and 2021 among repeatedly vaccinated healthcare workers** Jessica Hadiprodjo, University of Melbourne
09:45	**Mapping the Commitment of Haematopoietic Progenitors to the T Cell Lineage** Dhruti Parikh, University of Melbourne and St Vincent's Institute
09:49	**Unravelling the roles of haematopoietic thymic antigen presenting cells in unconventional T cell development** Yi Wang, University of Melbourne
10:00 – 10:45	**Session 3, Lecture Theatre** **Keynote Speaker 1: Prof Ben Cowie, The Peter Doherty Institute for Infection and Immunity** **Chairs: Linda Reiling, Burnet Institute**
10:45 – 11:25	**Morning tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**

11:25 – 12:05	**Session 4, Lecture Theatre** **Oral Presentations 2: Honours Students** **Chairs: Rhea Longley, Walter and Eliza Hall Institute and Stella Liong, RMIT University**
11:25	**Lifting the Break: Regulation of Programmed Death-Ligand 1 in Dendritic Cells** Delaram Babaei, Bio21, University of Melbourne
11:35	**An isothermal CRISPR-Cas12a point-of-care assay for rapid and sensitive detection of *N. gonorrhoeae*** Natasha Dyson, University of Melbourne and Walter and Eliza Hall Institute of Medical Research
11:45	**Investigating the effects of amino alcohol antimalarials on artemisinin activity in *Plasmodium falciparum*** Jennifer Le, Monash Institute of Pharmaceutical Sciences
11:55	**Identification of potential HLA-A*03:01 restricted CD8+ T cell epitopes conserved across influenza A and B viruses** Hannah Priest, Monash University
12:05 – 12:45	**Session 5, Lecture Theatre** **Oral Presentations 3: Early-Career Researchers** **Chairs: Ghizal Siddiqui, Monash Institute of Pharmaceutical Sciences and Stephen Scally, Walter and Eliza Hall Institute**
12:05	**Potent, HIV-specific latency reversal through CRISPR activation delivered by lipid nanoparticles exhibiting a high efficiency of transfecting resting CD4+ T cells with minimal toxicity** Paula Cevaal, Peter Doherty Institute of Infection and Immunity, University of Melbourne
12:15	**Olaparib attenuates bleomycin-induced pulmonary fibrosis in a sheep model** Habtamu Derseh, Federation University
12:25	**Multi-omic approaches define new functional roles for the transient receptor potential vanilloid 4 (TRPV4) ion channel in macrophages** Nathan Nguyen, Monash University
12:35	**Identifying antibody responses to PfEMP1 that protect children from severe malaria** Isobel Walker, Peter Doherty Institute for Infection and Immunity, University of Melbourne
12:45 – 14:00	**Lunch and Poster Session 1, Cossar Hall** **(includes transit time from and to Lecture Theatre)** **See below for more information**
14:00 – 15:10	**Session 6, Lecture Theatre** **Oral Presentations 4: PhD Students** **Chairs: Catarina dos Santos Sa e Almeida, University of Melbourne and Stephany Sanchez, WHO Collaborating Centre for Research and Reference on Influenza**
14:00	**Characterisation of Novel Cytokine Interferon Epsilon in the Murine Peritoneal Cavity** Jasmine Chuah, Hudson Institute of Medical Research
14:10	**Characterising host immune responses to invasive multi-drug resistant ST34 monophasic *Salmonella*** Sahampath Hettiarachchi, Monash University and Hudson Institute of Medical Research

14:20	**Partners in crime: investigation of influenza-group A streptococcus coinfection** Jenny Hua, Murdoch Children's Research Institute
14:30	**CD1c presentation of a branched ganglioside, GD1a** Guan-Ru Liao, Monash University
14:40	**Characterising the expression and signalling properties of NKp44-isoforms in Natural killer cell cytotoxicity** Alexander Sedgwick, The Peter Doherty Institute for Infection and Immunity and University of Melbourne
14:50	**Understanding persistence of *Streptococcus pneumoniae* serotype 6B in Mongolia following vaccine introduction** Paige Skoko, Murdoch Children's Research Institute
15:00	**Dysregulated immune responses via TLR7 drive maternal and foetal complications during gestational IAV infection** Gemma Trollope, RMIT University
15:10 – 15:50	**Afternoon Tea, Cossar Hall** **(includes transit time from and to Lecture Theatre)**
15:50 – 16:20	**Session 7, Lecture Theatre** **Keynote Speaker 2: Dr Kim O'Sullivan, Monash University** **Chair: Joshua Bourne, Monash University**
16:20 – 17:00	**Session 8, Lecture Theatre** **Science Bites 2** **Chairs: Praveena Thirunavukkarasu, Monash University and Joshua Bourne, Monash University**
16:20	**Differences in Fc-dependent functional activities of ChAdOx1-S and CoronaVac vaccine-induced antibodies against SARS-CoV-2** Alexander Harris, Burnet Institute
16:24	**Geospatial mapping of malaria and anaemia among mothers and infants in Papua New Guinea** Catherine Ives, Monash University
16:28	**The molecular definition of potent *Plasmodium falciparum* invasion inhibitory epitopes on PTRAMP-CSS** Pailene Lim, Walter and Eliza Hall Institute of Medical Research
16:32	**CD28 stimulation enhances CAR T cell differentiation, particularly in older patients, but CD28 abundance is predictive of CAR T cell product quality** Palak Mehta, RMIT University
16:36	**Functional antibody responses after quadrivalent HPV vaccination** Chau Quang, The University of Melbourne
16:40	**Bypassing antimalarial drug resistance by altering parasite redox mechanisms** Annie Roys, Monash Institute of Pharmaceutical Sciences
16:44	**Methylseleninic acid induces HIV viral reactivation from latently infected cells in vitro and ex vivo** Rory Shepherd, The Peter Doherty Institute of Infection and Immunity
16:48	**The lipidome of lipid droplets changes significantly during viral infection in the brain** Zahra Telikani, La Trobe University
17:00 – 18:00	**Evening networking and Poster Session 2, Cossar Hall**
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

POSTER 1

12:45 – 14:00	Lunch and Poster Session 1, Cossar Hall (includes transit time from and to Lecture Theatre)
1	**Fibroblastic Reticular Cells coordinate protective adaptive immune responses during viral infection in the spleen** Yannick Alexandre, University of Melbourne, Mid-career researcher
2	**Structural landscape of SARS-CoV-2 entry: Molecular interplay between spike glycoprotein human serine proteases** Naveen Vankadari, University of Melbourne, Mid-career researcher
3	**Identification of a potent dengue virus inhibitor using a virtual compound screen** Thomas Burton, University of Melbourne, Early-career researcher
4	**Investigating the monocyte inflammatory response to bacterial ligands in patients with Crohn's disease** Paul Gill, Monash University, Early-career researcher
5	**CD4+ T cell recognition of Haemagglutinin epitopes across different influenza strains** Emma Grant, La Trobe Institute for Molecular Science (LIMS), La Trobe University, Early-career researcher
6	**DISC-The Next Generation of African swine fever vaccine** Fan Jia, CSIRO, Early-career researcher
7	**Investigating the role of lipid droplets in the antiviral mechanism of *Wolbachia*** Robson Kriiger Loterio, Monash University, Early-career researcher
8	Withdrawn
9	**Discovery of new enzyme function in *Plasmodium falciparum*** Nadine Djunaedi, Monash University, Honours student
10	**Characterising the replication complex of a recently isolated novel Australian henipavirus** Emily Dowling, CSIRO Australian Centre for Disease Preparedness and Deakin University, Honours student
11	**Characterizing the immune memory response generated toward Group A Streptococcus** Selwyn Lo, Monash University, Honours student
12	**Regulation of thymocytes migration during T cell development by Coronin 2a, a putative actin-cytoskeleton regulator** Thuy Trang (Jenny) Lam, University of Melbourne, PhD student
13	**The role of the human kinase B-Raf in *Plasmodium falciparum* blood-stage infection** Adedoyin Akinware, RMIT University, Masters student
14	**Identification of novel malaria proteins involved in parasite-host cell interactions** Christina Dizdarevic, Deakin University, Masters student
15	**γδ T cell-derived IL-4 promotes cellular immunity to liver-stage malaria** Shirley Le, University of Melbourne, Masters student
16	**Host erythrocyte and reticulocyte cell signalling during infection with *Plasmodium spp.*** Mohammad Shuaib, RMIT University, Masters student

17	**Novel Drug Targets for Inhibiting Malarial Nuclear Import** Alec Wehmeier, Deakin University, Masters student
18	**Fc-dependent functional antibody responses in immunity to severe *Plasmodium falciparum* malaria in children** Grace Wright, Burnet Institute, Masters student
19	**Enzymatic processing in influenza antigen presentation** Hong Ngoc Le, Biomedicine Discovery Institute, Monash University, Research assistant
20	**Transmission dynamics and population structure of *P. falciparum* and *P. vivax* in Mondulkiri Province, Cambodia** Katelyn Stanhope, Burnet Institute, Research assistant
21	**Factors associated with infant carriage of antimicrobial resistance genes: a systematic review** Aseel Al-Araji, Deakin University, PhD student
22	**Interleukin-2, not chemokine receptors CXCR3, CXCR5 or CXCR6, controls Th1/Tfh fate bifurcation during blood-stage malaria** Takahiro Asatsuma, Peter Doherty Institute for Infection and Immunity, University of Melbourne, PhD student
23	**Anti-inflammatory effect of increased IgG4 following SARS-CoV-2 mRNA vaccine boosting is antigen dependent** Carissa Aurelia, Peter Doherty Institute of Infection and Immunity, University of Melbourne, PhD student
24	**Functional assessment of the NOD2 signalling pathway in patients with primary immunodeficiency** Ebony Blight, Monash University, PhD student
25	**Mechanism of self-ganglioside presentation by the antigen presenting molecule CD1b** Yanqiong Chen, Monash University, PhD student
26	**Defining targets and mechanism of action of immunity against *Plasmodium vivax* malaria** Rosy Cinzah, Burnet Institute, University of Melbourne, PhD student
27	**Investigating the chronic pulmonary and vascular consequences of influenza infection in atherosclerotic mice** Madison Coward-Smith, RMIT University, PhD student
28	**Response and resistance to combination immune checkpoint blockade associate with distinct baseline and on-treatment blood T-cell profiles in melanoma patients** Jack Edwards, Monash University, PhD student
29	**Restoring sensitivity of *Streptococcus pneumoniae* to tetracyclines with metal ionophores** Chloe Estoque, University of Melbourne, PhD student
30	**Investigating the role of cytokine signalling regulator CISH in T-cell responses to viral infections** Rifqa Fayaz, Deakin University, PhD student
31	**Attenuating inflammation-driven neural damage through the cGAS-STING pathway in a mouse-model of traumatic brain injury** Amelia Fryer, University of Melbourne, PhD student
32	**Investigating the frequency of T cell subsets in the ganglionic and aganglionic bowel in Hirschsprung Disease** Ashleigh Gould, Monash Institute of Pharmaceutical Sciences, PhD student

33	**Characterising Antibody Epitopes to a Malaria Parasite Protein to Inform Vaccine Design** Timothy Ho, Burnet Institute, PhD student
34	**Monocytes support antibody-mediated protection in malaria** Jessica Horton, Burnet Institute, PhD student
35	**Development of Real-Time Biosensors to Detect Airborne Allergens** Kira Hughes, Deakin University, PhD student
36	**Defining novel virus restriction factors for respiratory virus infections** Shirley Lin, University of Melbourne, PhD student
14:00	**[Return to main program above]**

POSTER 2

17:00 – 18:00	Evening networking and Poster Session 2, Cossar Hall (includes transit time from and to Lecture Theatre)
37	**Defining the fine specificity of antibody responses to polymorphic and conserved epitopes of the lead malaria vaccine antigen: *Plasmodium falciparum* circumsporozoite protein** Alessia Hysa, Burnet Institute and University of Melbourne, PhD student
38	**Elucidating the prognostic and functional roles of NK cell subsets in bladder cancer** Md Abdullah Al Kamran Khan, University of Melbourne, PhD student
39	**Plasma metabolomic variation in Juvenile idiopathic arthritis is not exclusively driven by chronic inflammation** Jooa Kwon, University of Melbourne and Murdoch Children's Research Institute, PhD student
40	**Comparison of Cellular Immunity in Fractional and Standard Dose of Pfizer-BioNTech (BNT162b2) in Healthy Mongolian Adults** Kiara Mangano, Murdoch Children's Research Institute, Honours student
41	**The lipid droplet recruits antiviral signalling proteins following cellular viral infection** Jay Laws, La Trobe University, PhD student
42	**Unveiling the interplay of *Coxiella burnetii* Dot/Icm effectors: insights into host immune subversion** Yi Wei Lee, University of Melbourne, PhD student
43	**MMV687794 impairs blood-stage *Plasmodium falciparum* invasion by perturbing lysophospholipids** Dawson Ling, Burnet Institute, PhD student
44	**Activity of bis-1,2,4-triazines, a novel potent antimalarial, requires parasite-mediated activation process** Peiyuan (Annie) Luo, Monash Institute of Pharmaceutical Sciences, PhD student
45	**Alteration of neuroimmune pathways rescues impaired intestinal permeability and behaviour in a mouse model of colitis** Samantha Matta, RMIT University, PhD student
46	**Discovering novel natural products to combat antibiotic resistant pathogens** Negero Negeri, Monash University, PhD student
47	**Withdrawn**
48	**Uncovering the regulatory networks for *Klebsiella pneumoniae* iron acquisition** To Nguyen, Monash University, PhD student
49	**BCL-2 and BCL-XL dynamically regulate LPS-primed macrophage survival and inflammation in the absence of BCL2A1** Ashlyn Pascoe, Monash University and Hudson Institute of Medical Research, PhD student
50	**Identifying Merozoite Surface Proteins as Targets of Protective Functional Antibody Responses against *Plasmodium falciparum* and *P. vivax* malaria** Kaitlin Pekin, Burnet Institute, PhD student
51	**Mucosal delivery of self-assembling nanoparticle vaccines against SARS-CoV-2 using recombinant influenza virus vectors**

	Devaki Pilapitiya, University of Melbourne and The Peter Doherty Institute for Infection and Immunity, PhD student
52	**TREML4 ablation in mice leads to the development of innate immune memory following polymicrobial sepsis** Corey Pollock, La Trobe University, PhD student
53	**Gasdermin D mediates the severity of influenza a virus-induced lung disease** Sarah Rosli, Hudson Institute of Medical Research, PhD student
54	**Understanding the protective role of goblet cells in Hirschsprung Disease** Matthew Rowe, Monash Institute of Pharmaceutical Sciences, PhD student
55	**Developing precision RNA therapeutics for Tuberculosis** Jan Schaefer, Walter and Eliza Hall Institute of Medical Research, PhD student
56	**Enhancing thermal stability of live virus vaccine using metal organic frameworks** Aimee Talbot, CSIRO, PhD student
57	**Towards the development of non-antibiotic based strategies to clear *Klebsiella pneumoniae* from the gut** Sher Maine Tan, University of Melbourne, Peter Doherty Institute for Infection and Immunity, PhD student
58	**Assessment of monovalent and bivalent SMAC mimetics to both shock and kill the HIV reservoir** Kiho Tanaka, Peter Doherty Institute for Infection and Immunity, PhD student
59	**The polyphenol rich sugarcane extract (PRSE) has potential antiviral activity against influenza A virus *in vitro*** Caolingzhi Tang, University of Melbourne, PhD student
60	**Redefining iNKT cells thymic development pathway in human** Naeimeh Tavakolinia, University of Melbourne, PhD student
61	**Understanding the role of rodent malaria clag genes in new permeation pathway formation** Mitchell Trickey, Deakin University, PhD student
62	**Promoting Extrinsic Apoptosis to Diminish Latent HIV Reservoir in Vivo** Le Wang, University of Melbourne, PhD student
63	**Exploring the Potential of *Plasmodium falciparum* Exportin-1 as a Target for 2- Aminobenzimidazoles through Nuclear Fractionation Coupled Proteomics** Yunyang Zhou, Monash Institute of Pharmaceutical Sciences, PhD student
64	**Antibody responses and B-cell memory formation after COVID-19 vaccination in patients with primary immunodeficiency** Jessica Canning, Monash University, Honours student
18:00 – 18:15	**Prize-giving, thank yous and conclusion, Cossar Hall**

ABSTRACTS
SESSION 1

Identification of monoclonal antibodies against *Plasmodium vivax* Apical Membrane Antigen 1 that promote functional responses

Chiara Drago*[1,2], Lee M Yeoh[1,3], Linda Reiling[1,2,3], Christopher L King[4], Lenore Carias[4] and James G Beeson[1,2,3].

[1]Burnet Institute, Melbourne, Australia. [2]Monash University, Clayton, Australia. [3]The University of Melbourne, Melbourne, Australia. [4]Case Western Reserve University, Ohio, USA.

* = presenting author

Monoclonal antibodies (mAbs) are a novel approach to malaria prevention and control. Recently, mAbs against *Plasmodium falciparum* malaria were found to be an effective therapeutic, suppressing parasitaemia and preventing clinical disease in infected individuals. However, the same strides have not been made for *Plasmodium vivax*. Whilst *P. falciparum* causes more deaths, *P. vivax* is more prevalent and severe than initially thought. Shortfalls in *P. vivax* research mean there is currently no vaccine in clinical trials. Here, we generated mAbs specific to *P. vivax* Apical Membrane Antigen 1 (*Pv*AMA1), which is an essential part of the parasite invasion machinery. We assessed their ability to initiate immune functions associated with malarial protection and aimed to quantify reactivity against AMA1 from other *Plasmodium* strains and species.

All mAbs greatly inhibited erythrocyte invasion by parasites expressing *Pv*AMA1 and exhibited high reactivity overall with various markers of humoral immunity. Specifically, plate-based assays were used to investigate initiation of the complement cascade and binding of Fc-receptors, which are found on various immune cells such as monocytes. Binding of mAbs to these markers suggests that downstream functional effects would be stimulated, and therefore, the immune system should be activated. One mAb, #838839, was the strongest at fixing C1q complement protein and binding to the Fc-receptor FcγRIIa. It also showed considerable binding to FcγRI and FcγRIIIa and was the best performing mAb overall. Furthermore, several species-transcending mAbs with substantial reactivity were identified by testing mAb binding to different recombinant AMA1 proteins. Two mAbs, #816817 and #818819, exhibited significant binding to AMA1 from two other species, *P. knowlesi* AMA1 and *P. falciparum* AMA1. As such, these mAbs may have the potential to confer cross-species protection, which would be beneficial in endemic areas where coinfections are especially prevalent.

To the best of our knowledge, this paper is the first to illustrate that anti-*Pv*AMA1 mAbs can mediate antibody effector mechanisms against *P. vivax*, suggesting that they may induce an array of protective immune responses. These results provide *in vitro* proof-of-concept that mAbs are potentially effective as a therapeutic to *P. vivax*, with specific mAbs identified as promising leads for further investigation.

The Omicron BA.1 bivalent COVID-19 booster vaccine enhances the capacity of SARS-CoV-2-specific memory B cells to recognise Omicron BA.5 and BQ.1.1.

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Background: To overcome the limited recognition of SARS-CoV-2 Omicron subvariants by the vaccine-elicited antibody response, bivalent COVID-19 mRNA vaccines comprising ancestral Wuhan-Hu-1 (WH1) and the Omicron BA.1 or BA.5 variant have been administered globally. We previously showed that monovalent 3rd dose boosters enhanced recognition of Omicron BA.2 and BA.5. Here, we compared monovalent and BA.1 bivalent 4th dose boosters in their capacity to enhance neutralising antibody (NAb) and memory B-cell (Bmem) recognition of the Spike receptor binding domains (RBD) of Omicron BA.1, BA.5 and BQ.1.1.

Objectives: 1) Quantify the serological response and 2) phenotype the RBD-specific Bmem compartment elicited by the monovalent 4th dose and the BA.1 bivalent 4th dose booster; and 3) determine the capacity of these Bmem to bind BA.5 and BQ.1.1 RBDs.

Methods: Peripheral blood was sampled before and 1 month after a 4th dose booster from healthy adult recipients of either a monovalent (n=18) or BA.1 bivalent mRNA vaccine (n=33). Plasma NAb against WH1, Omicron BA.1, and BA.5 were quantified with a plaque reduction neutralisation test. Fluorescent tetramers of in-house recombinantly produced RBDs of the WH1, BA.1, BA.5, and BQ.1.1 variants were incorporated in an extensive spectral flow cytometry panel to quantify the absolute numbers and immunophenotype of RBD-specific Bmem.

Results: Both the monovalent and bivalent vaccines similarly boosted NAb levels against WH1 and BA.5, whereas the response to BA.1 was significantly higher following the BA.1 bivalent booster. WH1-specific Bmem numbers were not boosted by either vaccine. Only the BA.1 bivalent vaccine significantly boosted the numbers of Bmem recognising BA.1, and moreover it enhanced the frequencies of WH1-specific Bmem that recognised BA.1, BA.5, and BQ.1.1.

Conclusions: The BA.1 bivalent booster can significantly boost NAb and Bmem specific for Omicron BA.1, and may be able to improve recognition of descendent subvariants beyond that of a conventional vaccine. This project provides new insights into the capacity of a bivalent Omicron-based booster to improve immune memory against emerging viral variants to protect against severe COVID-19.

Inflammatory profiles of vaginal *Gardnerella vaginalis* isolates from South African women with and without bacterial vaginosis

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Bacterial vaginosis (BV) is highly prevalent amongst women residing in sub-Saharan Africa, where HIV is also widespread. BV, characterised by the overgrowth of non-optimal vaginal anaerobic bacteria, most commonly *Gardnerella vaginalis*, is associated with genital inflammation which facilitates HIV acquisition. However, *G. vaginalis* is also found in healthy women with low levels of inflammation, suggesting strain-level differences that may influence inflammatory responses. Hence, this study aims to characterize vaginal *G. vaginalis* isolates from South African women to elucidate their role in inflammation.

Cervicovaginal fluid samples from 10 BV-negative and 10 BV-positive South African women (aged 16 – 25) were cultured on Columbia Blood agar to isolate single strains of *G. vaginalis*, followed by species-level identification via 16S rRNA Sanger sequencing. Isolates were then co-cultured with vaginal epithelial VK2/E6E7 cells and the concentrations of inflammatory cytokines previously associated with HIV risk were measured via Luminex assay. The protein profiles of the isolates were also analysed via liquid chromatography tandem mass spectrometry.

Thirty-nine isolates were acquired from all women, including n = 15 *G. vaginalis* and other bacterial taxa. Three and four *G. vaginalis* isolates from different BV-negative and BV-positive women, respectively, were selected for inflammatory profile assessment. Significant increases in interleukin (IL)-1β, IL-6, IL-8 and chemokine ligand (CCL)2, CCL4 and CCL5 were induced by isolates from BV-positive women compared to BV-negative women ($p < 0.05$). Proteomics analyses detected 2,139 proteins and although 125 proteins were significantly differentially abundant between isolates from BV-negative versus BV-positive women, only two remained significant after adjusting for multiple comparisons. This included chaperone protein ClpB and peptidase.

G. vaginalis isolates from BV-positive women elicited higher levels of inflammatory cytokines previously associated with increased HIV acquisition risk compared to those from BV-negative women. This suggests that strain-level differences may play an important role in genital inflammation and resultant HIV acquisition risk. Few proteins differed significantly between isolates from BV-negative versus BV-positive women, largely due to sample size and high variance between individual strains. Hence, the proteomic and genomic profiles of additional *G. vaginalis* isolates are being investigated to determine differences that may influence inflammation.

ABSTRACTS
SESSION 2

Long-lived anti-cytokine autoantibodies associated with post-COVID-19 sequalae

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Emerging COVID-19 serological studies have identified a link between the presence of autoantibodies and severe disease. These autoantibodies can interfere with essential immunological processes which impede pathogen defence response and exacerbate lung and vascular injuries. However, the presence of autoantibodies following a broader spectrum of COVID-19 disease severity and their relevance to developing post-COVID-19 sequalae remains less explored.

In this study, a custom developed multi-plex Luminex panel consisting of 20 autoantigens was used to screen for autoantibodies that target proteins involved in coagulation, complement activation, inflammation, cellular recruitment, and neutrophil extracellular trap formation. We analysed a total of 143 COVID-19 convalescent individuals (1-467 days from diagnosis) and 278 COVID-19 seronegative healthy controls. Compared to healthy individuals, COVID-19 convalescent participants had increased prevalence of autoantibodies for 14 out of 20 autoantibodies screened. Additionally, more than 30% of the convalescent population had detectable autoantibodies targeting at least one of the 20 autoantigens tested. In support of previous studies, individuals that experienced severe disease had the highest levels of autoantibodies, while younger people (18-30 years old) had the lowest abundance of autoantibodies during convalescence. We found that most detectable autoantibodies persist up to a year following infection, and in some cases even up to 400 days. Analysis in a subset of COVID-19 convalescent individuals with autoantibodies that were divided into whether they experienced prolonged symptoms lasting over four weeks (n=33) or not (n=9) revealed that specific autoantibodies (e.g. interferon omega, interleukin-17A, interleukin-17F, interferon alpha-1, interferon alpha-2, mannose-binding lectin-associated serine protease 2) were more prevalent in the group with ongoing symptoms. In conclusion, this study identified autoantibody profiles associated with COVID-19 disease, including their association with disease prognosis and with post-COVID-19 sequalae.

Modelling & Targeting Cytokine Storm in Dengue virus-infected Mice

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With an estimated 100 million symptomatic cases annually, Dengue is well recognised as the most common human arboviral infection globally. A subpopulation of infections exhibits debilitating symptoms of systemic inflammation and vascular leakage, which may be deadly unless monitored closely in hospital. This severe disease is characterised by a cytokine storm: a dysregulated host immune response in which overabundant immune signalling becomes harmful to the host. Dengue treatment is limited to fluid replacement therapy to replace lost blood volume, and Dengue vaccines have had a troubled development that limits public uptake. Novel therapeutic options are urgently needed.

To address this, I have optimised a Dengue mouse model that recapitulates the inflammatory disease observed in humans, where viremia peaks 1-2 days post symptom onset, before symptoms worsen simultaneously with immune activation and viral clearance. Type 1 interferon (IFN)-deficient mice infected with Dengue virus type 2 (DENV2) develop peak viremia at 2 days post-infection followed by weight loss and immune activation up to 4 days post-infection, at which point viremia reaches low or undetectable levels. 17/26 cytokines tested were upregulated in infected mouse plasma and spleen samples, including key inflammatory cytokines upregulated in human disease such as IL-6, TNF, and IL-1β.

Interestingly, COVID-19 and Dengue not only share many risk factors, but also immune signatures and inflammatory symptoms. Based on the recent progress in understanding cytokine storm in COVID-19, I am now targeting cytokine signalling with clinical-stage anti-inflammatory drugs to treat Dengue in my established mouse model. Preliminary data suggest that TNF blockade with etanercept did not mitigate disease outcomes. I am currently determining the efficacy of Dexamethasone (anti-inflammatory glucocorticoid) and GM-CSF blockade, and plan to assess Jak inhibition, IL-6 blockade and IL-1β blockade. Collectively, this work will provide crucial information for the development of treatment strategies for moderate/severe Dengue, and my discoveries here may also be applied to the significantly deadlier Dengue/COVID-19 coinfection.

The role of an innate-like T cell subset during *Plasmodium* sporozoite infection

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The *Helicobacter pylori* virulence factor, Tipα, is carried by bacterial extracellular vesicles to the nuclear compartment of host cells.

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The bacterium *Helicobacter pylori* tightly regulates the host immune response to dampen inflammation and promote persistence. Excessive inflammatory responses to the bacterium, however, promote gastric carcinogenesis. Several *H. pylori* proteins have been associated with an increased risk of gastric cancer. One of these proteins, tumour necrosis factor-α-inducing protein (Tipα), when produced in its recombinant form was reported to traffic to the host cell nucleus, leading to tumour necrosis factor (TNF) gene expression and carcinogenic effects. Although Tipα is secreted by the bacterium, we showed in proteomic studies that it is also released by bacterial extracellular vesicles (BEVs); these membrane "blebs" are highly efficient at entering and subverting host cell functions. We hypothesised that *H. pylori* BEVs carry Tipα to the nucleus, resulting in perturbed nuclear processes that promote carcinogenesis. To address this hypothesis, we generated *H. pylori tipA* mutants and complemented *tipA*/*tipA*+ strains. By immunoblotting, we confirmed that *H. pylori* BEVs harbour Tipα. In addition, the quantity of Tipα secreted into culture supernatants varied between *H. pylori* strains, but was not associated with disease outcome. Gastric epithelial cells (AGS) were treated with *H. pylori* OMVs or recombinant Tipα (rTipα). By immunoblotting and confocal microscopy, we showed that BEV-associated Tipα is present within the cytoplasm at 4 hours post-treatment and accumulates in the nuclear compartment by 18 hours. We then tested the ability of Tipα to induce pro-inflammatory signalling in AGS and THP-1-derived macrophages. Consistent with previous work, rTipα induced TNF production in THP-1 cells. Conversely, BEVs from *tipA* bacteria induced significantly more TNF than those from wild-type (WT) or *tipA*/*tipA*+ bacteria ($p = 0.0007$), as well as significantly more IL-8 production in AGS ($p < 0.0001$) and THP-1 cells ($p = 0.0127$). Taken together, we propose that BEV-associated Tipα may dampen pro-inflammatory responses but promote carcinogenesis in *H. pylori* infection.

300 words

Influenza vaccine responses to A(H1N1)pdm09 antigens in 2020 and 2021 among repeatedly vaccinated healthcare workers

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Background

Repeated administration of influenza vaccines appears to incrementally attenuate immunogenicity and effectiveness, especially when successive vaccines are antigenically similar. Although these effects appear to be worse for A(H3N2), they are also observed for A(H1N1)pdm09, which has shown increasing antigenic diversity in recent years.

Methods

A cohort of Australian health care workers (HCWs) was followed for post-vaccination antibody responses across two years during which influenza did not circulate (2020-2021). Vaccine administered in 2020 contained an A/Brisbane/02/2018-like H1N1 antigen, while in 2021 an antigenically distinct A/Victoria/2570/2019-like antigen was included. Pre-vaccination, 14 days and 7 months post-vaccination sera were assessed in haemagglutination inhibition (HI) assay against influenza A(H1N1)pdm09 vaccine viruses from the corresponding years to assess pre/post vaccination antibody titres. Differences in titre were compared by prior vaccination history.

Results

A total of 1384 HCWs contributed sera in the two years. Among them, 96 were previously unvaccinated (vaccinated in 0/5 prior years) and 778 were frequently vaccinated (≥5/5 prior years). While frequent vaccination attenuated titres and titre rises in both years, the effect was substantially diminished in 2021. Notably, only 16% of frequently vaccinated versus 80% of previously unvaccinated HCWs seroconverted in 2020 versus 80% and 86%, respectively in 2021.

The 2021 vaccine strain differed from all prior H1N1pdm09 vaccines at HA positions N129D, K130N and N156K, which are within prominent antigenic sites. Additionally, only the 2021 vaccine strain had 185I, which was present in seasonal H1N1s. We are currently investigating whether these substitutions facilitated a stronger or more specific immune response through mechanisms such as escape from memory dominance or recall of memory against prior seasonal strains. Sera are being titrated against viruses from the alternate year, and against reverse genetics viruses bearing single substitutions. PBMC's are being assessed to compare the frequency and phenotype of H1 HA reactive B cells induced.

Conclusions

The H1N1 vaccine antigen used in 2021 induced substantially greater antibody responses than the 2020 antigen, particularly among frequently vaccinated HCW. Investigations are underway to understand how antigenic changes in the 2021 antigen may have enhanced immunogenicity.

Mapping the Commitment of Haematopoietic Progenitors to the T Cell Lineage

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T cells develop in the thymus from bone marrow-derived haematopoietic progenitors that migrate via the bloodstream. Multiple haematopoietic progenitors with T cell lineage potential have been identified in the bone marrow, but the identity of the populations that actually colonise the thymus remains unclear. The characterisation of these thymus-seeding progenitors and their immediate progeny, the early thymic progenitors, has been challenging due to their rarity. Moreover, it is thought that these progenitors, at least at a population level, remain multipotent after entering the thymus. Thus, they can differentiate into non-T cell lineages until the thymic microenvironment drives their commitment toward the T cell lineage.

To characterise the earliest stages of T cell development in the thymus, $CD4^{-}CD8^{-}$ double negative thymocytes were profiled in depth by single-cell RNA-sequencing, which allows for identifying distinct and potentially rare populations based on their transcriptional profiles. Within these double-negative thymocytes, we identified a novel and transcriptionally discrete population that expressed multiple multipotency-associated markers. However, the overall transcriptional profile of this population did not align with the previously identified “traditional” early thymic progenitor. This novel population also differed in cell surface marker expression. In particular, it expressed CD25, which the “traditional” early thymic progenitor does not express. Still, the two populations had similar expression of the multipotency-associated transcription factor *Spi1* and haematopoietic stem and progenitor-associated marker CD34. These findings suggest that there may be more than one population of thymic progenitors, and these may be derived from different bone marrow progenitors that seed the thymus. Future work will determine these thymic progenitors' non-T cell lineage potential and bone marrow-derived precursors *in vitro* and *in vivo*.

Unravelling the roles of haematopoietic thymic antigen presenting cells in unconventional T cell development

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Mucosal-associated invariant T (MAIT) and natural killer T (NKT) cells are unconventional T cells that recognise non-peptide antigens presented on non-classical MHC molecules MR1 and CD1d, respectively. They have important roles in microbiota homeostasis, infection, cancer, and autoimmunity. These cells are thymus-derived, where they branch off from conventional T cell development at positive selection. While there are clearly major differences in MAIT and NKT development such as intrathymic effector differentiation, little is understood about how this process is orchestrated.

We postulate that MAIT and NKT development is driven by signals received by physically interacting with thymic antigen presenting cells (APCs). To study physical interactions in the thymus, we exploited thymic rosettes, which are complexes of developing T cells interacting with a central APC. We found that MAIT and NKT cells, particularly in later stages of development, are enriched in rosettes compared to total thymus cells. This suggests that they likely interact with APCs during their final stages of maturation, such as effector differentiation. To identify which APCs are interacting with MAIT and NKT cells, we thoroughly characterised thymic APCs using single cell RNA sequencing. We identified previously underappreciated complexity in hematopoietic APCs. Thymic DCs and macrophages consist of five distinct subsets: immature conventional DC1s (cDC1s), immature *Cd209*+ cDC2s, *Ccr7*+ mature DCs, plasmacytoid DCs and monocyte-derived cells (MC). Of these populations, we demonstrate that a subpopulation of MCs express *Mr1* and *Cd1d1,* and *Cd1d1* is additionally expressed in cDCs. This suggests that these cells are capable of interacting with MAIT and NKT cells via their T cell receptor, and potentially be the APCs that are necessary for their development. With the identification of the potential roles of these APCs, the extrinsic molecular signals that are involved in unconventional T cell maturation and acquisition of effector functions can now be elucidated.

ABSTRACTS
SESSION 4

Lifting the Break: Regulation of Programmed Death-Ligand 1 in Dendritic Cells

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The complex relationship between cancer and the immune system has been a subject of intense research, with the surface protein programmed death-ligand 1 (PD-L1) emerging as a key player. PD-L1 overexpression in cancer cells can hinder the antitumor response of CD8+ T cells, which can lead to unchecked tumour growth[1]. Despite the existence of anti-PD-L1 treatments, their limited efficacy necessitates a deeper comprehension of PD-L1 regulation[2]. Intriguingly, PD-L1 expression by dendritic cells (DCs), which are pivotal initiators and shapers of the immune response against cancer, can also contribute to the suppression of antitumor immunity [3,4]. This can make dendritic cells appealing targets for novel therapeutic approaches.

B-cell lymphoma 6 (BCL6) was identified as a potential regulator of PD-L1 in a CRISPR/Cas9 screen performed in MuTu dendritic cell line. This project aims to further validate the role of BCL6 in regulating PD-L1 using previously generated BCL6-knockout MuTu dendritic cells. A retroviral vector was used to reconstitute the wild type and N21K loss-of-function mutant BCL6, which was created using site-directed mutagenesis, into the BCL6-knockout MuTu dendritic cells. Subsequently, through a multifaceted approach involving flow cytometry, western blot, and cytometric bead array a broader role for BCL6 in these cells was characterised.

As predicted, BCL6 inhibited surface and total PD-L1 expression at resting conditions and lowered the increase of PD-L1 following stimulation with poly I:C, IFN-γ, CpG and CpG+IFN-γ. Beyond PD-L1, BCL6 promoted expression of the antigen-presenting molecule MHCII and downregulated IL-6 secretion under resting and the mentioned activation conditions. BCL6 additionally exhibited the ability to downregulate the related costimulatory molecule PD-L2, when cells were stimulated with CpG and CpG+IFN-γ. Finally, BCL6 increased the resting levels of FLT3, a receptor critical for dendritic cell development and homeostasis. Future analysis will generate BCL6 gene-deleted murine bone marrow derived DCs to examine the role of BCL6 in primary dendritic cells.

By exploring PD-L1 expression mechanisms in DCs, we seek vital insights into the interplay of cancer and immunity. This could catalyse improved diagnostics and treatments, ameliorating patient outcomes and easing the personal and socioeconomic burdens of cancer.

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An isothermal CRISPR-Cas12a point-of-care assay for rapid and sensitive detection of *N. gonorrhoeae*

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The sexually transmitted infection, gonorrhoea impacts over 87 million individuals every year and the rising rates of antimicrobial resistance in the causative agent, *Neisseria gonorrhoeae* pose a major threat to public health. Prevention and control of gonorrhoea depend on accessible, rapid, and accurate diagnosis, however, existing diagnostic methods are expensive, time-consuming, and require specialised expertise, hindering accessibility, especially in low- and middle-income countries where gonorrhoea is most prevalent. Therefore, there is an urgent need for novel diagnostics. Exploiting the capabilities of isothermal recombinase polymerase amplification (RPA) in combination with CRISPR-Cas12a technology, our study aimed to develop a rapid point-of-care assay for *N. gonorrhoeae* detection. To achieve this, a panel of 22 primer/guide sets was screened for sensitive and specific detection of *N. gonorrhoeae,* against panels of serially diluted *N. gonorrhoeae* genomic DNA (gDNA), non-gonococcal *Neisseria* gDNA, and non-gonococcal sexually transmissible pathogens, using a fluorescence-based readout. The best-performing primer/guides were then tested in combination on six *N. gonorrhoeae* strains from the World Health Organization reference panel, in a dual-target approach. The developed assay delivers results within 45 mins, has a limit of detection of 4 copies/µL, and demonstrates 100% specificity against other pathogens, while the dual-target design enhances assay robustness and offers a minimised risk of diagnostic escape. These results were clinically validated in a blind concordance study of 60 clinical samples, comparing assay results to 'gold-standard' quantitative PCR, where a fluorescence signal exceeding 10 standard deviations above the mean fluorescence of the no template control, was defined as positive. This novel diagnostic approach has the potential to overcome current limitations in gonorrhoea management, improving access to quality diagnosis and facilitating effective care within a single appointment, to assist in the global elimination strategy.

Investigating the effects of amino alcohol antimalarials on artemisinin activity in *Plasmodium falciparum*

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Malaria is a major global health concern with drug resistance to all currently available antimalarials. Infection with *Plasmodium falciparum* parasites is the main cause of malaria-associated morbidity and mortality. Artemisinin-based combination therapies (ACTs) are the recommended first-line treatment against *P. falciparum*, which combine an artemisinin derivative with a partner antimalarial. The most widely used ACT partner drugs globally are the amino alcohol class, including lumefantrine, mefloquine and amodiaquine. However, the mechanism of action of these drugs remains largely unknown. Additionally, resistance has now emerged to the artemisinin component, which manifests in parasites at the very early stage of its asexual lifecycle known as the ring stage, and it is unknown how amino alcohols interact with artemisinins in ring-stage parasites. This work investigates the relationship between amino alcohols and artemisinins, and the implications for artemisinin resistance.

We performed ring stage survival assays with a combination of dihydroartemisinin (DHA) and amino alcohols, as well as a range of other antimalarials. Parasite cultures were treated with 700 nM DHA alone or in combination for 3 hours. Clinical plasma concentrations of the partner drugs were used, which causes no ring-stage parasite killing under these conditions. Results showed a significant survival increase for *Pf*3D7 rings treated with DHA and the amino alcohols mefloquine or quinine compared to DHA alone (16-27% vs 3-5%). This effect was not seen with other antimalarials such as pyrimethamine and chloroquine. In artemisinin resistant parasites (Cam3.II^{R539T}), amino alcohol combinations with DHA further potentiated artemisinin resistance (20% vs 50-70%). As artemisinin activity is dependent on activation by haem released by parasite haemoglobin digestion, we performed haem fractionation assays to determine if amino alcohols decrease the availability of haem for artemisinin activation. Mefloquine-treated *Pf*3D7 parasites showed a 25% decrease in haem levels, suggesting that amino alcohols antagonise artemisinin activity by decreasing activation. Further studies will use high resolution mass spectrometry to investigate whether amino alcohol antimalarials directly impact artemisinin activation in parasites. Future research may be needed to establish whether the antagonistic relationship exhibited between amino alcohols and DHA at the early ring-stage will potentiate further progression of artemisinin resistance in the field.

Identification of potential HLA-A*03:01 restricted CD8$^+$ T cell epitopes conserved across influenza A and B viruses

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Influenza viruses cause annual epidemics and sporadic pandemics of acute respiratory illness. Current vaccines stimulate antibody-based immunity against surface antigens of the virus. These surface antigens have highly variable structures and evolve to evade detection by the immune system. Thus, current vaccines are reformulated annually in anticipation of the circulating strains. When the strains included in the influenza vaccine match the circulating strains, vaccine efficacies can be as high as 60% however this drops to 10% in a mismatched season.

Instead of targeting B cells, alternative influenza vaccines could target CD8$^+$ T cells using the antigen processing and presentation pathway. Antigen processing and presentation samples proteins of the cell, including influenza proteins if the cell is infected, and loads their peptide products onto Human Leukocyte Antigen class I molecules (HLA-I) that traffic to the cell surface to be surveyed by CD8$^+$ T cells. CD8$^+$ T cells therefore have access to all influenza proteins, including the more conserved internal proteins.

HLA-I are genetically diverse, and different HLA variants will present different arrays of peptides. This study aimed to identify influenza peptides presented by the common HLA-I molecule HLA-A*03:01 that are conserved between strains and could act as immune targets. To achieve this, cells expressing HLA-A*03:01 were infected with influenza A or B viruses, the HLA-A*03:01 molecules extracted, and the bound peptides eluted and analysed by mass spectrometry. This analysis identified over 90 influenza derived HLA-A*03:01 ligands per virus. This included peptides covering two regions of PB1 conserved between Influenza A and B viruses. These peptides represent candidates that can be investigated for immunogenicity and vaccine development. The identification of conserved T cell targets has the potential to drive generation of vaccines protective against diverse influenza strains.

ABSTRACTS
SESSION 5

Potent, HIV-specific latency reversal through CRISPR activation delivered by lipid nanoparticles exhibiting a high efficiency of transfecting resting CD4⁺ T cells with minimal toxicity

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Introduction: Activation of HIV transcription through LTR-targeted CRISPRactivation (CRISPRa) provides a promising strategy of reversing HIV latency without affecting host-cell transcription. However, the advancement of this novel latency-reversing agent is hampered by the lack of a delivery vehicle for the CRISPRa machinery to resting CD4⁺ T cells. We hypothesized that targeted mRNA-lipid nanoparticles (LNPs) could be used to advance CRISPRa as a next-generation latency-reversing agent.

Methods: Fluorescently labelled standard and modified LNPs encapsulating reporter mCherry mRNA (mCherry-LNP) or co-encapsulating the dCas9-SAM CRISPRa system (CRISPRa-LNP) were formulated through microfluidic mixing using two different lipid mixes. T cell-targeting antibodies were captured onto the LNPs following controlled-orientation conjugation of anti-IgG1 nanobodies to the LNP surface. Transfection efficiency and associated toxicity were assessed in non-stimulated or αCD3/αCD28 pre-stimulated primary CD4⁺ T cells or PBMCs from HIV-negative donors. Reactivation of HIV transcription was assessed in J-Lat LTR-Tat-IRES-GFP reporter cells.

Results: Transfection efficiency of pre-stimulated CD4⁺ T cells using standard LNPs was moderate (mean±SEM mCherry⁺ cells 29±5%) but toxic (43±5% viability) after 72h. In non-stimulated cells, few cells were transfected (2±0.3%) with lower toxicity (68±8% viability), which coincided with a 20-fold reduction in LNP association. In contrast, transfection of non-stimulated CD4⁺ T cells with modified LNPs resulted in a striking 92±2% efficiency at minimal toxicity (88±3% viability) within 72h. Similarly, treatment with modified but not standard CRISPRa-LNPs induced potent HIV transcription with all five targeting guideRNAs, reaching up to 76±13% GFP⁺ J-Lat cells compared with 0.89±0.1% using non-targeting guideRNA, both at viabilities >90%. Functionalization of the modified LNPs with T cell-targeting antibodies enhanced the T cell transfection efficiency in the presence of bystander cells from below 1% to above 50%.

Conclusions: We developed a novel LNP formulation capable of delivering nucleic acid-based therapeutics to resting CD4⁺ T cells. Antibody-functionalization of the modified LNP surface further enhances the specificity towards T cells with great potency. The three-component dCas9-SAM CRISPRa system can be co-encapsulated into one LNP and can induce strong latency reversal in a cell line model for HIV latency. These results provide compelling justification for the further assessment of CRISPRa-LNP as a 'shock and kill' strategy.

Olaparib attenuates bleomycin-induced pulmonary fibrosis in a sheep model

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Introduction:
Poly (ADP-ribose) polymerase 1 (PARP-1) is a constitutive enzyme involved in the regulation of various biological processes that are implicated in pulmonary fibrosis, including DNA repair, cell death, metabolism and inflammation. The aim of this study was to investigate whether the clinically used PARP inhibitor drug Olaparib can attenuate pulmonary fibrosis in a large animal model.

Methods:
Sheep (n=12) received two fortnightly instillations of bleomycin (3U) or saline (control) into separate lung segments. Two weeks after the second bleomycin/saline administration, sheep were randomly assigned into 2 groups and treated orally, twice/week for 4 weeks, with either Olaparib (10 mg/kg in 10% DMSO/10% 2-hydroxy-propyl-β-cyclodextrin/PBS) or vehicle alone (controls). Lung function analysis and bronchoalveolar lavage fluid collection were performed in all sheep at baseline, two weeks after the second bleomycin/saline administration, and at the end of the experiment. The degree of inflammation, fibrosis and overall pathology was determined by the semiquantitative histopathology scoring method.

Results:
Olaparib significantly reduced bleomycin-induced lung injury and fibrosis in sheep. In vehicle-treated control animals, bleomycin-infused lung segments had prominent fibrosis, whereas saline-infused internal control lung segments in the same animal had normal lung architecture with no signs of lung injury and fibrosis. The overall pathology, inflammation, and fibrosis scores were all significantly reduced in bleomycin-infused segments of sheep treated with Olaparib, compared to bleomycin-administered lung segments of vehicle-treated control sheep (overall pathology: bleomycin-vehicle 18.57 ± 1.80 vs bleomycin-Olaparib 6.58 ± 1.60, p= 0.0043; inflammation scores: bleomycin-vehicle 9.77 ± 0.30 vs bleomycin-Olaparib 3.52 ± 0.76, p= 0.0081; fibrosis score: bleomycin-vehicle 8.80 ± 0.85 vs bleomycin-Olaparib 3.06 ± 0.87, p= 0.0103). While there were no changes observed in whole lung function measures, Olaparib significantly reduced neutrophil and lymphocyte numbers in bronchoalveolar lavage fluid of bleomycin-infused lung segments as compared to bleomycin-infused lung segments of vehicle-only treated animals.

Conclusion:
Inhibition of PARP provided significant resolution of bleomycin-induced pulmonary fibrosis, suggesting that the activation of PARP plays a key role in the pathophysiology of pulmonary fibrosis in sheep. Our data suggest the possibility of repurposing PARP inhibitors for pulmonary fibrosis.

Multi-omic approaches define new functional roles for the transient receptor potential vanilloid 4 (TRPV4) ion channel in macrophages

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Transient Receptor Potential Vanilloid 4 (TRPV4) is a polymodal ion channel that is expressed by a diverse range of cell types including macrophages (1-3). TRPV4 responds to environmental cues such as mechanical stimulation (e.g. cell swelling, shear stress and extracellular matrix stiffness), temperature and endogenous lipids (e.g. arachidonic acid derivates). In macrophages, TRPV4 facilitates innate immune functions such as cytokine release, phagocytic activity and cell motility (1, 4). Although established, current understanding of how TRPV4 contributes to these processes is limited by the use of highly targeted methods to detect specific proteins, lipids and transcriptional endpoints. However, these approaches may not accurately reflect the true complexity of how TRPV4-dependent calcium signalling influences these pathways (5). We applied a multi-omics approach including proteomic, metabolomic and lipidomic profiling to provide novel insights to clarify how TRPV4 mediates these established roles. Functional knockout of TRPV4 in macrophages resulted in a 20% change in the proteome. It revealed unappreciated roles for TRPV4 in pathways involved in innate immunity and metabolism. Together, these findings significantly advance our understanding of how TRPV4 functions as a key integrator of diverse signalling processes that are central to macrophage function and likely to be more broadly applicable to TRPV4 signalling in other cells.

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Identifying antibody responses to PfEMP1 that protect children from severe malaria

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Severe malaria leads to approximately 600,000 deaths annually and predominantly affects children under five[1]. A key factor in pathogenesis of severe malaria is the sequestration of *Plasmodium falciparum* parasite infected erythrocytes (IEs) in the microvasculature, which is mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the IE surface. Each parasite expresses one of approximately 60 PfEMP1 variants, which contain multiple variant subdomains. Several variants have been associated with severe disease, including those that bind to the endothelial receptor ICAM-1. PfEMP1 is the major target of antibodies on the IE, however details of the subdomain targets and biophysical features of antibodies that confer protection from severe malaria are unclear. We performed a detailed characterization of the PfEMP1 targets and antibody Fc features in a case control study of Papua New Guinean children that included 157 severe malaria cases and 82 with uncomplicated malaria. We measured multiple antibody Fc features (class, subclass, Fc receptor interactions and C1q fixation) for antibodies targeting 33 recombinant PfEMP1 domains that have previously been associated with severe or uncomplicated malaria. We used machine learning with regularized logistic regression to identify eight antibody features that could differentiate between severe and uncomplicated malaria with 76% accuracy. The selected features included FcγRIIIb binding antibodies targeting DBLβ domains that bind to ICAM-1, along with FcγRIIb, FcγRIIIa and IgG4. To confirm the antibody target and role of FcγRIIIb binding we evaluated antibody dependent neutrophil phagocytosis of IEs that bind to ICAM-1. We found that phagocytosis of IEs that bind to ICAM-1 was higher when opsonized with plasma from children with uncomplicated malaria compared to severe malaria. Our results suggest that antibodies targeting ICAM-1 binding DBLβ and which induce neutrophil phagocytosis protect children from severe malaria. This work may lead to development of a PfEMP1 based therapeutic that induces a targeted functional immune response, or development of sensitive prognostic indicators for severe malaria.

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ABSTRACTS
SESSION 6

Characterisation of Novel Cytokine Interferon Epsilon in the Murine Peritoneal Cavity

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The novel type I interferon, interferon epsilon (IFNε) possesses a unique manner of spatiotemporal expression and regulation. Largely studied in the female reproductive tract (FRT), it has been shown to maintain homeostatic conditions and mediate protective immunity against common FRT pathogens. Many FRT pathologies including cancer, endometriosis, and infection can extend detrimentally into the peritoneal cavity, and are characterised by dysregulated immune responses. Therefore, it is hypothesised that IFNε may have protective immunomodulatory effects that influence the phenotype, activity and composition of peritoneal immune cells under both steady state and inflammatory conditions.

Previous research utilizing syngeneic mouse models of high grade serous ovarian cancer (HGSOC) has indicated that IFNε administration alleviates tumour burden and ascites development, accompanied by changes in the activity of peritoneal immune cell populations. Following these observations, the peritoneal immune cells present in both male and female wild-type (WT) and *Ifnε-/-* mice in steady-state conditions and during inflammation were investigated via immunophenotyping. The results indicated that in homeostatic states, endogenous IFNε maintains a basal immunity in the peritoneal cavity by regulating the peritoneal myeloid cells through their recruitment into the peritoneal cavity, differentiation and proliferation, as well as through the modulation of the activation states in peritoneal lymphoid cells.

Administration of exogenous IFNε as a pretreatment prior to the induction of peritonitis in WT mice showed that IFNε may act similarly to IFNβ in the peritoneal cavity during infection, potentially through regulating the migration and activation of the peritoneal immune cells in response to infection. Collectively, these data underline the role of IFNε in the murine peritoneal cavity to maintain basal immunity, which may prompt the peritoneal immune response during bacteria-induced inflammation. Thus, IFNε may have potential as a future therapeutic in peritoneal pathologies featuring aberrant activity in the peritoneal immune cells, primarily peritonitis, endometriosis and HGSOC metastases.

Characterising host immune responses to invasive multi-drug resistant ST34 monophasic *Salmonella*

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Non-typhoidal *Salmonella* (NTS) is one of the major causes of foodborne disease globally with an estimated incidence of 93.8 million cases per year. The large number of animal reservoirs and high foodborne transmission rates contribute to this distribution. In immunocompetent patients, NTS clinically manifests as gastroenteritis to which there are no gold-standard therapies.

Sequence type 34 (ST34) monophasic *Salmonella* isolates are known to cause an invasive form of disease with a fatality rate 200 times higher than caused by typical NTS. Treatment for this invasive disease requires antibiotics. However, the presence of antibiotic resistance genes and mechanisms of immune evasion present a significant barrier to treatment. These characteristics coupled with the lack of a specific therapy for NTS distinguish these ST34 monophasic isolates as a severe public health problem.

Further understanding of ST34 monophasic isolates is crucial to establishing avenues for combatting these pathogens.This project aims to outline the cellular responses to these ST34 monophasic isolates and elucidate the impact that antibiotics have on their infective ability. This was done by investigating the intracellular replication, cytotoxicity, and the activation of inflammatory pathways in host cells upon infection. The ST34 monophasic isolates exhibit increased replication in macrophages and induce less cytotoxicity compared to typical NTS. Using phosphoproteomics, we found several host kinases involved in cell cycle regulation and inflammation that were differentially altered by ST34 isolates in comparison to other circulating clinical *Salmonella* isolates. Additionally, most ST34 isolates were determined to be phenotypically resistant to four classes of antibiotics.

Future work will involve quantifying the effect of antibiotics on bacterial replication and cytotoxicity. Our study will be the first to analyse the host cellular mechanisms these ST34 isolates alter to elicit their disease. Our findings will help determine potential host protein targets for the development of novel therapeutics against ST34 isolates and give insight into how intracellular pathogens cause disease.

Partners in crime: investigation of influenza-group A streptococcus coinfection

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The upper respiratory tract harbors a diverse community of microbes including the bacterium group A streptococcus (also known as strep A) and influenza virus. Epidemiological evidence suggests an association between influenza coinfection and severe strep A disease. However, little is known about influenza-strep A interactions that underly enhanced disease.

Here, we examined influenza-strep A coinfection by establishing an air-liquid interface model using primary human nasal epithelial cells. These fully differentiated cultures of airway epithelial cells are composed of diverse cell types, such as mucous-producing and ciliated cells, closely resembling the human upper airway epithelium in vivo. In this study, we infected these primary epithelial cells with influenza A virus (subtype H3N2), followed by strep A. We examined the effect of the virus on strep A and compared the effects of coinfection and single infections on the epithelial cells. Prior influenza infection did not affect bacterial load immediately after bacterial inoculation but increased bacterial density of strep A (serotype M75) by approximately 100-fold at 24 hours post-bacterial infection ($n=20$, $p<0.0001$). Likewise, this effect of prior viral infection was also observed with several other strep A strains of differing serotype. Coinfection also led to a higher proportion of intracellular bacteria ($n=7$, $p=0.03$), measured by antibiotic protection assay. Additionally, we evaluated the effect of coinfection on airway integrity and airway epithelial innate defense mechanisms. Coinfection reduced transepithelial electrical resistance ($n=4$) and cilia beating frequency ($n=4$-5) compared with strep A ($p=0.004$ and 0.1, respectively) or influenza ($p=0.003$ and 0.02, respectively) single infections.

This study presents novel experimental data demonstrating that coinfection with influenza A virus facilitates strep A replication in the upper respiratory tract. In contrast, contrary to previous research conducted with submerged cell cultures, coinfection does not enhance initial bacterial adhesion to nasal epithelial cells. Enhanced damage to nasal epithelium and impaired mucociliary transport suggest that influenza virus may have a role in facilitating strep A dissemination within the host. We are currently investigating the bacterial and host transcriptomic responses during coinfection to understand the biological processes involved.

CD1c presentation of a branched ganglioside, GD1a

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Antigen presentation is key in initiating T cell-mediated adaptive immunity in disease progression such as viral infection or autoimmune diseases. Well-known antigens, peptides, are presented to receptors on T cells (TCRs) via antigen presenting molecules known as major histocompatibility complex (MHC) proteins [1]. The CD1 family of proteins are MHC-like proteins that present lipid antigens to T cells and consists of 5 isoforms in humans. One member, CD1c, can present microbial lipid-based antigens from Mycobacterium tuberculosis (Mtb) in protective immunity [2], as well as self-phospholipids and acylglycerides that are recognised by autoreactive T cells [3].

Gangliosides are a class of sphingolipids enriched in the brain and nerve tissues, with studies showing that self-ganglioside presentation by CD1c to T cells play an aberrant role in mediating autoimmune disorders such as multiple sclerosis (MS) [4]. However, the molecular mechanisms that mediate this mode of activation in autoimmunity remain unknown. To answer this, we determined the crystal structure of CD1c in complex with a branched ganglioside, GD1a, by first producing CD1c recombinantly and loading GD1a into the CD1c antigen binding cleft in vitro. The two fatty acid tails of GD1a are anchored in F' pockets with the ganglioside head group protruding out of G' portal. Here, as opposed to standard lipid presentation by other CD1 members such as CD1d, where the branched headgroup is presented upwards towards the typical TCR recognition site, the ganglioside headgroup is presented uniquely sideways, representing a novel mechanism of antigen presentation. In summary we demonstrated, for the first time, the mechanisms of branched ganglioside presentation by CD1c. Future work will investigate CD1c-ganglioside autoreactive T cell receptor repertoire in healthy donor and patient with disease of interest.

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Characterising the expression and signalling properties of NKp44-isoforms in Natural killer cell cytotoxicity

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Natural killer (NK) cells can restrict tumour growth by secreting proinflammatory cytokines and direct lysis of malignant cells. While NK cells readily recognise molecules upregulated to the tumour cell surface, whether NK cells can sense tumour-secreted factors has been largely ignored. The NK cell receptor NKp44 binds to the secreted mitogen Platelet Derived Growth Factor-D (PDGF-D). Tightly regulated during homeostasis, PDGF-D overexpression drives pro-tumour pathways by binding to PDGF receptor-β. That NKp44 binding to PDGF-D triggered NK cell secretion of proinflammatory cytokines challenges the prevailing dogma that activating receptor ligands shed into the tumour microenvironment suppress NK cell anti-tumour function. However, whether this interaction can trigger NK cell cytotoxicity was not shown. Using a flow cytometric killing assay we show that recombinant PDGF-D can enhance NK cell lysis of tumour cell lines *in vitro*.

Still, how the different NKp44-isoforms might regulate NK cell activation and trigger cytotoxicity remains controversial. NKp44 isoform-1 (NKp44-1), but not NKp44-2 or NKp44-3, encodes a putative tyrosine-based inhibition motif. Using green fluorescent protein (GFP) reporter T cells genetically engineered to express one NKp44-isoform each, we show that all three receptors can stimulate GFP expression upon binding to PDGF-D. Moreover, co-crosslinking of NKp44-1 with the T cell receptor does not reduce reporter cell activation, indicating that NKp44-1 does not act as an inhibitory receptor. Intriguingly, NKp44-1 expressing reporter cell clones display significantly reduced surface expression compared to NKp44-2 and NKp44-3. This suggests this cytoplasmic tyrosine-motif may function to regulate NKp44-1 internalisation rather than inhibit NK cell activation.

To date, detection of NKp44-isoform expression has been limited to the transcript level. We have generated novel antibodies that can specifically detect NKp44-isoform proteins in immortalised and primary NK cells by flow cytometry, confocal microscopy and western blot. These reagents will facilitate the tracking of NKp44-isoforms through the cell upon binding to PDGF-D and visualisation of their role in facilitating tumour cell lysis. Overall, this research will improve our understanding of the molecular machinery that underpins NK cell surveillance of PDGF-D in anti-tumour immunity.

Understanding persistence of *Streptococcus pneumoniae* serotype 6B in Mongolia following vaccine introduction

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Pneumonia is the leading cause of death for children under five years of age and *Streptococcus pneumoniae* (the pneumococcus) is the leading bacterial cause. Pneumococcal conjugate vaccines (PCVs), targeting a subset of pneumococcal capsule polysaccharides (serotypes), are effective at reducing the disease burden. However, some vaccine-serotypes persist, a phenomenon that is poorly understood, particularly in Asia. Here, we investigate vaccine-serotype persistence post-PCV introduction in Mongolia.

Hospital-based pneumonia surveillance (2015-2021) was conducted across four Mongolian districts to measure the effect of vaccine introduction in 2016. Nasopharyngeal swabs (n=15,183) were collected from children with pneumonia and a subset (n=6,545) were screened for pneumococci and serotyped using DNA microarray. To understand the genetic background of circulating serotypes, we used DNA microarray to infer genetic lineage (using Global Pneumococcal Sequence Cluster, GPSC) and examined lineage changes post-PCV. Using an infant mouse model of disease, we explored differences in colonisation and disease dynamics between lineages, by infecting C57BL/6 mice (five days old) intranasally with pneumococci (one isolate per lineage).

Overall, there was a reduction in the prevalence of vaccine-serotypes post-PCV. However, some individual vaccine-serotypes (6A, 6B, 19F and 23F) persisted and remain a likely cause of pneumonia in Mongolian children. Analyses show lineage replacement among 6B, where the dominant lineage pre-PCV, GPSC23 (16/21, 76% in 2015), was replaced by GPSC6 post-PCV (15/18, 83% in 2020; $p<0.0001$). In our mouse model, at seven days post-infection, GPSC23 colonised to a higher pneumococcal density in the nasopharynx than GPSC6 ($p=0.03$). Over the 15 days post-infection, GPSC23 had a higher tendency to transition to disease, with 86% (12/14) of mice becoming moribund when compared with the GPSC6 lineage (14%, 2/14 mice becoming moribund at 20 days of age; $p<0.0001$). In the moribund mice, GPSC23 had disseminated throughout the body to the lungs (n=6 mice), blood (n=5 mice), brain (n=1 mouse) and middle ear (n=1 mouse).

Despite persistence of vaccine-serotype 6B, our mouse model showed the replacement lineage GPSC6 was less likely to transition to disease, potentially adding confidence to the current vaccine program in Mongolia. Future experiments will examine other phenotypes to better understand lineage replacement.

Dysregulated immune responses via TLR7 drive maternal and foetal complications during gestational IAV infection

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Background: Influenza A virus (IAV) is responsible for ~1-billion infections and up-to 650,000 global deaths annually. Whilst typically considered to be self-limiting, certain populations are at risk of severe complications, including pregnant people. Gestational IAV infection causes increased morbidity and mortality, driven by acute cardiopulmonary events in the mother, which results in poor foetal outcomes including pre-term birth, intrauterine growth restriction, and altered neurodevelopment. These complications arise despite a lack of vertical transmission of the virus *in utero* and is evidenced to be related to a pregnancy-specific inflammatory vascular storm within the maternal aorta, characterised by increased viral dissemination and inflammatory cell infiltration. Here, we propose the pattern recognition receptor, Toll-like receptor 7 (TLR7), which senses ssRNA viruses such as influenza, drives the hyper-immune phenotype, which disrupts the delicate immune balance required for healthy pregnancies, ultimately leading to poor maternal and foetal outcomes following infection.

Results: Using a mouse model of gestational IAV infection we show that TLR7-deletion ($TLR7^{-/-}$) reduces disease severity, as evidenced by restored bodyweight growth in infected dams across the pregnancy. Additionally, we show $TLR7^{-/-}$ prevents viral dissemination into the heart and aorta of infected dams. $TLR7^{-/-}$ confines infection to the respiratory tract, whilst simultaneously preserving the integrity of the alveoli and peri-bronchiolar space. Reducing viral dissemination prevents a cardiovascular manifestation of gestational infection, bradycardia, $TLR7^{-/-}$ animals exhibited normal pulse rate compared to WT animals. The reduced disease severity in the maternal cardio-respiratory systems prevented foetal morbidity and mortality. Offspring born to $TLR7^{-/-}$ IAV-infected dams did not exhibit growth restriction, neuro-inflammation, cell death or hypoxia compared to their WT counterparts, indicating foetal growth and neurodevelopment is preserved in the absence of TLR7.

Conclusion: We have for the first time shown that the exacerbated disease pathogenesis of gestational IAV infection is driven by TLR7. In the absence of TLR7 systemic disease severity is reduced to uninfected levels, both lung pathology and cardiovascular manifestations are significantly improved. By preventing systemic disease maternal health and foetal development are preserved. These data offer insight into the pathogenic mechanisms of gestational IAV infections and indeed presents TLR7 as a viable therapeutic target.

ABSTRACTS
SESSION 8

Differences in Fc-dependent functional activities of ChAdOx1-S and CoronaVac vaccine-induced antibodies against SARS-CoV-2

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The development and deployment of efficacious vaccines formulated on the original Wuhan (Wuhan-Hu-1) strain has decreased the global burden of COVID-19, however this progress is threatened by the emergence of variants of concern (VOCs). The protective role of neutralising antibodies induced through vaccination has been well documented; however, antibodies can also mediate other arms of the immune system by binding to Fcγ receptors (FcγR) on immune cells or by fixing complement and activating the classical pathway of the complement system, facilitating phagocytosis and cell-killing. These Fc-dependent responses induced following vaccination are less well defined, especially against VOCs such as the Omicron variant currently in circulation worldwide.

We aimed to determine the magnitude of FcγR-binding and complement-fixing antibodies targeting the Wuhan, Delta, and Omicron SARS-CoV-2 spike proteins, elicited through natural infection or vaccination with the AstraZeneca or Sinovac vaccines, which have been extensively used globally. We also quantified the influence of prior SARS-CoV-2 exposure on vaccine-induced antibody Fc-mediated functional activities. Serum samples were collected from Brazilian adults attending the UERJ Hospital with a SARS-CoV-2 infection (n=200; median of 67 days post infection) or for vaccination (n=222; median of 34 days post dose two) with either the AstraZeneca or Sinovac vaccines (46.8% had a prior infection). Samples were tested for IgG, IgA, and IgM against the spike protein, and their ability to engage FcγRI, FcγRIIa, and FcγRIIIa, and fix complement.

AstraZeneca vaccination generally induced higher Fc-mediated functional responses compared to Sinovac vaccination. Induction of complement-fixing antibodies was generally low. Previously exposed vaccinees had substantially higher Fc-mediated functional antibodies than those who were SARS-CoV-2 infection naïve. Previously exposed AstraZeneca vaccinees had significantly higher antibody Fc-mediated responses compared to previously exposed Sinovac vaccinees, with notably elevated levels of complement-fixation. IgG magnitude and FcγRI-binding activity were generally well maintained against variant S proteins across all vaccine groups. However, antibody FcγRIIa and FcγRIIIa-binding and complement-fixation was significantly reduced against the Omicron S protein. These findings are important in understanding Fc-functional antibody responses following SARS-CoV-2 vaccination, thereby informing future vaccine design and optimal booster policy.

Geospatial mapping of malaria and anaemia among mothers and infants in Papua New Guinea

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Background

Papua New Guinea (PNG) has the highest incidence of malaria cases in the Western Pacific region. Pregnant women and infants are particularly vulnerable to infection. Women who contract malaria during pregnancy are at higher risk of maternal mortality and poor outcomes for the newborn infant. Anaemia is also high prevalent and is partly caused by malaria. This study describes the geospatial distribution of these conditions among women during pregnancy and the first 12-months post-partum, as well as anaemia and low birth weight among their infants (0-12months), in the East New Britain Province of Papua New Guinea.

Methods

A secondary data analysis was performed from the longitudinal observational cohort study (N = 699). Data was collected at the first antenatal care (ANC) clinic, birth, and 1, 6 and 12-months post-partum. Prevalence estimates and heatmaps illustrating the geospatial distribution of these conditions at each time-point were produced for malaria, anaemia, and low birth weight differences.

Results

Maternal malaria prevalence (including both *P. Falciparum* and *P. Vivax*) at ANC was 12.3%, but did increase to 37.6% at the 12-month follow-up. There was also a high burden of maternal anaemia throughout the study peaking at ANC, 83.2%. The lowest prevalence of maternal anaemia was 69.5% at the 6-month follow-up. Maternal malaria and anaemia were both widespread across the study region, with a greater density of cases occurring in the central region. However, major hotspots for each condition did differ at the 12-month follow-up. In infants, there was a high burden of anaemia, 59.8% at 6 months and 57.4% at 12 months. This was widespread across the study region with no significant hotpots. The prevalence of low birth weight was 12.1%. While this showed a relatively even distribution, a potential hotspot was identified.

Conclusion

There is a high burden of disease among women and infants and geospatial mapping suggests future initiatives could target interventions in the highest burden areas.

The molecular definition of potent *Plasmodium falciparum* invasion inhibitory epitopes on PTRAMP-CSS

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Plasmodium falciparum invasion of human erythrocytes is a complex multi-step process that can be targeted with therapeutics to reduce global malaria burden. The leading blood-stage vaccine candidate Rh5 has a significant role during invasion by forming a pentameric complex with CyRPA, Ripr and the disulphide-linked heterodimer PTRAMP-CSS. PCRCR binding to basigin is essential for invasion, and neutralising biologics to all members have been identified. Recently described nanobodies to PTRAMP and CSS showed moderate growth inhibition in comparison to antibodies developed against Rh5, Ripr and CyRPA. Further development of these blood-stage immunogens is required to induce a more potent immune response. Through antigen optimisation we have extended this campaign by screening a larger nanobody library specific to the PTRAMP-CSS heterodimer to determine more relevant inhibitory epitopes. We have identified four potent inhibitory nanobodies against PTRAMP-CSS and have elucidated the molecular structure of these novel neutralising and non-neutralising epitopes using X-ray crystallography. Through understanding the molecular definition of potent inhibitory epitopes on PTRAMP-CSS, a rational basis is provided for structure-guided development of a next-generation malaria vaccine.

CD28 stimulation enhances CAR T cell differentiation, particularly in older patients, but CD28 abundance is predictive of CAR T cell product quality.

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Aim: Chimeric antigen receptor (CAR) T cell therapy (CTT) is an autologous T cell-based treatment for B cell-based malignancies. During CTT, the patient's T cells must first be activated *in vitro* with either anti-CD3 or anti-CD3/CD28 monoclonal antibodies (mAb). Dysfunctional T cells, such as terminally differentiated CD28- T cells, could hinder the efficacy of CTT and are found at higher frequencies in older patients. This study aims to define the optimal activation protocol for CAR T cell generation with older donors and to test whether CD28 expression on patient PBMCs can predict CAR T cell product quality.

Methods: Samples from young (20-30yo) and aged (60+) healthy donors were used to generate CAR T cells using both anti-CD3 and anti-CD3/CD28 mAb activation protocols. CD28 expression was assessed pre-activation and CAR T cell quality was tracked, in terms of yield, phenotype and function. Correlative analyses were performed to determine whether CD28 expression could predict CAR T cell product quality with either anti-CD3 or anti-CD3/CD28 mAb activation.

Results: Aged PBMC samples generated more differentiated CAR T cell products than young samples, and anti-CD3/CD28 mAb activation further exacerbated differentiation as compared to anti-CD3 mAb activation in both young and aged donors. Anti-CD3/CD28 mAb activation also generated a higher frequency of IFNγ- and TNF-producing CAR T cells, increased killing capacity and augmented proliferation potential for both donor groups. Finally, we defined a novel metric of CD28 abundance with high predictive value for CAR T cell product quality.

Conclusions: In summary, anti-CD3/CD28 mAb activation protocols exacerbate T cell differentiation and may not be optimal for CAR T cell generation with aged samples. Accordingly, CD28 abundance on patient PBMCs can predict CAR T cell product quality and may be used to identify patients who would benefit from anti-CD3/CD28 mAb activation protocols.

Functional antibody responses after quadrivalent HPV vaccination

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Human papillomavirus (HPV) vaccines are effective at preventing cervical cancer, the 4th most common cancer in women. Originally licensed as a 3-dose schedule, a 1-dose HPV vaccine schedule is now provisionally recommended for girls aged 9-20 years. There is no identified correlate of protection for HPV, but neutralising antibodies (NAb) are thought to play a major role in immune protection. Despite this, a single dose of HPV vaccine has been shown to provide similar protection while inducing significantly less NAb. Apart from neutralisation, antibodies can engage Fc receptors (FcR) on immune cells to mediate protective effector functions, such as antibody-dependent cellular phagocytosis (ADCP). However, the role of functional antibodies in vaccine immunity against HPV is unclear. To better understand how HPV antibodies mediate protection, we used systems serology approaches to comprehensively characterise the functional antibody response following HPV vaccination.

We used serum samples previously collected from Fijian girls who received 1, 2 or 3 doses of quadrivalent HPV vaccine (4vHPV) 6 years earlier and after a bivalent HPV vaccine booster. Serum antibodies in a subset of samples (n=160, 20/group) were assessed for FcR binding (FcγRIIa, FcγRIIb, FcγRIIIa) against 7 oncogenic HPV types (HPV16, 18, 31, 33, 45, 52, 58) using a multiplex immunoassay. Additionally, we optimised an ADCP assay to measure the phagocytic capacity of THP-1 cells, a human leukemia monocytic cell line, following stimulation with beads conjugated to biotinylated HPV16 or HPV18 antigen.

Girls who received 1 dose of 4vHPV 6 years earlier had antibodies that bound to FcγRIIa and FcγRIIIa significantly less compared to 2, but not 3 doses. However, these levels remained significantly higher than in unvaccinated girls and there was no difference in FcγRIIb binding. Following a booster dose, similar binding to FcγRIIa, FcγRIIb and FcγRIIIa was observed in girls who received 1, 2 or 3 doses 6 years earlier, suggesting sufficient immune memory following 1 dose of 4vHPV. Preliminary data indicates that serum antibodies from vaccinated individuals can trigger ADCP, although analyses are ongoing. This work will improve our understanding of the HPV vaccine immune mechanisms and provide immunological evidence to inform HPV vaccine schedules.

Bypassing antimalarial drug resistance by altering parasite redox mechanisms

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Resistance has emerged for every class of antimalarial, including artemisinin combination therapies (ACTs), the current first line. Drug resistant parasites have been reported to have an increased ability to manage oxidative stress and maintain redox homeostasis following drug treatment, possibly due to an enhanced antioxidant system. We hypothesised that disrupting this redox balance by targeting the parasites' glutathione pathway will make parasites more susceptible to oxidative stress, and therefore re-sensitise them to existing antimalarials. This work aims to tackle resistance by identifying redox-modifying drugs that can be combined with artemisinin derivatives.

Growth inhibitory studies and ring-stage survival assays were used to determine the antimalarial activity of different redox-active compounds and to identify compounds that could be synergistic with artemisinin *in vitro.* Real time analysis of intracellular glutathione was performed using the redox reporter *P. falciparum* NF54*attB*$^{[hGrx1\text{-}roGFP2]}$ parasite line and LCMS-based thiol analysis, alongside untargeted metabolomics to identify metabolic changes in drug treated parasites.

We identified sulforaphane (SFN) to be a promising candidate, which alters parasite redox status and potentiates the activity of artemisinin. The combination of 15µM SFN with 700nM dihydroartemisin (DHA) in early ring-stage parasites resulted in a decrease in parasite survival compared to DHA alone (41% ± 7.3).15µM SFN induced an increased oxidative burden within parasites after 1 h incubation. Untargeted and targeted thiol metabolomics confirmed that SFN's antimalarial activity is redox mediated and not as a result of other major metabolic changes within the parasite

This study suggests that the addition of SFN to existing antimalarial therapies could re-sensitise resistant parasites to existing antimalarials, thereby extending their life span. Ongoing studies will investigate the applicability of sulforaphane with other antimalarials and determine the safety and efficacy of this approach in drug-resistant malaria *in vivo*.

Methylseleninic acid induces HIV viral reactivation from latently infected cells in vitro and ex vivo

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Background: HIV continues to be a pathogen of global importance, with 40 million people currently living with the virus. Preventing cure is the reservoir of latently infected cells which persist despite antiretroviral therapy (ART). The 'shock and kill' approach to HIV cure relies on reversing latency and inducing death of infected cells. Existing latency reversing agents (LRAs) have been insufficient to reduce the reservoir. Thus, novel agents which can achieve the "kill" are of interest. The organic selenium compound Methylseleninic acid (MSA) has been previously characterised as an apoptosis sensitiser. Here we investigate MSA as an LRA, alone and in cotreatment with previously characterised LRAs.

Methods: Latent cell lines containing an integrated HIV provirus with a green fluorescent protein (GFP) reporter (J-LAT10.6), and CD4+ T-cells isolated from peripheral blood mononuclear cells from people living with HIV (PLHIV) on ART were cultured with 10uM MSA. Viral reactivation was measured by GFP expression or cell-associated unspliced (US) and multiply spliced (MS) HIV RNA in CD4+ T-cells. Surface expression of activation markers CD38 and CD69 were quantified by flow cytometry. RNA sequencing was performed on MSA-treated CD4+ T-cells isolated from people living with HIV (PLHIV) on ART. Synergy with LRAs was measured by Bliss Independence (BI).

Results: MSA potently induced HIV protein in J-LAT10.6 cells (23.12-fold increase in GFP expression cf. unstimulated cells, $p<0.001$). In CD4+ T-cells from PLHIV MSA induced US HIV RNA (4.4-9 fold-increase $p=0.016$) but not MS RNA; increased CD69 expression (6.8-52.5 fold-increase, $p=0.016$) and decreased CD38 expression (4.21-39.6 fold-decrease, $p=0.016$); RNA sequencing revealed that MSA significantly upregulated pro-apoptotic genes (SMAC, BIM, BAK1) and downregulated anti-apoptotic genes (XIAP, BCL2). In J-LAT10.6 cells, MSA significantly synergised with the bromodomain inhibitor, JQ1 (BI = 0.09, $p=0.0418$) and SMAC mimetic, AZD5582 (BI = 0.5, $p=0.0109$) in the induction of GFP.

Conclusions: MSA induced HIV reactivation in latently infected cell lines, and in CD4+ T cells from PLHIV in the absence of sustained T-cell activation. Interestingly, MSA treatment also led to a pro-apoptotic phenotype suggesting it may induce cell death in HIV-infected cells alongside its latency reversal function. MSA demonstrates promise as a dual 'shock and kill' agent.

The lipidome of lipid droplets changes significantly during viral infection in the brain

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The CNS is made of numerous cell types (neurons, astrocytes and microglia) and emerging evidence suggests heterogenous antiviral responses adopted by specific cell types within the CNS. We have demonstrated for the first time, that lipid droplets (LDs) play vital roles in facilitating the magnitude of the early antiviral immune response, in particular the production of interferons and control of viral replication in an astrocyte model; however, the mechanisms at play remain unknown. This study sought to utilise a combination of *in vitro* and an *in vivo* brain infection models to further elucidate LD roles in viral infection via examination of LD dynamics and structure following activation of antiviral responses.

Brain sections harvested from LCMV (lymphocytic choriomeningitis virus) infected mice were stained using antibodies for the main CNS cell types (neurons, astrocytes and microglia) and LDs. The upregulation of LDs in vivo was significantly localised to astrocytes, which was also further confirmed utilising both human and rodent astrocytic and neuronal cell lines, in vitro. LDs were then isolated from *in vivo* and *in vitro* models using optimised methods to determine both the LD lipidome and proteome prior to and following viral infection via mass spectrometry. Lipidomics of viral mimic stimulated astrocytic cell lines revealed that although minimal changes were observed in lipid species at the whole cell level, there was a significant shift in the lipidome of LDs. This shift was underpinned by (1) a global upregulation of long, and very long polyunsaturated fatty acids, including docosahexaenoic acid and arachidonic acid, known antiviral lipids; and (2) a significant change in the LD structural lipids towards a heightened ability to accommodate protein cargo on the LD surface. Significant lipidomic alterations were also supported by the upregulation of enzymes essential for this process.

These findings not only unveil remodeling of the LD`s lipidome during an effective antiviral response but also suggest a distinct lipidomic composition for LDs with high potency to recruit antiviral proteins. This knowledge will be deployed to design lipidomic platforms; artificial LDs (aLDs), as a novel strategy towards enhancing antiviral responses within different cell type populations.

ABSTRACTS
POSTER 1

Fibroblastic Reticular Cells coordinate protective adaptive immune responses during viral infections in the spleen

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Subsets of lymphoid stromal cells, including Fibroblastic Reticular Cells (FRCs) support the architecture of lymphoid tissues by constructing microanatomical compartments. Stromal cells also express chemokines to attract naïve T cells and dendritic cells and orchestrate immune responses. Contrary to lymph nodes that sample pathogens localised in peripheral tissues, the spleen samples pathogens in the blood and is important for many immune responses. The subsets of FRC residing in the spleen are poorly characterised, and the mechanisms by which FRCs influence immune responses to infection remains unclear. We have previously identified significant heterogeneity amongst splenic FRCs that responded dynamically to viral infection. Ablation of CCL19+ FRCs during viral infection impaired T cell priming in the spleen, leading to poor T cell activation and expansion. In the absence of CCL19+ FRCs, virus-specific T cells aberrantly clustered with dendritic cells and virus-infected cells at the margins of the white pulp. Impaired maturation of dendritic cells suggest that CCL19+ FRCs are required to coordinate dendritic cell activation and interactions with T cells. Importantly, impaired T cell activation resulted in the development of defective memory T cells that were unable to protect against secondary viral infection. Overall, these data demonstrate that diverse populations of splenic stromal cells orchestrate immune responses by responding dynamically to infection and facilitating immune cell interactions.

Structural landscape of SARS-CoV-2 entry: Molecular interplay between spike glycoprotein human serine proteases

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Abstract:

The intricate mechanism governing SARS-CoV-2 cell entry causing COVID-19, primarily medicated by the spike glycoprotein, holds significant implications for potential therapeutic interventions. The spike glycoprotein of SARS-CoV-2 binds to the host receptor ACE2 and is activated by the host serine protease Furin and TMPRSS2 via proteolytic activation for subsequent entrance. Here, we present how TMPRSS2 and Furin recognize and activate the SARS-CoV-2 spike using structural, molecular, clinical, and computational studies. Foremost, we report the structure of Furin in complex with SARS-CoV-2 spike glycoprotein, demonstrating how Furin binds to the S1/S2 region of spike glycoprotein and eventually cleaves it. Second, we discovered TMPRSS2 cleavage sites in the S2 domain of the SARS-CoV-2 spike and described the structure as a complex including the catalytic triad. Then, using whole-exome sequencing, we identified a mutation rs12329760 (V160M) in the TMPRSS2 gene that results in a decreased infection rate in clinically diagnosed COVID19 patients. Similarly, our whole-exome sequencing analysis revealed that genetic variants/alleles in Furin modify the binding affinity for viral spike glycoprotein and speculate the possible cause for the diversity in the infection. We also present the structures of possible protease inhibitors in association with TMPRSS2 and Furin, which could disrupt interaction with spike protein. The findings of the structural studies contribute to our understanding of the mechanism and mode of action of Furin and TMPRSS2, which are hallmarks of increased virulence or invasion, as well as insight into the highest quality intervention options.

Keywords: Spike glycoprotein, protease, TMPRSS2, Furin, SARS-CoV-2, Genetic alleles

Identification of a potent dengue virus inhibitor using a virtual compound screen

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Abstract:

Dengue virus (DENV) is the most common mosquito-borne viral disease, imposing a substantial public health burden in tropical and subtropical regions worldwide. Despite this, there are no approved antiviral therapeutics available. DENV non-structural protein 1 (NS1) is required for viral RNA replication and infectious particle production, and is a significant contributor to the pathogenesis associated with dengue illness. These functions are dependent on the oligomeric state of NS1. We sought to identify novel inhibitors of NS1 using the cloud network infrastructure-based warpDOCK pipeline(1). After computational identification of binding pockets within the NS1 dimerisation interface, we virtually screened 7.8 million drug-like compounds for a potential interaction with NS1. From the array of top hits, we screened 24 compounds for inhibition of DENV growth and cytotoxicity, via live cell imaging of an mScarlet-based reporter virus. In follow up experiments, the most promising antiviral compound, Compound V2.3, exhibited strong inhibition of infectious particle production (IC_{50}: 599 nM), with little impact on viral RNA replication and minimal cytotoxicity. Employing a medicinal chemistry approach, we have identified a structural analogue of Compound V2.3 with improved antiviral efficacy and reduced cytotoxicity. Future studies will focus on exploration of the chemical space of this group of inhibitors to identify compounds with enhanced antiviral activity, and characterisation of the interaction of this novel group of antiviral compounds with NS1.

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Investigating the monocyte inflammatory response to bacterial ligands in patients with Crohn's disease

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Background and Aims: There is currently no cure for Crohn's disease (CD). Therefore, treatments focus on inducing disease remission by controlling the inflammatory response. It has been observed that the innate immune response fails in some CD patients, resulting in a loss of immune homeostasis in the bowel. This study compared the inflammatory response to stimulation with bacterial ligands in primary monocytes collected from a cohort of patients with CD and healthy controls (HC), to identify defects in immune-signaling pathways.

Methods: Blood samples were collected from HC and CD patients recruited from University College Hospital and The Royal Free hospital in London. Baseline plasma cytokine levels were quantified using a multiplex assay. Primary monocytes were immediately isolated from blood samples using MACS and cultured for 24 hours with bacterial ligands MDP and LPS. Post-stimulation levels of secreted inflammatory cytokines were quantified using ELISA. Monocyte metabolic activity post-stimulation was assessed using WST-1 assay. Immunophenotyping of primary monocytes was performed using flow cytometry.

Results: A total of 21 HC and 13 CD patients completed the study. The majority of CD patients (8/13) exhibited L3 Ileocolonic disease. Plasma IL-1β (0.15 vs 0.06 pg/mL) and IFN-γ (3.82 vs 2.46 pg/mL) was significantly higher in CD patients compared to HC. CD patients had a higher proportion of $CD14^+$ classical monocytes (90.1 vs 87.6%) and lower $CD16^+$ non-classical monocytes (4.1 vs 6.3%) compared to HC. Monocyte cytokine production and WST-1 metabolic activity in response to stimulation was similar between HC and CD. However, plasma IL-10 concentration was inversely correlated to the amount IL-8 (r= -0.42, *P=0.01*) and IL-1β (r=-0.36, *P*=0.04) produced by stimulated monocytes across the study cohort. A sub-cohort of 4 CD patients with mutations in NOD2 receptor had significantly lower WST-1 metabolic activity in response to MDP stimulation when compared to NOD2 wild-type CD patients.

Conclusion: Monocyte inflammatory responses to bacterial ligands MDP and LPS were similar in CD patients compared to HC, despite altered inflammatory profiles at baseline. Further studies examining the inflammatory response in CD patients with NOD2 mutations are needed to assess if NOD2 is a useful therapeutic target.

CD4$^+$ T cell recognition of Haemagglutinin epitopes across different influenza strains

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The ongoing SARS-Cov2 pandemic is a modern-day reminder of the impact that novel viral strains can have on a population without pre-existing immunity. The influenza A virus (IAV) has caused five pandemics in the last 150 years and is particularly prone to mutations in the surface glycoprotein haemagglutinin (HA) which is the primary target of the CD4$^+$ T cell and antibody responses.

CD4$^+$ T cells recognise peptide presented by human leukocyte antigen class II (HLA-II) molecules with their T cell receptor (TCR). Although the pHLA-II-TCR interaction is extremely specific, it may cross-recognise similar peptides leading to a cross-reactive response, and providing broad protection against different IAV strains. The ability of CD4$^+$ T cells to cross-react with variants of a HA peptide from different IAV strains, especially those not in circulation, is not well understood. Furthermore, individuals expressing certain HLA-II molecules are known to be more susceptible to severe viral infections. In the context of IAV, HLA-DRB1*07:01 has been associated with poor response to the influenza vaccine whereas HLA-DRB1*11:01 has been associated with protection from viruses such as HIV and HCV. Investigation into the molecular basis underlying this association is warranted.

Using intracellular cytokine staining and HLA-II tetramer staining, we found that HLA-DRB1*11:01$^+$ individuals have more cross-reactive responses towards HA peptide derived from different IAV strains than HLA-DRB1*07:01$^+$ individuals, and that the cross-reacting CD4$^+$ T cells generally have lower polyfunctionality than specific CD4$^+$ T cells. Moreover, we provide the first insight into the molecular and functional basis of IAV epitope presentation by different HLA-II molecules.

DISC – The Next Generation of African swine fever vaccine

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African swine fever (ASF) is a contagious disease of pigs that is currently causing a global pandemic[1, 2]. With a nearly 100% fatality rate, ASF has a drastic impact on pig populations, posing a threat to biodiversity and biosecurity[3]. The causative agent of this devastating disease, ASF virus (ASFV) is a large, enveloped double-stranded DNA virus with a genome length varying between 170 and 190 kilobases. The genome contains between 151 and 167 open reading frames encoding for proteins involved in host infection, viral replication, and host immunity evasion[4]. ASFV has a cell tropism for monocytes and macrophages[5].

The ASFV is known as one of the most challenging pathogens to produce a safe vaccine against because of its complexity. The most promising ASFV vaccine candidates to date are live-attenuated vaccines (LAV), which have been developed by deletion of genes associated with virulence. There are concerns about the use of ASF LAV because of the potential to revert to a virulent phenotype, post vaccination shedding and poor protection in immunocompromised pigs[6]. Therefore, there is a recognised need for a safe vaccine that can provide effective protection against ASFV for pigs. Defective Infectious Single Cycle (DISC) virus vaccines could be the solution that fulfills both safety and immunogenicity profiles[7]. Infection with DISC virus is limited to just one round within host cells, with no further spread, while it allows expression of an array of the viral proteins that are required for the generation of protective immune responses.

Herein, we proposed to select gene(s) of the ASFV genome for generating deletion mutants using innovative genetic engineering tools. Production of a DISC vaccine will be complemented with a DIVA (Differentiating Vaccinated from Infected Animals) strategy to augment disease diagnosis[8]. Complementary cell lines expressing the missing gene(s) in the DISC candidates will enable a defined and scalable cell substrate for downstream manufacture. This work will advance our knowledge in ASF virology, and in a broader context, form the basis for new capabilities that can be applied to other challenging vaccine targets for human and animal health.

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Investigating the role of lipid droplets in the antiviral mechanism of *Wolbachia*

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Aedes aegypti mosquitoes are the major vector for human pathogenic viruses such as dengue virus (DENV). Over the past decade, the insect endosymbiotic bacterium *Wolbachia pipientis* has been introduced in *Ae. aegypti* populations as an antiviral biocontrol tool, significantly reducing the mosquito-human transmission of *Aedes*-borne viruses in regions where these mosquitoes are established [1, 2]. Despite the success of this intervention, we still do not fully understand how *Wolbachia* restricts viruses. We recently demonstrated *Wolbachia*-ER interactions and triacylglycerol biosynthesis for lipid droplet (LD) formation as key host cell modifications that contribute to the *Wolbachia*-induced antiviral state. Here we extend this work by investigating the role of LDs in contributing to *Wolbachia*'s antiviral effects. LDs have been shown to facilitate immune signaling in mammalian cells. We compared expression levels of a panel of innate immune genes in *Ae. aegypti*-derived cell lines (*Aag2)* stably infected with antiviral (*w*Mel-*Aag2*, *w*AlbB-*Aag2*) or non-antiviral (*w*Pip-*Aag2*) *Wolbachia* strains. We did not identify a consistent trend in immune gene modulation in cell lines with antiviral *Wolbachia* strains compared to *Wolbachia*-free-*Aag2* or *w*Pip-*Aag2*, suggesting increased LD induction by antiviral strains does not facilitate immune activation. Since lipid peroxidation can induce the formation of LDs, we next hypothesized that *Wolbachia*-induced oxidative stress stimulates LD synthesis in *Aag2* cells. To test this, we compared levels of reactive oxygen species (ROS) in antiviral (*w*Mel-*Aag2*, *w*AlbB-*Aag2*) or non-antiviral (*w*Pip-*Aag2*) cell lines. Interestingly, none of the *Wolbachia* strains induced higher levels of ROS compared to the matched *Wolbachia*-free line. However, when we restricted LD formation by adding the small molecule C75 we measured an increase in ROS levels in cells harboring antiviral strains. Thus, these findings suggest that LD accumulation, triggered by ROS produced by resident antiviral *Wolbachia* strains, protects the cells against lipid peroxidation. Simultaneously, LD formation may sequester lipids away from membranes where they are usually exploited by viruses for their lifecycle. These findings provide further molecular understanding into organelle modification by antiviral *Wolbachia* strains and demonstrate that *Wolbachia*-induced lipid redistribution is an important contribution to the antiviral state in *Ae. aegypti*.

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Antibody responses and B-cell memory formation after COVID-19 vaccination in patients with primary immunodeficiency

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Background: Primary immunodeficiencies (PIDs) are rare genetic diseases causing immune dysfunction[1]. As the majority of patients have an antibody deficiency, they are susceptible to recurrent infections and poor vaccination responses, posing them at greater risk of developing severe COVID-19 disease. In healthy individuals, COVID-19 vaccination effectively elicits neutralising SARS-CoV-2-specific antibodies and durable memory B cells (Bmem) that recognise circulating SARS-CoV-2 variants[2,3]. It remains incompletely understood if the heterogeneous PID population mount antibody and Bmem responses that recognise SARS-CoV-2 variants and protect against severe disease.

Objective: To evaluate the capacity of PID patients to mount IgG and Bmem responses to ancestral and omicron sub-lineages BA.2, BA.5, BQ.1.1 and XBB.1.5 after two and three COVID-19 vaccine doses.

Methods: 31 PID patients and 43 controls were sampled one-month after doses two and three. Recombinant spike receptor-binding domains (RBD) of ancestral and omicron sub-lineages were utilised for ELISA-based quantification of IgG responses. RBDs were biotinylated and tetramerised with fluorochrome-conjugated streptavidin's for enumeration of absolute numbers and immunophenotypes of variant RBD-specific Bmem using flowcytometry.

Preliminary results and expected outcomes: The median concentration of ancestral-specific IgG was boosted from 11 µg/ml following dose two to 23 µg/ml following dose three in PID patients. In contrast, the controls were boosted from 34 µg/ml to 160 µg/ml respectively. Moreover, levels of IgG specific for all omicron variants was significantly increased following dose three in both cohorts. Reactivity of ancestral IgG to all omicron variants was below 40% for PID patients compared to ≥55% in the controls. Flowcytometric assessment of the magnitude and capacity of Bmem cells to recognise omicron variants in PID patients is ongoing and will be presented at the meeting. Circulating RBD-specific Bmem potentially provide a better marker of immune competence, as antibody measurement is confounded by antibody replacement therapy in this cohort.

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Discovery of new enzyme function in *Plasmodium falciparum*

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Malaria is a life-threatening disease causing over half a million deaths and nearly 250 million cases globally.[1] *Plasmodium* parasites are the etiologic agent of malaria which are transmitted through the bites of female *Anopheles* mosquitoes with *Plasmodium falciparum* being the deadliest species.[2] Despite the *P. falciparum* genome being sequenced nearly a decade ago, over 40% of the protein-encoding genome remains unannotated and a significant fraction of annotated metabolic genes have yet to be assigned to specific metabolic pathways.[3,4] In this study, we looked closely at the PF3D7_1126600 gene, putatively annotated as a steryl ester hydrolase. A previous study showed that sphingolipid metabolism was significantly perturbed in PF3D7_1126600 knockout mutants when compared against wildtype NF54 parasites whereby an increase in sphingoid bases and a decrease in ceramide levels were observed indicating a possible ceramide synthase function.[5] In this study, we looked at examining the discrepancy between the annotated steryl ester hydrolase function of the PF3D7_1126600 gene and the newly predicted ceramide synthase function by implementing an omics approach to identify changes in the mutant's lipidome and proteome. Our results were inconsistent with previous findings in that knockout of the PF3D7_1126600 gene did not result in significant perturbation of sphingolipid precursors, and ceramide depletion was not extensive, suggesting this may be a secondary response to the knockout. As the knockout had minimal impact on asexual blood stage parasites, we are now investigating sexual stages of the parasites, where we are examining changes in gametocyte development, exflagellation, and changes in lipid profile as a result of the gene knockout.

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Characterising the replication complex of a recently isolated novel Australian henipavirus

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Henipaviruses are zoonotic, single-stranded, non-segmented, negative-sense RNA viruses belonging to the *Paramyxoviridae* family (Chua et al., 1995). Hendra virus (HeV) and Nipah virus (NiV) are the most notable henipaviruses, with a high lethality rate of approximately 70 percent (Lamb et al., 2007). Recently, there have been multiple novel henipa-like viruses isolated across the globe, including Mojang virus (MojV) from cave rats in China (Wu et al., 2014), Daeryong virus (DARV) isolated from shrews in the Republic of Korea (Lee et al., 2021) and Langya virus (LayV) isolated from a human-obtained throat swab in China (Zhang et al., 2022). A novel Australian henipavirus has recently been isolated at CSIRO ACDP from Pteropid bat urine collected in Queensland (unpublished data), and named Salt Gully Virus (SGV). The minigenome system is a reverse genetics system that uses complementary DNA to assess the life cycle of a virus throughout its transcription and translation without requiring the full-length infectious virus (Khattar et al., 2000). It involves transfection of gene-specific plasmids into a permissive cell line, enabling the expression of the viral replication proteins that make up the Ribonucleic protein (RNP) complex. These are the nucleocapsid (N), phosphoprotein (P) and RNA-dependent RNA polymerase (L) (Freiberg et al., 2008). The minigenome system also requires a plasmid encoding a reporter gene such as green fluorescent protein (GFP) or firefly luciferase flanked by the 5' and 3' non-coding regions of the genome (Yun et al., 2015). This allows us to measure the replication efficiency of viruses, without the need for infectious virus which is restricted to handling at biosafety level 4.This project aims to characterize SGV through the development and analysis of a minigenome system specific to the virus. We will also assess the cross-complementarity of the SGV replication system with replication proteins belonging to the other Australian henipaviruses: HeV and CedV. Understanding the replication cycle of SGV is an essential step in the characterization of this virus, of which pathogenicity and infectivity is currently unknown.

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Characterizing the immune memory response generated toward Group A Streptococcus

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Streptococcus pyogenes, also known as Group A Streptococcus (GAS), causes either mild (e.g. pharyngitis, impetigo) or severe infections (e.g. necrotizing fasciitis), and is implicated in the development of autoimmune sequelae (e.g. acute rheumatic fever). These conditions collectively contribute to over half a million deaths annually worldwide, leading to increased urgency for the development of a GAS-based vaccine. However, whilst several new vaccine candidates are approaching clinical trials, very little is currently known about the levels of pre-existing immunity present within the community to this ubiquitous pathogen, which may influence vaccine-induced responses.

Therefore, to inform vaccine development of possible pre-existing immunity, we have investigated what degree of humoral immunity is present in healthy adults from the community, using buffy coat samples obtained from the Australian Red Cross Lifeblood. Here, we have focused our efforts on two leading GAS vaccine candidates, streptococcal C5a protease (ScpA) and *S. pyogenes* adhesion and division protein (SpyAD), and measured plasma antibodies by ELISA and antigen specific memory B-cells by 17-colour spectral flow cytometry. We find that of the samples tested via flow cytometry, 45% had memory specific to ScpA, whilst 86% had memory specific to SpyAD. This contrasts with the ELISA findings, where antibodies specific to both proteins were detected in all serum samples. Further investigation revealed that CD21+ CD27+ classical memory along with IgG1 B-cell receptor isotype is the predominant phenotype expressed by memory B-cells specific to both proteins, with smaller IgM- and IgA-expressing populations also present. There was a moderate positive correlation for between SpyAD-specific serum antibodies and memory B-cell frequency expressing IgG, but not IgA, whilst there was generally no correlation observed between serum antibodies and memory B-cell frequency specific to ScpA for both IgG and IgA.

Altogether, our findings inform the development of GAS vaccines as we have demonstrated that pre-existing immunity is prevalent within the community, which will likely influence the outcome of vaccination. Given the recent progress of new vaccine candidates towards clinical trials, time will tell whether pre-existing memory helps or hinders GAS vaccine efficacy.

Regulation of thymocytes migration during T cell development by Coronin 2a, a putative actin-cytoskeleton regulator

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T cell development occurs in the thymus and thymocytes follow a highly ordered migratory pattern through the organ as they develop. Regulation of the actin cytoskeleton is expected to control both cell movement and responses to extracellular signals. Coronin protein was selected because Coronin 1a (Coro1a) has been implicated in the regulation of mature T cell migration, activation, and trafficking process. The knockout of Coro1a, however, did not disturb T cell development. Preliminary data indicated that the knock of Coronin 2a (Coro2a) affected the early stage of T cell development and the migration of cancer cells.

To study the role of Coro2a in the development process, we developed a knockout mouse model where the coding region of *Coro2a* was replaced by a *LacZ* reporter. Using this reporter, we showed that Coro2a is upregulated from the double negative DN1 stage, with the highest expression at the DN2 stages and is then downregulated. *Coro2a*$^{-/-}$ mice significantly reduced thymus size and total thymocyte numbers. This decrease was due to an accumulation of thymocytes at the DN2 and DN3 stages with a subsequent loss of cells at the double positive (DP) stage. Analysis of mixed bone marrow chimera mice indicated that the effect of *Coro2a* deficiency is intrinsic to thymocytes. *Coro2a*$^{-/-}$ DN cells exhibited impaired migration towards certain chemokines *in vitro*. Preliminary confocal microscopy indicates that Coro2a co-localises with F-actin but is excluded from the cell's leading edge. Together, this suggests that Coro2a may indeed be an important regulator of the actin cytoskeleton in DN cells, which in turn is important for their migration toward the outer cortex and subcapsular zone. Further investigation aims to determine the precise molecular function of Coro2a within thymocytes.

The role of the human kinase B-Raf in *Plasmodium falciparum* blood-stage infection.

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Abstract

Malaria, a disease caused by the infection of parasites from the genus Plasmodium, remains a primary global health concern. The selection of mutations under drug pressure have resulted in emergence of strains resistant to treatment, contributing to resurgences and waning efficacy of anti-malarial medications. Hence, there is a need for the development of alternatives to traditional therapeutics. All available malaria chemotherapeutics target parasite-encoded enzymes or parasite-controlled processes such as heme polymerization. Recent studies show that a number of host erythrocyte signalling kinases are required for parasite growth and survival, suggesting that an approach known as Host-Directed Therapy can be implemented. This approach is refractory to the most direct pathway to resistance since the target proteins are not under the parasite's genetic control. Possible targets for this approach include host cell B-Raf, c-MET, and MEK kinases. We have shown that inhibitors of these kinases impair parasite proliferation and survival. More specifically, the B-Raf inhibitors SB-590885, PLX8394, and Dabrafenib, which all inhibit B-Raf activation through unique mechanisms, have antimalarial properties. As expected, drug-selection experiments were unable to generate parasites with resistance to SB-590885 or Dabrafenib. However, we isolated parasites which showed a 2x shift in IC50 value of wildtype 3D7 parasites, indicating a reduced susceptibility to PLX8394. We now intend to investigate the role of host cell B-Raf in *Plasmodium falciparum* blood-stage infection and the mechanism of PLX8394-resistance. This will shed light on host-parasite interaction at the molecular level and provide a basis for the development of host-directed therapy for malaria treatment.

Keywords: B-Raf kinase, Host-directed therapy, *Plasmodium falciparum*, anti-malarial drugs.

Identification of novel malaria proteins involved in parasite-host cell interactions

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Malaria is an infectious disease transmitted by *Anopheles* mosquitoes and caused by protozoan parasites of the *Plasmodium* genus, of which *P. falciparum* is the most lethal. In a human host, *Plasmodium* parasites invade and remodel red blood cells (RBCs) to grow and obtain essential nutrients. One organelle implicated in invasion, parasite establishment within the RBC, and remodelling is the rhoptry. To date, only 30 *Plasmodium* rhoptry proteins have been identified using empirical approaches. Elucidating the rhoptry proteome will be crucial to understanding the diverse processes that rhoptry proteins contribute to in mediating host-parasite interactions. This may also reveal novel potential therapeutic targets, which are desperately required due to rising drug resistance of *Plasmodium* parasites.

Novel techniques in proteomics, such as proximity labelling, provide new approaches in identifying proteins that localise to a particular cellular region. Proximity labelling involves the fusion of a proximity labelling enzyme to a gene of interest to label proteins in close proximity. One such enzyme is TurboID, a promiscuous biotin ligase that biotinylates proximal proteins by catalysing their covalent attachment to biotin-AMP. Proximity labelling studies in *P. falciparum* are limited.

The aim of the study was to identify the rhoptry proteome by fusing TurboID to proteins localising to different regions in the rhoptry. This body of work demonstrates successful fusion of TurboID to *P. falciparum* CERLI, a protein that localises to the rhoptry cytoplasmic face, and *P. berghei* RON3, a rhoptry bulb-localising protein. Biotinylation, affinity purification, and identification of proteins proximal to CERLI by mass spectrometry have been performed, revealing 129 proteins significantly enriched in TurboID-expressing parasites when compared to a wildtype negative control. In particular, 14 of these are known rhoptry-localising proteins. Gene ontology analysis revealed the most significantly enriched proteins localise to apical cellular compartments or to membranous structures, and include SNARE and adaptor proteins that participate in vesicle-mediated transport in the secretory pathway. In addition, proteins that participate in cellular homeostasis and other diverse processes were enriched. Identification of these proteins provides the basis for understanding rhoptry protein trafficking through the secretory pathway and evaluation of their potential as future therapeutic targets.

γδ T cell-derived IL-4 promotes cellular immunity to liver-stage malaria

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A direct correlation between γδ T cells and protection against liver stage malaria has been observed in mice[1] and in humans[2,3], but the precise molecular mechanisms by which γδ T cells exert their protective effect are undefined. In mice, intravenous injection with radiation-attenuated sporozoites (RAS), confers sterile protection against challenge infection through expansion and proliferation of antigen-specific $CD8^+$ and $CD4^+$ T cells in the spleen. Expanded cells migrate to the liver wherein a small subset will remain as resident-memory T cells (T_{RM}). Here, the $CD8^+$ T_{RM} provide long-lived sterile protection from reinfection. In the absence of γδ T cells, protective $CD8^+$ liver T_{RM} are not generated, leaving mice susceptible to reinfection. Using *Plasmodium*-specific T cells as a readout for effective immunity, we have determined that $CD8^+$ and $CD4^+$ T cells show equal levels of activation in the spleen following RAS injection, but they do not accumulate to the same degree in the absence of γδ T cells. Antibody-mediated blockade of the δTCR confirmed a role for γδ T cells in the first 24 hours after antigen exposure. Further investigation revealed that IL-4 is crucial in this system, and mixed bone-marrow chimeras showed that γδ T cells are the critical source of that IL-4. We are now using CRISPR-edited primary dendritic cells for reconstitution of DC-deficient mice, to investigate the mechanism by which γδ T cell derived IL-4 leads to $CD8^+$ T cell activation and expansion for memory development. This project has revealed a novel pathway for collaboration between γδ T cells and dendritic cells, hinting at potential for utilizing γδ T cells in the context of vaccination.

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Host erythrocyte and reticulocyte cell signalling during infection with *Plasmodium* spp.

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Intracellular pathogens, such as *Plasmodium* parasites, modulate their host phosphorylation signalling pathways for survival and proliferation during infection. However, signalling information is complex and challenging to comprehend holistically. In this project, we implemented an antibody microarray approach to evaluate the signalling environment during *Plasmodium knowlesi* infection of human reticulocytes. This allowed us to measure the difference in kinase expression and phosphorylation levels between the infected and uninfected samples. A comprehensive analysis identified the top 10 signals (in terms of fold-change from the uninfected control) with consistent patterns across two biological replicates and specific parasite exposure times. Specifically, UBS (Upstream Binding Factor), RSK (Ribosomal s6 Kinase), and MEK exhibited consistent activation at12-hour post-infection time. HGK, TrkB, and c-Jun N-terminal kinases (JNKs) were also prominently activated in the 24-hour time post-infection. Many of these top signals (such as UBS and RSK) correspond to signalling elements implicated in haematopoiesis. Additional analysis will be done to identify other top-consistent signals prior to validation through biochemical and pharmacological methods.

Keywords: malaria, protein kinase, kinomics, host-directed therapy HDT, host-pathogen interactions.

Novel Drug Targets for Inhibiting Malarial Nuclear Import

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Malaria continues to be a global health concern, responsible for 627,000 deaths worldwide in 2020 alone(1); the vast majority of which are due specifically to *P. falciparum (2)*. Currently the gold standard of malaria treatments are artemisinin-based combination therapies in which artemisinin, or a derivative of artemisinin, is combined with a partner antimalarial drug and administered together. Unfortunately, drug resistant strains of *P. falciparum* have emerged with resistances to artemisinin and five of the six partner drugs in use today (3-15). Thus, the discovery of novel antimalarial drugs is paramount. Optimum drug targets are those which disrupt/inhibit a unique fundamental pathway, ideally such a pathway which is required for both sexual and asexual stages of *P. falciparum.* One such target which meets these pre-requisites is the nuclear import pathway(s)(16). Herein, a novel screen is developed for the detection of drug compounds which disrupt nuclear import, an essential fundamental pathway in *P. falciparum.* Parasites expressing a florescent reporter fused with a nuclear localization sequence are treated with drug compounds from the Medicines for Malaria Venture organization's drug collections. Nuclear import is then observed by florescent microscopy and compounds which disrupt the trafficking of the florescent marker across the nuclear envelope are submitted to a counter screen comprising of nuclear fractionation and analysis by western to confirm import disruption. The target of passing anti-nuclear import compounds is then investigated via *in vitro* resistance generation and analysis of single nucleotide polymorphisms and protein stability target identification.

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Fc-dependent functional antibody responses in immunity to severe *Plasmodium falciparum* malaria in children

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Plasmodium falciparum is one of the most widespread *Plasmodium* species and causes the majority of malaria cases and deaths worldwide. Young children are particularly susceptible to severe and fatal forms of malaria caused by *P. falciparum*. Progress in malaria control has stalled in recent years, highlighting the need for vaccines as key interventions to help reduce the global burden of malaria. The World Health Organisation has recommended the development of highly efficacious vaccines against malaria to prevent severe illness and deaths. However, no vaccines currently target *P. falciparum* merozoites, which is the *P. falciparum* parasite form associated with clinical symptoms and severe disease following invasion of and replication within red blood cells. This is partly due to a limited understanding of the targets and mechanisms of action of immunity to *P. falciparum* merozoites that contribute to protection from severe disease. Antibodies play a key role in immunity against malaria, and likely function by directly blocking invasion and mediating Fc-dependent functional antibody responses. These Fc-dependent responses include complement activation and fixation and cross-linking with Fc-receptors on immune cells leading to antibody-dependent opsonic phagocytosis, antibody-dependent cellular cytotoxicity or antibody-dependent cellular inhibition. Emerging research suggests such Fc-dependent functional antibody responses to *P. falciparum* merozoites are associated with reduced disease severity, but the specific antigenic targets remain unknown. Using a high throughput bead-based multiplex assay we have developed, we will evaluate functional antibody responses (complement fixation and Fcγ-receptor binding) to 35 merozoite antigens in a cohort of children from Papua New Guinea with severe or uncomplicated clinical *P. falciparum* malaria. We will identify important *P. falciparum* merozoite antigens that are targets of antibody responses associated with protection against severe clinical *P. falciparum* malaria and the mechanisms of action involved. This work will inform the development of highly efficacious vaccines to reduce the global burden of malaria and prevent severe disease, thereby reducing hospitalisations and deaths.

Enzymatic processing in influenza antigen presentation

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The current influenza vaccines protect against the virus by stimulating antibody responses against highly variable HA and NA surface proteins. However, CD8+ cytotoxic T cells can target more conserved influenza peptides presented on HLA molecules to achieve long-lasting immunity. These peptides are the result of proteasomal processing of proteins which, in addition to generating linear peptides, can also ligate two originally distant peptide fragments to create 'spliced' peptides. Although the estimated proportion of spliced peptides in the immunopeptidome has been controversial (ranges from 1-40% (1-5)), the identification of key spliced epitopes in cancer promotes the need for further exploration of their contribution to immunity in cancer and infectious disease.

Currently, the roles of spliced peptides in immunity against influenza infection are not known. We used mass spectrometry to identify potential influenza-derived spliced peptides presented by the HLA-A*02:01 molecule as described previously (1), and identified a T cell receptor (TCR) specific for a putative influenza A PB2 spliced peptide, termed 2071. Subsequently, we characterised the presentation conditions of the immunodominant HLA-A*02:01 restricted influenza A M1 peptide, $M1_{58-66}$, alongside 2071, by investigating the responses of TCR transduced reporter cell lines. Influenza A virus (IAV) infection of different antigen presenting cell (APC) lines successfully elicited strong responses from SKW3.GIL (reporter cell line expressing a TCR for $M1_{58-66}$). Whilst the mirrored stimulating conditions revealed selective responses of SKW3.2071 (reporter cell line expressing a TCR for the 2071 peptide) to infected B cells only. Moreover, the failure of APCs overexpressing PB2 protein and IAV infected non-B cells to stimulate SKW3.2071 suggests further validation of the proposed epitope's origins is required. Interestingly, influenza B virus (IBV) infection of different APCs also revealed weak responses from SKW3.2071 to B cells only, suggesting either cross-reactive recognition of IAV and IBV epitopes due to high sequence similarity, or recognition of a commonly upregulated B-cell-derived peptide. Even though the mystery of source and enzymatic processing of the 2071 spliced peptide is yet to be fully unveiled, this study acts as a starting point for investigation of spliced influenza peptides as a means to diversify the immune response to influenza infection.

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Transmission dynamics and population structure of P. falciparum and P. vivax in Mondulkiri Province, Cambodia

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For many decades, stakeholders have worked tirelessly towards malaria reduction, and elimination has been successful in some countries. The incidence of *P. falciparum* malaria infections in Cambodia has decreased markedly in the recent years and the country is on track to achieve elimination of this species by 2025. However *P. vivax* infection burden continues to increase, accounting for approximately three-quarters of all Plasmodium infections in Cambodia. A cross-sectional survey conducted in Kaev Seima District, Mondulkiri Province in Cambodia by the Asia Pacific International Centre for Malaria Research (ICEMR) has demonstrated that living and working in the forested areas is a risk factor for both *P. falciparum* and *P. vivax* malaria. This survey identified infections of *P. falciparum* and *P. vivax* outside the forests however it is not known whether these are driven by infections acquired in the forest. Population genetic analyses can be used to understand transmission dynamics and population structure, as well as the origins and flow of infections between villages. This project aimed to apply SNP barcoding to *P. falciparum* and *P. vivax* isolates from the cross-sectional study to allow population genetic analysis. This SNP barcoding of 176 informative, validated SNPs in 34 *P. falciparum* isolates resulted in 127 successfully genotyped SNPs, 9 of which were polymorphic in this Cambodia population. For *P. vivax*, SNP barcoding of 178 informative, validated SNPs in 65 isolates resulted in 53 successfully genotyped SNPs, 19 of which were polymorphic.

The analysis revealed low population diversity for both species with no evidence of clustering or population structure between village and forest. Further, genotypes originating from different geographical locations were seen to be highly related. These results support the hypothesis that parasites originate in forest areas, and are the likely source of infections in villages outside the forest as demonstrated by the high gene flow to and between areas. This information may be used by malaria programs to successfully interrupt and monitor impacts of control efforts on malaria transmission.

Factors associated with infant carriage of antimicrobial resistance genes: a systematic review.

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Background

In recent years, the carriage of antimicrobial resistance (AMR) genes has increased dramatically among infants. The reasons for this, however, remain poorly understood. Here we aim to identify potential determinants of AMR, the sites in which AMR genes are carried, and the most prevalent resistant infections.

Methods

Following PRISMA guidelines, we performed a systematic review using PubMed and Web of Science databases covering the years 2000 to 2021. We included studies which investigated AMR genes in infants using next-generation sequencing. Our search yielded 1840 articles, of which 32 were included in the final sample.

Results

The most common identified determinants of AMR were delivery in neonatal intensive care units (n=4), antibiotic exposure in infants' gut microbiome (n=7), caesarean section as mode of delivery (n=3), and mother-to-child bacterial transmission (n=3). 9 of the 32 studies identified two main biological reservoirs of AMR including gut and the nasopharyngeal microbiota. The most common antibiotic resistance classes identified were aminoglycoside, β-lactams, macrolide, and tetracycline. Last-resort antibiotic resistance genes were also detected in infants' guts. Finally, we found the bacteria from order Enterobacterales and Bacteroidetes are commonly identified as carrying AMR genes.

Conclusion

Neonatal intensive care unit, antibiotic use, caesarean section, and maturity of the infant's gut are each independently associated with increased AMR carriage. More comprehensive studies regarding healthy infants and factors such as antibiotic use, mode of delivery and other factors are required. Furthermore, uniformity of AMR databases and bioinformatics pipelines should be addressed to promote inter-study comparisons.

Interleukin-2, not chemokine receptors CXCR3, CXCR5 or CXCR6, controls Th1/Tfh fate bifurcation during blood-stage malaria.

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$CD4^+$ T cells are protective against blood-stage infection with malaria parasites. Two subsets, T-helper 1 (Th1) and follicular helper T cells (Tfh), control infection through IFNγ secretion and by supporting high-affinity antibody production [1]. Previously we observed early co-expression of CXC chemokine receptors (CXCR) 3 and CXCR5 by *Plasmodium*-specific TCR transgenic PbTII cells, prior to their bifurcation towards either Th1 or Tfh fates, which had been supported by CXCL9/10 expressing monocytes or B cells [2]. This led us to hypothesize that competition between CXCR3 and CXCR5 influences Th1/Tfh fate in malaria. To test this, genes encoding CXCR3, CXCR5, or CXCR6 were disrupted in naïve PbTIIs via CRISPR/Cas9 knockout methods and examined for differentiation *in vivo.* Strikingly, none of these chemokine receptors, either alone or in combination, substantially influenced either PbTII expansion or Th1-differentiation. Instead, by disrupting *IL2ra,* we determined that early IL-2 signaling, most likely within the first two days of infection, plays a critical role in supporting Th1 differentiation, but not clonal expansion. Thus, during malaria, the initial balance between the emergence of humoral and cellular immunity is partly mediated by Interleukin-2.

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Anti-inflammatory effect of increased IgG4 following SARS-CoV-2 mRNA vaccine boosting is antigen dependent

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Antibody Fc-mediated responses, such as antibody dependent cellular cytotoxicity and phagocytosis, play an important role in maintaining vaccine-induced protection against SARS-CoV-2, especially against variants of concern (VOCs). These Fc effector functions are driven by binding of immunoglobulin G (IgG) to Fc-gamma receptors (FcγRs) expressed on innate immune cells. IgG subclasses (IgG1-4) have different affinities for FcγRs. IgG1 and IgG3 have higher affinity to FcγRs and mediate more potent Fc effector functions, whereas IgG2 and IgG4, often described as anti-inflammatory subclasses, have weaker affinity. To date, few studies have explored subclass composition against SARS-CoV-2 VOCs following vaccination and boosting and their consequence on Fc-mediated responses. Here, we profiled the IgG subclass composition to different VOCs following mRNA vaccination and the impact on Fc effector functions.

Plasma (n=46) was collected following two and three doses of mRNA vaccination. Using a multiplex assay, we profiled these samples for SARS-CoV-2 specific subclass titres and ability to bind FcγRIIIa as a surrogate for Fc effector functions. IgG responses against ancestral Spike and receptor binding domain (RBD), and the RBDs of VOCs Delta and Omicron (BA2 and BA5) were investigated.

SARS-CoV-2-specific IgG1 dominated the response following the second mRNA vaccine dose and showed positive correlations with FcγRIIIa engagement ($0.92<\text{Spearman } r<0.97$, $p<0.0001$ for all antigens). A modest boost in IgG1 against all antigens was seen following boosting (1.1- to 4.5-fold). In contrast, ancestral Spike and RBD-specific IgG4 was increased 70- and 100-fold respectively by vaccine boosting ($p<0.001$). However, smaller fold-increase in IgG4 to Delta, BA.2 and BA.5 RBD was induced by boosting (ranging 7- to 17-fold, $p<0.0001$ for all). Intriguingly, FcγRIIIa binding was negatively correlated with Spike-specific IgG4 (Spearman $r=-0.56$, $p<0.001$), but did not correlate with IgG4 titres to any RBD tested ($-0.21<\text{Spearman } r<-0.07$., $0.25<p<0.71$).

These preliminary findings suggest repeated mRNA vaccination skews towards anti-inflammatory subclasses, but the consequence on Fc effector function is antigen dependent. Mechanistic studies on the consequence of subclass composition on Fc-mediated responses to VOCs are ongoing. A greater understanding in the balance of IgG subclasses required for optimal Fc-mediated responses may provide strategies to modulate Fc effector functions against VOCs by vaccination.

Functional assessment of the NOD2 signalling pathway in patients with primary immunodeficiency

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Background: Despite advances in genomics, many patients with primary immunodeficiency (PID) remain genetically undiagnosed, thereby limiting their access to targeted therapeutics. In diagnosed patients with antibody deficiency, 70% of affected genes function in one of 5 critical immune signaling pathways. One pathway is downstream of Nucleotide-binding oligomerization domain 2 (NOD2), a cytosolic receptor for bacterial muramyl dipeptide (MDP). We here aimed to establish a robust laboratory assay to functionally assess pathogenicity of 3 variants of unknown significance (VUS) in components of this pathway.

Methods: Whole-exome-sequencing was performed on gDNA of 114 PID patients. To evaluate NOD2 signalling, patient immune cells were evaluated by flow cytometry for production of intracellular TNFα in response to L18-MDP stimulation (NOD2-dependent). LPS-stimulated (NOD2-independent positive control) and unstimulated (negative control) samples were run concurrently.

Results: Following LPS-stimulation, the majority (range 82.4-99.5%) of monocytes from 18 healthy donors and 5 patients produced TNFα. In healthy donors, 52% (range 27.9-95.9%) of monocytes produced TNFα in response to L18-MDP. Two patients with X-linked lymphoproliferative disease due to an *XIAP* mutation had no NOD2-dependent TNFα production. In contrast, a patient with a heterozygous *TNFAIP3* (A20) VUS (p.Q150R) had high TNFα production (82.8%) whilst in a patient with a heterozygous *XIAP* VUS (p.Y139C; 32%) and a patient with a heterozygous *NOD2* VUS (c.2546+2dupT; 29%) TNFα production were low, but all within the healthy control range.

Conclusion: Here we show assessment of NOD2-dependent TNFα production is able to identify patients with a complete loss-of-function phenotype. Due to the large spread in healthy controls, other read-outs might be required for interpretation of subtler defects, such as L18-MDP-induced p38 and p65 phosphorylation. This *ex vivo* functional evaluation of immune pathway analysis could provide rapid insights into pathogenicity of VUS, thereby expediting genetic diagnosis and targeted treatment in patients with PID.

Mechanisms of self-ganglioside presentation by the antigen presenting molecule CD1b

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T cells play a critical role in adaptive immunity, mediated by receptors on the surface of T cell (TCR) that recognise antigens presented by antigen presenting molecules. Studies on T cell immunity are centred on classical major histocompatibility complex (MHC) that present antigenic peptides to TCRs. However, non-classical antigenic molecules such as lipids can also be presented by antigen presenting proteins CD1s, which in comparison to peptide-MHC, the mechanisms of CD1 proteins presenting antigenic lipids to TCRs remain largely unknown.

The human CD1 family are classified as Group 1 (CD1a, CD1b and CD1c) and Group 2 (CD1d) based on gene sequence and function. Compared to peptide-MHC, CD1 molecules in humans are non-polymorphic, as such, the mechanisms of T cell activation by CD1 proteins can be broadly applicable to all humans. CD1b, which is well-known for presenting lipids from the *Mycobacterium tuberculosis* cell wall (1), has recently been shown to play a role in autoimmunity, such as in multiple sclerosis, cellular stress, and cancer. However, the molecular mechanisms of lipid presentation by CD1b, and mechanisms of modulating autoimmunity, largely remain unexplored.

Significant structural studies into the presentation of self-phospholipids by CD1b have previously been conducted, however mechanisms of self-ganglioside presentation, which include ganglioside-monosialic acid (GM1) found to induce T cell mediated autoimmune responses in multiple sclerosis (2), remain unknown. To characterise this, a panel of gangliosides (CD1b -GM1, GM2, GM3 and sulfatide) previously identified as CD1 antigens were manually loaded into CD1b *in vitro*, and their structures were determined via x-ray crystallography. The ganglioside sphingosine and fatty acid tails are sequestered into the C' and A' portals of CD1b, respectively, while the ganglioside head group is positioned above the CD1b surface. CD1b presents gangliosides and phospholipids in a similar manner, however ganglioside head groups are distinctly larger and therefore protrude further above the CD1b surface than phospholipids. These findings provide novel molecular insights into the mechanism of antigenic lipid presentation by CD1b, and led to future understanding of their role in immune activation.

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Defining targets and mechanism of action of immunity against *Plasmodium vivax* malaria.

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Malaria is a mosquito-borne disease caused by the parasite *Plasmodium*, responsible for >200 million cases and ~500,000 deaths annually. *Plasmodium falciparum* and *P. vivax* account for most of the cases globally. Currently, only one malaria vaccine is licensed with moderate efficacy that wanes quickly and only targets *P. falciparum.* No vaccines are available against *P. vivax,* the most geographically widespread *Plasmodium* species and limited candidates are under development threatening malaria elimination efforts. A major bottleneck in developing *P. vivax* vaccines has been a limited knowledge of the targets and mechanisms of action of protective *P. vivax* immunity.

The *P. vivax* circumsporozoite protein (*Pv*CSP) is essential for establishing a successful infection and therefore a promising vaccine target. Current studies focus on the most common allelic variants of the highly immunogenic and polymorphic central repeat region (CRR) of *Pv*CSP. Antibodies play a key role in immunity against malaria and have been shown in *P. falciparum* to act through different mechanisms including complement-fixation and engaging Fcγ-receptors (FcγR) on immune cells. However, knowledge is limited on the functions of antibodies targeting *P. vivax* and *Pv*CSP specifically. To address these gaps, we measured antibody magnitude (IgG) and function (complement-fixation and binding of FcγRI, FcγRIIa, FcγRIIIa/b) to full-length *Pv*CSP, the CRR of the two major allelic variants (*Pv*CSP-VK210 and *Pv*CSP-VK247), as well as the conserved N- and C-terminal regions. We used antibody samples from adults in Papua New Guinea and those generated in rabbits following vaccination against the two *Pv*CSP alleles.

We observed significant antibody responses against both variants of *Pv*CSP, including the CRRs, and antibodies showed some degree of allele-specificity. There was also antibody reactivity to the conserved N- and C-terminal regions among malaria-exposed adults and rabbit vaccine samples regardless of the variant used for vaccination. FcγRI binding was consistently high across all *Pv*CSP regions tested, followed by FcγRIIIa/b binding. Whereas FcγRIIa binding, and complement-fixation were significantly lower. These results provide new insights into the important roles of different regions and variants of *Pv*CSP as targets of *P. vivax* antibody responses including mechanisms of action involved. This knowledge will inform future vaccine design based on *Pv*CSP.

Investigating the chronic pulmonary and vascular consequences of influenza infection in atherosclerotic mice

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Introduction: Influenza A virus infection (IAV) in patients with cardiovascular disease, such as atherosclerosis, increases their risk of plaque destabilization, myocardial infarction, and death. The mechanisms for this are largely unknown and the chronic complications caused by infection are poorly understood.

Aim: To determine if IAV infection acutely exacerbates pulmonary and vascular dysfunction in atherosclerotic mice ($APOE^{-/-}$) and whether this accelerates atherosclerosis and triggers chronic pulmonary remodelling.

Methods: Male 5-week-old $APOE^{-/-}$ mice were placed on a high-fat diet for 7 weeks. At the beginning of week 8, mice were intranasally inoculated with 10^3 plaque-forming units of a mouse-adapted IAV strain (Hk-x31). Mice were then allowed to continue on a high-fat diet until cull, 7 weeks post-infection. Throughout the protocol, blood pressure and pulse rate were taken weekly. At experiment endpoints, Aortic function was assessed by dissecting aortic rings and using the thromboxane-mimetic U46619, acetylcholine, and sodium nitroprusside to assess smooth muscle contraction, endothelial-dependent and independent relaxation, respectively. Lung function was also assessed examining FEV0.1 and FVC as well as the mean linear intercept method to determine airway damage. Right ventricles were also collected from mice.

Results: At 7-weeks post-infection, $APOE^{-/-}$ infected mice showed significantly reduced maximal contractions to U46619 and relaxation to acetylcholine, indicating significant endothelial and smooth muscle damage in the vasculature of infected mice. Additionally, right ventricular hypertrophy was observed in infected mice, which may be indicative of pulmonary hypertension. Impairment in pulmonary function was also observed with infected mice having significantly decreased FEV0.1/FVC ratios when compared to controls. Finally, we found IAV infection also caused significant systolic hypotension and bradycardia and long-term elevation in inflammatory cell infiltration in the airways.

Discussion: Our study demonstrates the significant impact that IAV infection has on cardiovascular disease severity in atherosclerotic-prone mice long after viral clearance. Furthermore, infected $APOE^{-/-}$ mice demonstrated long-lasting respiratory complications and features of pulmonary hypertension which may sensitise them to future respiratory complications of future respiratory infections and suggests that viruses may be drivers of cardiovascular disease.

Response and resistance to combination immune checkpoint blockade associate with distinct baseline and on-treatment blood T-cell profiles in melanoma patients.

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Introduction.

Despite the success of immune checkpoint blockade (ICB), a majority of melanoma patients fail to respond or experience severe treatment-related toxicity. Currently, there are no biomarkers available to predict these events. We here performed in-depth evaluation of the blood T-cell compartment to identify reliable predictive biomarkers to rationalise ICB use.

Methods.

Peripheral blood samples were collected from 30 patients with stage III-IV melanoma at baseline and after one cycle of combination PD-1/CTLA-4 blockade, and 21 age-matched healthy controls. Patients were classified as responders or non-responders by best overall response to treatment, as well as grouped on the occurrence of severe toxicity. Absolute immune cell counts were obtained at the time of sampling, and PBMCs were cryopreserved prior to spectral flow-cytometric analysis of the T-cell compartment.

Results.

At 6 months post ICB commencement, 14 patients (47%) failed to respond to treatment and 15 (50%) experienced severe toxicity. At baseline, non-responders had fewer circulating T cells than controls (median, 804 vs. 1297 cells/μL), mainly due to lower $CD4^+$ (p=0.0047) and $CD8^+$ (p=0.0031) naive T cells, as well as higher proportions of $CD8^+$ (p<0.0001) and $CD4^+$ (p=0.0003) T cells expressing Ki67, and increased highly-suppressive T-regulatory cells compared to responders. One cycle of ICB selectively expanded existing T-cell memory in all patients, and responders showed significantly greater upregulation of Ki67 expression in $CD4^+$ central memory (p=0.0043) and regulatory (p=0.0065) cells than non-responders. Severe toxicity associated with small but significant changes to $CD8^+$ T effector memory phenotype.

Discussion.

Response to ICB was associated with distinct T-cell profiles before and after one cycle of ICB, but toxicity was linked to only minor differences. Hence, pre- and early on-treatment immunophenotype are a promising source of response biomarkers, but further work is required to identify drivers of toxicity.

Restoring sensitivity of *Streptococcus pneumoniae* to tetracyclines with metal ionophores

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Streptococcus pneumoniae (the pneumococcus) is a globally significant bacterial pathogen that can cause considerable mortality particularly in young children (≤5 years), the elderly (≥65 years), and the immunocompromised. Despite the success of vaccines in reducing the incidence of pneumococcal disease and death, antibiotics remain critical in treating active infections. However, the rise in multidrug resistant (MDR) isolates seriously limits available and efficacious treatments, with novel and innovative therapies urgently needed to address the ongoing crisis.

Metal intoxication is a well-established, host-mediated, antimicrobial strategy against invading pathogens. Previous studies in *S. pneumoniae* and other human pathogens have shown that metal intoxication can result in increased susceptibility to current antibiotics.

Here, we investigate ionophores, a class of compound that facilitates the unregulated shuttling of metal ions across the bacterial cell membrane to stimulate metal intoxication and rescue the efficacy of the frontline antibiotic, tetracycline (TET). Using minimal inhibitory concentration (MIC) assays, we show restored clinical susceptibility to TET in MDR *S. pneumoniae* clinical isolates upon co-administration with the ionophore. Furthermore, bactericidal efficacy (≥3-log reduction in colony forming units (CFU) compared to controls) was achieved within 4 hours of treatment at clinically relevant antibiotic concentrations with the ionophore.

To elucidate the molecular mechanisms mediating this ionophore-antibiotic (ionobiotic) synergy, inductively coupled plasma mass spectrometry (ICP-MS) was conducted revealing highly dysregulated metal accumulation which is further potentiated upon the addition of the antibiotic. This hyperaccumulation corresponded with increased expression of the metal-specific membrane efflux pump and oxidative stress response genes, indicating intracellular metal stress.

Morphological investigation using scanning electron microscopy (SEM) showed visible and distinct disruptions to the cell membrane and cellular aggregation under individual and combined ionobiotic treatment. Analogous assays quantifying peptidoglycan production, membrane fluidity, and zeta potential showed increased membrane rigidity and peptidoglycan synthesis, and a slightly positive surface charge under ionobiotic treatment. The latter which has implications for effective immune clearance by positively charged cationic antimicrobial peptides.

Future assays will involve other '-omic' analyses such as metabolomics and generating *in vivo* data to thoroughly assess this therapeutic approach in overcoming antibiotic resistance and aid in reducing the global burden of pneumococcal disease.

Investigating the role of cytokine signalling regulator CISH in T-cell responses to viral infections

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The current resurgence of influenza cases and influenza-related deaths is a major healthcare concern, with approximately 200,000 lab-confirmed cases and 194 deaths recorded to date in Australia. The influenza virus rapidly accumulates mutations on its surface proteins, resulting in the emergence of new strains. This causes seasonal influenza epidemics and takes advantage of the lack of pre-existing host immunity. To circumvent this, influenza vaccines need to be reformulated annually. In addition, there is potential for current antivirals to develop resistance. Therefore, alternative strategies must be developed to improve disease outcomes.

Following infection, cytokine release by immune cells promotes an antiviral response through activation, clonal expansion and proliferation of immune cell subsets. However, excessive cytokine production may result in heightened inflammation and tissue damage. Therefore, this process must be closely regulated, and one of the proteins that carries out this role is Cytokine-inducible SH2-containing protein (CISH). CISH is involved in the regulation of several key cytokines including GM-CSF, IL-2, IL-15 and IFN-γ. This is carried out through several negative feedback mechanisms, such as competitive inhibition and proteasomal degradation. To date, CISH's role in clearance of a viral infection such as influenza has not been established. Our unique approach to improve disease outcomes focuses on host factors such as CISH, as opposed to current interventions that target the virus.

My project aims to elucidate the exact mechanisms by which CISH regulates $CD4^+$ and $CD8^+$ T-cell responses following influenza A virus (IAV) infection, using a CISH knockout C57BL/6 mouse model. Previous studies in our lab determined that CISH knockout mice exhibit reduced weight loss compared to wild-type mice, enhanced $CD8^+$ T-cell immunity, and improved recovery from IAV infection. Utilising *in vitro* stimulation and proliferation assays, *in vivo* infections, viral quantification studies and molecular techniques, our findings will allow us to better understand antiviral immunity mediated by CISH following IAV infection, including its interactions and pathways within innate and adaptive immune cell subsets. These findings may unveil CISH's potential as a new therapeutic target, promoting long-lasting and effective recovery and viral clearance – eliminating the need for frequent reformulation of therapeutics.

Attenuating inflammation-driven neural damage through the cGAS-STING pathway in a mouse-model of traumatic brain injury.

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Traumatic brain injury (TBI) remains a major public health concern worldwide with unmet medical need. We previously confirmed the adaptor protein stimulator of interferon genes (STING) and downstream type-I interferon (IFN) signaling are key modulators of the detrimental neuroinflammatory response after TBI (Abdullah et al., 2018). Therefore, pharmacological inhibition of STING presents a viable therapeutic opportunity in combating this damaging neuroinflammatory response. This study investigated the neuroprotective effects of the small-molecule STING inhibitor C-176 in the controlled-cortical impact (CCI) mouse model of TBI in 10–12-week-old male mice. 30 minutes post-CCI surgery, a single 750nmol dose of C-176 or saline (vehicle) was administered intravenously. Analysis was conducted 2h- and 24h-post TBI (n=7-9 for each group). C-176-treated mice displayed a significant reduction in striatal gene expression of pro-inflammatory cytokines TNF-α, IL-1β and CXCL10 compared to their vehicle-treated counterparts at 2h post-TBI (29.87±18.47 vs 118.87± 25.05, p=0.008, 8.98±4.81 vs 36.36±6.17, p=0.001, 10.43±2.02 vs 20.89±4.15, p=0.004, respectively. Values expressed as fold-change relative to sham). Mice administered C-176 had significantly smaller cortical lesion areas when compared to vehicle-treated mice 24h post-TBI (4.68±0.44 mm^3 vs 6.60±0.53mm^3, p=0.036). Quantitative temporal gait analysis conducted using DigiGait™ showed C-176 administration attenuated TBI-induced impairments in gait symmetry (1.0±0.017 vs 1.1±0.026, p=0.02), stride frequency (3.0±0.068 vs 3.6±0.15 strides per second, p=0.001) and forelimb stance width (1.7±0.050cm vs 1.9±0.038cm, p=0.025). This study demonstrates the neuroprotective activity of C-176 in ameliorating acute neuroinflammation and reducing neural injury post-TBI, supporting the therapeutic potential of small-molecule STING inhibitors for improved functional outcomes following TBI.

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Investigating the frequency of T cell subsets in the ganglionic and aganglionic bowel in Hirschsprung Disease

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Hirschsprung disease (HSCR) is a congenital neuropathy characterised by the incomplete migration of neural crest cells in the colon during embryonic development. This results in an aganglionic region that causes a functional bowel obstruction and a seemingly "normally" innervated ganglionic region of the bowel. A significant proportion of HSCR patients develop Hirschsprung-associated enterocolitis (HAEC), a severe inflammation of the colonic mucosa. HSCR treatment assumes the ganglionic region is healthy. However, the persistence of HAEC after surgical removal of the aganglionic bowel challenges this. Keck et al.[1] demonsrated patients with low cholinergic innervation in the aganglionic bowel had an increased likelihood of HAEC. Analysis of T cell subsets in these patient specimens showed increased Th17 frequency compared to the ganglionic region. In contrast, patients with high cholinergic innervation were shown to have increased TReg frequency compared to the ganglionic region[1]. We hypothesize that T cell subset frequency in the ganglionic region is no different from the aganglionic region and this contributes to HAEC susceptibility.

This study investigated the frequency of CD4+ and CD8+ T cell subtypes within mucosal lymphocyte populations from ganglionic and aganglionic regions of HSCR colon.

Colon specimens from 8 HSCR patients (6-20 months: 6 males, 2 females) were analysed. Intraepithelial (IEL) and lamina propria lymphocytes (LPL) were isolated and analysed using flow cytometry.

In IEL populations, comparable CD4+ Th1 (CXCR3+ CCR4- CCR6-), Th2 (CXCR3- CCR4+ CCR6-), Th17 (CXCR3- CCR4+ CCR6+ CCR10-), Th1/17 (CXCR3+ CCR4- CCR6+) and Th22 (CXCR3- CCR4+ CCR6+ CCR10+) cell frequencies were observed in both the ganglionic and aganglionic regions. Additionally, equivalent CD4+ tissue resident cells (CD103+CD69+) and TRegs (CTLA4+FoxP3+) were observed. Both CD4+ and CD8+ LPLs exhibited equivalent frequencies. In CD8+ IELs, there was a decrease in the frequency of CXCR3+ CCR4- CCR6- cells but an increase in CXCR3- CCR4+ CCR6+ CCR10+ cells in the aganglionic region compared to the ganglionic.

These findings establish similar frequencies of CD4+ and CD8+ T cell subtypes in the ganglionic and aganglionic regions of the HSCR colon. This further supports the hypothesis that these two regions are no different and therefore may provide some insight into post-surgical HAEC development.

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Characterising Antibody Epitopes to a Malaria Parasite Protein to Inform Vaccine Design

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The only malaria vaccine recommended for widespread use has shown moderate efficacy in endemic settings. Therefore, better vaccines are strongly needed. Here, we closely examine regions and epitopes of a *Plasmodium* surface protein that induces protective functional antibodies to inform the development of vaccines with high protective efficacy.

The *Plasmodium* parasite expresses numerous surface proteins at the merozoite blood stage, with merozoite surface proteins (MSPs) being an abundant class. MSPs play a role in red blood cell attachment and invasion, subsequent intracellular replication then leads to clinical illness. Therefore, vaccine-induced immunity towards MSPs may be able to prevent malaria illness. The protein MSP2 is a major protein abundant on the surface of merozoites with limited genetic variation, making it an attractive target for vaccine development.

Emerging evidence has shown that antibodies with specific functions can effectively target malaria parasites and are associated with protection. For MSP2, naturally acquired antibodies opsonise merozoites and can crosslink Fc receptors on innate cells, which activate phagocytosis and cellular cytotoxicity. Moreover, fixation and activation of serum complement proteins by antibodies can lyse merozoites and inhibit replication. However, the specific epitopes of these functional antibodies have not been defined, but this knowledge is needed to inform optimal vaccine design.

We identify regions within MSP2 that are strongly targeted by functional antibodies. Peptides were designed based on antigenic regions of MSP2 and were tested against sera from malaria-exposed Kenyan adults. We found that the cohort displayed high IgG reactivity against all peptides across MSP2. We then identified MSP2 domains that potently bound complement-fixing antibodies. Lead antigenic regions and epitopes will be further characterised to measure Fc receptor engagement and opsonic phagocytosis by neutrophils and monocytes.

Overall, our results will demonstrate that certain regions within MSP2 are highly targeted by functional antibodies. These regions will then be evaluated in vaccine designs using pre-clinical animal studies. Incorporating these regions in a vaccine may direct the humoral response towards generating highly functional antibodies and afford better protection against malaria. The fine-tuning of protective epitopes can also be applied to other MSPs to design an effective multi-antigen vaccine.

Monocytes support antibody-mediated protection in malaria

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Development of a vaccine that elicits long-lasting protection to malaria is hindered by an absence of established correlates of immunity. Increasing evidence suggests that antibodies capable of binding to serum complement proteins or receptors on innate immune cells are associated with malaria immunity, but the specific immune cells that support this protection are poorly characterised. Monocytes are critical effector cells and have demonstrated efficient phagocytosis of malaria parasites *in vitro*, however, as active malaria infection may impair their function, the role of monocytes during malaria is not well defined. To investigate the activity of monocytes in malaria, and how they support protective antibodies, we characterised the expression of functional surface receptors on monocytes collected from individuals in Papua New Guinea. Immune cells from children infected with the malaria parasites *Plasmodium falciparum* or *P. vivax*, as well as from children and adults without malaria infection, were assessed using spectral flow cytometry. Our results suggest monocytes are important effector cells of antibody-mediated phagocytosis during malaria. We found elevated expression of Fcγ-receptors in children with *P. falciparum* infection, suggesting monocytes enhance opsonic phagocytosis to support parasite clearance. In contrast, no such changes were evident in children with *P. vivax,* who generally showed a lower intensity of infection. Expression of complement receptors was not altered during infection with either malaria parasite. Age-dependent differences in innate cells were also evident, with expression of functional markers greater in uninfected adults than children, which may reflect a heightened inflammatory environment resulting from previous malaria infection. There was no evidence that antigen-presentation, vital to support B-cell activation and antibody production, was impaired by malaria, as expression of relevant surface markers was not altered on monocytes or dendritic cells in children with parasite infection. Our results improve the characterisation of monocytes in malaria and suggest they contribute to the protective associations of Fcγ-receptor-binding antibodies observed in malaria vaccine trials. A comprehensive understanding of the immune features involved in malaria protection is essential to support targeted vaccine design in order to enhance immunity and achieve long-lasting protection.

Development of Real-Time Biosensors to Detect Airborne Allergens

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Hirst-type traps are the most common sampling machines implemented at aeroallergen monitoring stations worldwide[1,2]. While Hirst-type traps can effectively collect airborne particulates over a long time, limitations arise from these lengthy and labour-intensive collection times, and can be error prone by an inexperienced counter. Current sampling can be significantly improved by developing "real-time" automatic monitoring methods to assist researchers with detecting sudden fluctuations in aeroallergen concentrations over shorter periods and provide valuable data for ETSA forecasts as thunderstorm asthma events are unfolding. One example of "real-time" automated sampling is biological immunosensors (biosensors), which are based on reactions between antigens and antibodies, coupled with electrical or chemical components, to produce an analytical signal[3]. The formation of antibody-antigen complexes can be measured with a level of high sensitivity to determine the presence or concentration of specific molecules[3]. Mass-sensitive biosensors were developed to detect allergens Lol p 5/Phl p 1 and Alt a 1 found in prominent allergenic pollen and fungi, respectively[4,5]. 1:3 11-MUA and 9-MNL thiols were modified via NHS/EDC activation to enable antibody attachment[6]. Antibody-antigen complexes were successfully formed in a simulation chamber under controlled conditions. The development of these biosensors are the first of its kind, as none had yet been built to detect pollen or fungal spore allergens. This research demonstrates that biosensors may become a viable alternative to conventional methods for monitoring airborne allergens in "real-time". Future studies should focus on testing biosensors in field conditions to validate their real-world usage.

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Defining novel virus restriction factors for respiratory virus infections

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Influenza A virus (IAV) is a respiratory virus of global health significance and can cause outbreaks of seasonal influenza disease. The IAV hemagglutinin (HA) is a key viral protein which not only determines the subtype of the virus but also mediates attachment and entry into the host cells. HA and other viral proteins are known to interact with a variety of host proteins during infection. These viral and host protein interactions can lead to either pro or antiviral outcomes – where antiviral activity can be attributed to the action of host interferon stimulated genes (ISGs). As both IAV HA and ISGs play an important role modulating IAV replication, we wanted to investigate whether there was an association between viral HA and these host proteins during infection. Herein, we conducted a mass spectrometry screen and identified novel host protein binding partners of IAV HA. Viral HA protein was found to interact with the host immunoresponsive gene 1 (IRG1) ISG protein, which is a mitochondria enzyme involved in regulating the production of reactive oxygen species. It is well characterized in bacterial infections as IRG1 produces the metabolite itaconate that can inhibit bacterial metabolism. However, the role for IRG1 in modulating viral infection is less understood. We validated the interaction between IRG1 and IAV HA and confirmed this interaction for multiple IAV HA proteins of different subtypes (H1, H3 and H5). Following validation of the association, an overexpression system was used to investigate whether IRG1 displayed antiviral activity against seasonal strains of IAV. We identified that IRG1 did not impact the early stages of infection, as the IRG1 overexpressing cells were as equally susceptible to IAV infection as the control cells. However, there were lower viral titers released from the IRG1 overexpressing cells compared to the control cells, suggesting that IRG1 did modulate the late stages of IAV infection. Our findings show that IRG1 protein associates with IAV HA and inhibits IAV infection. Ongoing studies will explore the mechanism of IRG-1 mediated antiviral activity and will also assess antiviral activity to other respiratory viruses. Enhancing IRG1 activity could present a novel “host-directed” antiviral therapy.

ABSTRACTS
POSTER 2

Defining the fine specificity of antibody responses to polymorphic and conserved epitopes of the lead malaria vaccine antigen: *Plasmodium falciparum* circumsporozoite protein

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Malaria caused over 240 million infections resulting in over 600,000 deaths in 2022. In 2021, RTS,S became the first malaria vaccine recommended for susceptible young children, however, it confers modest efficacy (~30–50%). The mechanistic basis of RTS,S-induced immunity remains unclear, but antibodies are known to play a key role in mediating protection. Improving RTS,S efficacy requires understanding the specific epitopes within the vaccine antigen targeted by highly protective antibodies and how polymorphisms in the vaccine antigen impact antibody recognition and vaccine efficacy.

RTS,S vaccine is a virus-like particle expressing a truncated form of the major *P. falciparum* parasite circumsporozoite protein (*Pf*CSP) of one strain only (3D7). The vaccine construct includes a highly immunogenic *Pf*CSP central repeat region which is a predominant antibody target; however, antibody responses to this region are only moderately associated with protection against clinical malaria. RTS,S also includes a polymorphic C-terminal region; the strain used in the RTS,S vaccine represents less than 10% of parasites circulating in Africa. It is unclear how well vaccine-induced antibodies recognise the C-terminal region of other polymorphic strains circulating in malaria-endemic populations. Furthermore, RTS,S excludes highly conserved *Pf*CSP junctional epitopes recently identified as targets of potent neutralising antibodies and it is unknown if RTS,S-induced antibodies cross-react with these epitopes.

Using a study population of 737 children from an RTS,S phase IIb clinical trial and studies in an animal model, we defined the fine specificity of RTS,S-induced antibodies to the central repeat, polymorphic C-terminal and conserved junctional epitopes of *Pf*CSP. Through this study we identified antibodies to specific epitopes that associated with protection from malaria. RTS,S-induced antibodies demonstrated reduced binding to polymorphic C-terminal regions of vaccine-dissimilar strains, which might explain low vaccine efficacy. Additionally, a proportion of children had promiscuous antibodies that cross-reacted with junctional epitopes excluded from the vaccine construct. Our findings elucidate the specificity of protective RTS,S antibody responses, the impacts of polymorphisms on antibody binding and reveal how targeting conserved epitopes could achieve highly effective next-generation malaria vaccines.

Elucidating the prognostic and functional roles of NK cell subsets in bladder cancer

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Abstract

Anti-tumour immune responses from NK cell were reported in bladder cancer (BLCA), however, the distinct anti-tumor functionalities of $CD56^{bright}$ and $CD56^{dim}$ NK subsets against BLCA remain largely unexplored. In this study, we elucidated the prognostic implications of these NK subsets in BLCA through a computational approach. Unique molecular signatures of $CD56^{bright}$ and $CD56^{dim}$ NK cells dissected the relative abundances of these subsets along with 3 stromal and 18 other immune cell types in the patient tumour transcriptomes from TCGA-BLCA dataset. $CD56^{bright}$ NK cells was predicted to be the abundant tumour-infiltrating NK subsets, which is also associated with improved patient prognosis. A similar favorable survival trend was projected for the mature-myeloid-dendritic (mDC) cells and CD8+-effector-memory-T cells. Furthermore, a positive correlation between these cell types with the NK cell subsets hints a potential NK-mDC-CD8+T cell cross-talk in the tumor microenvironment. Expression of transcripts encoding the activating NK cell receptors such as NKG2D, NKp44, CD2, and CD160 showed positive survival trends in combination with $CD56^{bright}$ NK cell infiltration. Transcription factors such as HOBIT, IRF3, and STAT2 were also correlated with $CD56^{bright}$ NK cell abundance. Additionally, HOBIT-dependent tissue-residency program was found correlated with NK/T cell signatures. Expression of different immune checkpoint receptors was found to be associated more with T cell signatures than the NK cell subsets. Finally, another indipendent single cell RNAseq dataset recapitulated the findings from the bulk transcriptomes. Overall, this study highlights the functional significance of $CD56^{bright}$ NK in BLCA patient prognosis that can potentially facilitate a better understaing of the NK cell's anti-tumour responses, which can ultimately lead to the development of promising NK cell therapies against BLCA.

Plasma metabolomic variation in Juvenile idiopathic arthritis is not exclusively driven by chronic inflammation.

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Background and knowledge gap

Juvenile idiopathic arthritis (JIA) is an autoimmune disease of unknown cause, characterised by inflammation of the joints in children. Although generally classified into subtypes based on clinical features, diagnosing JIA is challenging as symptoms are shared across subtypes and with other inflammatory diseases. Despite limited metabolomic analysis in JIA, the potential of metabolomics to improve diagnosis has not been fully explored.

Method

A total of 73 children with JIA and 18 age/sex matched controls were selected from the CLARITY (ChiLdhood Arthritis Risk factor Identification sTudY) cohort. The metabolomic profile of circulating plasma was generated using NMR (Nightingale Health, Finland) and the association with JIA, JIA subtype, and inflammatory status (Glycoprotein acetyls, GlycA) was tested using linear regression models.

Results

We characterised the metabolomic profiles in four JIA subtypes; oligoarticular, RF-positive and RF-negative polyarticular, and systemic JIA. In total 2/249 metabolomic biomarkers were significantly different in plasma from children with JIA compared to controls. No subtype-specific metabolomic variation was detected other than for 17 biomarkers in systemic JIA. 11/249 metabolomic indicators were associated with GlycA. Only one biomarker was both JIA-specific and GlycA-associated. Subtype-specific correlation analysis showed 4/17 GlycA-associated systemic JIA biomarkers.

Conclusion

The identification of JIA subtype-specific biomarkers in circulating plasma supports the heterogeneity of systemic and non-systemic JIA. While GlycA is elevated in JIA plasma, the low overlap between GlycA-associated and JIA-specific biomarkers, suggests that GlycA, the hallmark of chronic inflammation, is not the exclusive factor that contributes to the altered metabolic profile in children with JIA.

Comparison of Cellular Immunity in Fractional and Standard Dose of Pfizer-BioNTech (BNT162b2) in Healthy Mongolian Adults

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Background:

As vaccine induced immunity wanes over time, COVID-19 booster vaccines are recommended to protect against newly emerging variants of concern. Fractional dose booster vaccination is an important strategy to improve vaccine access in low- and middle-income countries, however, more data on its efficacy is needed. To address this, we undertook a phase 3 randomised clinical trial in healthy Mongolian adults (≥18 years of age), given either a fractional or standard dose of Pfizer-BioNTech (BNT162b2) booster vaccine.

Methods:

We have recruited 601 adults, which for this study, 60 participants who have received either standard (30μg) (N=30) or fractional (15μg) (N=30) Pfizer-BioNTech third dose have been selected for analysis. Peripheral blood mononuclear cells were collected prior to third dose (booster) vaccination (day 0) and 28 days post-vaccination. Cell-mediated immunity was measured by flow cytometry using an activation induced marker (AIM) and intracellular cytokine staining assay in response to stimulation with ancestral (WA.1) full length spike peptides. Wilcoxon testing was used for statistical significance ($p<0.05$) between day 0 and day 28. Statistical analysis between fractional and standard dosing will be conducted using a Friedman test.

Results:

In response to WA.1 stimulation, we observed an increased production of IFN-γ, IL-2 and TNF-α in memory CD4 T-cells following booster vaccination at day 28. Additionally, we found increased AIM+ CD4 and CD8 memory T-cells identified through CD69, CD137 and/or OX-40 co-expression following booster vaccination. Results will be unblinded and formal analyses between fractional and standard dose groups will be undertaken for presentation at the conference.

Conclusion:

Understanding how fractional dosing impacts cellular responses can help guide low- and middle-income countries with future COVID-19 vaccine strategies to improve vaccine access and immune protection.

The lipid droplet recruits antiviral signalling proteins following cellular viral infection

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Virally infected host cells trigger early innate signalling pathways to upregulate high levels of the anti-viral cytokine, interferon, in the first hours following infection to assist in host cell survival. Recent work from our laboratory has discovered that lipid droplets (LDs) are essential for the heightened production of interferon following viral infection and this study examined their role in recruiting antiviral signaling proteins following viral infection, which may assist in signalosome formation.

Proteomic analysis of isolated LDs from mock, or infected (viral mimic and Zika virus) astrocyte cells revealed key players of the antiviral signalling pathway, including RIG-I, STAT1, ISG15 and viperin localized to LDs following activation of antiviral signaling pathways. Using a novel developed technique, we could localize fluorescently labelled proteins to extracted LDs and determine their localization percentage. All targets were observed on a subset of LDs ranging from 80% for a LD resident protein (Viperin) and ~10% for novel antiviral LD proteins, including STAT1, STAT2 and MX1. Western blotting and confocal microscopy also confirmed the localisation of these novel proteins to LDs.

In addition to key antiviral proteins, proteomic analysis also revealed that approximately 10% of LD proteins are involved in regulating post translational modifications (PTMs) including phosphorylation (41.30%), ubiquitylation (33.57%) and ISGylation (1.22%), with ISGylation regulatory proteins also upregulated following viral mimic infection.

The protein RNF213 has been described to act as a sensor for ISGylated proteins on the LD, however, which proteins it is sensing following viral infection has yet to be explored. Preliminary fluorescent microscopy suggests that RNF213 localises to astrocytic LDs and interacts with antiviral signalling proteins such as MX1 and viperin which may act as a signalosome to enhance host immunity following viral infection.

Here, we demonstrate that there are important antiviral immune signalling proteins that localise to the LD following viral mimic stimulation, indicating that the LD may act as a signalling platform for signalosome formation to aid host immunity for the first time.

Unveiling the interplay of *Coxiella burnetii* Dot/Icm effectors: insights into host immune subversion

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Coxiella burnetii, the causative agent of human Q fever, is a unique bacterial pathogen that obligatorily replicates within a lysosome-derived intracellular space. Central to the establishment of this niche and ensuing pathogenesis is the Dot/Icm type IV secretion system encoded by *C. burnetii*. This system translocates approximately 150 distinct effector proteins directly into the host cytosol, with several playing indispensable roles in bacterial virulence by subverting various host cellular processes to enable *C. burnetii* intracellular proliferation. However, the functional roles of most of these effectors remain undefined. We harnessed the budding yeast *Saccharomyces cerevisiae* as a practical heterologous system to query the functions of *C. burnetii* effectors within a eukaryotic cellular context. Analysing the impact of each effector ectopically expressed in the yeast marked 11 as 'toxic' due to their capacity to perturb yeast growth. We describe a nuclear-targeting *C. burnetii* effector protein, NceA (nuclear *Coxiella* effector protein A), whose expression induces growth defects in *S. cerevisiae*. A functional role for NceA within the host nucleus is indicated by the presence of a nuclear localisation signal that is required for optimal targeting into the nucleus and NceA-induced yeast toxicity. A yeast toxicity suppressor screen identified nuclear activities, including regulation of gene transcription, chromatin remodelling, and ribosome biogenesis, as subverted by NceA. A subsequent system-wide screen using a modified Synthetic Genetic Array approach discovered two other effectors capable of alleviating NceA-induced yeast toxicity, indicating them as potential regulators of NceA host cellular functions (RonA and RonB) during infection. Further validation in tissue culture infection models demonstrated an antagonistic functional interplay between NceA and RonA during *C. burnetii* infection, influencing the activation of NF-κB—a pivotal transcriptional regulator in cellular immune and inflammatory responses. This study advances our comprehension of *C. burnetii* effectors and their intricate coordination in undermining host immune signalling to ensure successful infection.

MMV687794 impairs blood-stage *Plasmodium falciparum* invasion by perturbing lysophospholipids

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Responsible for parasite proliferation and symptomatic malaria, parasite invasion of erythrocytes represents an attractive novel drug target. Our group discovered a compound that specifically blocked invasion from schizonts, MMV687794. Genomic analysis of MMV687794-resistant parasites unveiled mutations in an alpha/beta hydrolase enzyme containing a lysophospholipase (LysoPL) motif we termed ABH-83: 1) Single amino acid substitution from the highly-conserved position 36 cysteine to tryptophan (C36W) 2) Single amino acid deletion in its promoter region. To validate ABH-83 as the drug target, the C36W mutation was engineered into wild-type parasites using CRISPR/Cas9, which recapitulated the MMV687794-resistant phenotype. An epitope tag and a GlmS riboswitch were also introduced into these parasites, enabling a closer examination of the role(s) of ABH-83. ABH-83 has been visualised by microscopy at the rhoptry surface, organelles that secrete important invasion-related proteins during erythrocyte invasion. By conducting western blot-based assays on the transgenic parasites: 1) Time-course series, we found that LysoPL ABH-83 is most highly expressed in schizonts, concordant with a role in invasion. 2) Sodium carbonate extraction, ABH-83 is shown to be membrane-associated (likely through its N-myristoylation motif), and this association is not altered by the C36W mutation. 3) Rhoptry/microneme protein processing assay, ABH-83 is shown to function downstream of protein kinase G, and reduced ABH-83 expression decreases rhoptry protein (Rh5) processing. Interestingly, reduced ABH-83 expression had no observable defect in parasite growth or morphology, but in wild-type transgenic parasites, it recapitulated the previously observed MMV687794-resistance phenotype. Furthermore, lipidomics data indicate that parasites with C36W ABH-83 have broad perturbations in lipids when compared to wild-type parasites and MMV687794-treated schizonts have elevated lysophospholipids, an effect less pronounced in parasites with C36W ABH-83. Overall, these results suggest that while ABH-83 may have a role in rhoptry lipid metabolism important for its functioning and morphology, MMV687794 likely has a different target, and the C36W mutation in ABH-83 likely arose as a compensation mechanism to counteract the lipid changes induced by the compound. Further lipidomic investigations aim to dissect this and the specific role(s) of ABH-83 in lipid metabolism.

Activity of bis-1,2,4-triazines, a novel potent antimalarial, requires parasite-mediated activation process

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Malaria remains a threat to global health and is responsible for more than half a million deaths worldwide per annum. Resistance to frontline antimalarials highlights the urgent need for the discovery of antimalarials with a novel mechanism of action. Bis-1,2,4-triazines are a class of fast-acting antimalarial candidates with low-nanomolar potency against the entire asexual blood stage and early sexual blood stage *Plasmodium falciparum* parasites. Their mode of action, despite unknown, appears to be novel. The bis-1,2,4-triazines display a short *in vivo* exposure in mice, but are able to clear malaria infection rapidly, highlighting a gap of knowledge about the relationship between the stability and antimalarial activity of this compound series. We used an LC-MS methodology to show that the rate of bis-1,2,4-triazine degradation in an *in vitro* cell culture system was correlated to the number of parasites per mL, indicating that a parasite-dependent mechanism is responsible for compound degradation. Analysis of a range of bis-1,2,4-triazines showed that *in vitro* stability was inversely related to antimalarial activity, suggesting that their antiparasitic activity requires activation, or that they irreversibly inhibit their drug target in the parasite. Ongoing work is dedicated to investigating their mode of action and exploring whether potent analogues can be developed with greater stability.

Alteration of neuroimmune pathways rescues impaired intestinal permeability and behaviour in a mouse model of colitis.

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Background: Individuals with autism spectrum disorder (ASD; autism) are more susceptible to inflammatory disorders and gastrointestinal dysfunction, but how interactions between the brain, gut, and immune system are altered in this group is unclear. We studied effects of an inflammatory insult on *Nlgn3*^{R451C} mice expressing an autism-associated mutation in Neuroligin-3, located at neuronal synapses. We previously reported increased microglial density in the brain, and increased numbers of smaller, more spherical intestinal macrophages in the caecum of *Nlgn3*^{R451C} mice, suggesting elevated immune activity. Mice lacking the interferon alpha/beta receptor subunit 1 (*IFNAR1*$^{-/-}$) show improved behavioural and functional outcomes in preclinical models of chronic inflammation but the combined impact of the *Nlgn3*^{R451C} and *IFNAR1*$^{-/-}$ mutations is unknown. **Methods:** We bred *Nlgn3*^{R451C} and *IFNAR1*$^{-/-}$ mice to produce *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mice. Mice were treated with 3% DSS (Dextran-Sodium-Sulfate) for 7 days to induce ulcerative colitis. Colitis symptoms (weight loss, bloody diarrhea, and rectal bleeding), colon length, and intestinal motility and permeability were assessed. Mouse behaviour was measured via an open field test on day 7. DNA was extracted from fecal pellet samples to analyse microbial communities. **Results:** All DSS-treated mice showed colitis symptoms, however *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mice recovered faster than wild-type mice. DSS treatment resulted in shorter colons in *Nlgn3*^{R451C}*, IFNAR1*$^{-/-}$ and wild-type mice. Although DSS-treated wild-type mice had increased ileal permeability, this was unchanged in *Nlgn3*^{R451C}, *IFNAR1*$^{-/-}$, and *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mice. DSS-treated mutant mice (i.e., *Nlgn3*^{R451C}*, IFNAR1*$^{-/-}$, and *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mice) exhibited reduced locomotor impairments in the open field test compared to wild-types. **Conclusion:** Here we show that modulating Neuroligin-3 in addition to disrupting the type-I interferon pathway, prevents some DSS-induced colitis phenotypes and provides resistance to this inflammatory insult. These findings suggest that both the *IFNAR1* and *Nlgn3* genes interact to improve behavioural outcomes in response to DSS-induced colitis.

DISCOVERING NOVEL NATURAL PRODUCTS TO COMBAT ANTIBIOTIC RESISTANT PATHOGENS

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Mounting pressure from antimicrobial resistant organisms threatens our ability to control infections. It is therefore vitally important to identify new sources of antimicrobials (1-3). Soil bacteria have been an excellent source of antibiotics; however, standard cultivation techniques are only capable of cultivating less than 1% of soil microbes. Here we have used microbial diffusion chambers to isolate and screen antimicrobial producing bacteria from Australian soils. We recovered 141 bacterial isolates and screened these for antimicrobial activity against multidrug-resistant clinical pathogens. We found 48% of isolates inhibited at least one organism in our test panel. Using high-resolution mass spectrometry (HRMS) attempted to identify and dereplicate known antibiotics produced by our isolates in conjunction with the GNPS database and annotation with Dereplecator+ (4). Whilst we have detected several known bioactive compounds in these crude extracts, the majority of the isolates show no hits to the known antimicrobial compounds, highlighting the potential of those isolates to biosynthesise new compounds. Analysis of the genomes of 8 selected bioactive isolates using antiSMASH and PRISM identified 244 BGCs encoding secondary metabolites. Further characterization of these BGCs and linking genomic information with metabolites detected in by mass spectrometry is underway, which will help us select promising BGCs for cloning and heterologous expression. In conclusion, this study highlights that Australian soil bacteria represent an untapped resource for the discovery of potentially new bioactive molecules.

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Uncovering the regulatory networks for *Klebsiella pneumoniae* iron acquisition

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Iron is an essential nutrient for bacterial life but it is not highly bioavailable. Bacteria secrete small, high-affinity iron chelating molecules called siderophores to overcome the iron limitation. Though siderophores are crucial for bacterial iron acquisition, they have the potential to cause adverse effects such as iron toxic overload. A tight regulatory control for siderophore production is essential to maintain an appropriate amount of intracellular iron. Currently, our understanding of siderophore regulation is not complete as it is mostly gathered from single gene studies in distantly-related bacteria. Recent innovations in high-throughput functional genomics technology provides a new tool for studying gene expression and regulation in bacteria.

In this study, we aimed to decipher the regulatory networks governing bacterial iron acquisition in hypervirulent *Klebsiella pneumoniae*, a critical public health threat in which siderophores are a major virulence factor. Hypervirulent *K. pneumoniae* produces four different siderophores – aerobactin, yersiniabactin, enterobactin and salmochelin – which make distinct contributions to infection. Transcriptomics showed numerous genes were differentially expressed on iron starvation, including all siderophore genes, other genes for anaerobic stress response, and acquisitions of other metals. Growing the strain under increasing levels of iron starvation showed sequential siderophore induction, with aerobactin produced first followed by enterobactin, yersiniabactin and, lastly, salmochelin. Expression of the plasmid siderophores salmochelin (*iroB*) and aerobactin (*iucA*) seemed plateau a lower level of expression than the chromosome-encoded ones (enterobactin/*entC* and yersiniabactin/*irp2*). The mice infected with the hpv *K. pneumoniae* strain rapidly developed illness, with bacteria disseminating to multiple sites within the host. The *in vivo* expression of the four siderophores was investigated and they were found to be differentially expressed in the different host niches.

Overall, our findings support the hypothesis that *K. pneumoniae* uses distinct regulatory mechanisms to control each of its four siderophores. Further work will explore the mechanisms underpinning their differential expression in different environmental and niches.

BCL-2 and BCL-XL dynamically regulate LPS-primed macrophage survival and inflammation in the absence of BCL2A1

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Intrinsic apoptosis is a cell death mechanism that is an important host response to combat pathogen infection. While traditionally thought to be immunologically silent, recent studies have demonstrated crosstalk between intrinsic apoptotic cell death signalling and inflammation via caspase-3/-7-dependent NLRP3 inflammasome activation. However, little is known about the regulation of intrinsic apoptosis during bacterial infection, and whether this inflammatory crosstalk is protective. Moreover, whether inducing cell death in infected macrophages, which act as a replicative niche for a growing number of antimicrobial resistant bacteria, can be harnessed to promote pathogen clearance remains unclear.

It is well established that bone marrow derived macrophages (BMDMs) rely on the BCL-2 family pro-survival proteins BCL-XL and MCL-1 for their survival. Our unpublished findings also reveal an essential role for the inducible, short-lived, BCL-2 family member BCL2A1 (A1) in delaying apoptosis upon Gram-negative bacterial LPS exposure and BCL-XL and MCL-1 targeting. Interestingly, we now find that targeting BCL-2, in conjunction with MCL-1 inhibition and A1 loss, also triggers late stage cell death and NLRP3 inflammasome activity in LPS-primed macrophages. Finally, we show the potential physiological relevance of A1 in limiting cell death and inflammation using the Gram-negative intracellular bacteria *Legionella pneumophila*. Collectively, our data suggest that pathogens may modulate BCL-2 family member expression and function to elude the host immune system.

Identifying Merozoite Surface Proteins as Targets of Protective Functional Antibody Responses against *Plasmodium falciparum* and *P. vivax* malaria

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Malaria is responsible for a major global health burden with 247 million cases and 619,000 deaths in 2021. Two major parasite species, *Plasmodium falciparum* and *P. vivax*, account for nearly all cases globally. *P. falciparum* has a high density of cases in Africa and *P. vivax* cases are distributed across the globe. Reduction in the burden of malaria has stalled in recent years, highlighting the need for efficacious vaccines against *P. falciparum* and *P. vivax* to support malaria elimination. Despite one *P. falciparum* vaccine recently being approved for use, its efficacy is modest and short-lived, and there are no *P. vivax* vaccines available, with limited candidates under development. Antibodies play an important role in immunity against malaria, but a better understanding of the targets and mechanisms of action of protective antibodies is needed to develop highly efficacious *P. falciparum* and *P. vivax* vaccines. The extracellular merozoite stage of the *Plasmodium* parasite invades and replicates in red blood cells causing clinical disease. Therefore, targeting merozoites is an attractive vaccine approach. However, there are many antigens expressed on the merozoite surface, presenting challenges for vaccine development. We are investigating merozoite surface proteins (MSPs) as major targets of protective functional antibody responses to *P. falciparum* and *P. vivax.* We are using antibody samples from naturally exposed individuals in malaria endemic regions and vaccines studies. These are analysed in novel high throughput assays to investigate the role of antibodies targeting MSPs, including assays to quantify: complement activation mediated merozoite invasion inhibition and erythrocyte replication, and to engage Fcγ-receptors to promote opsonic phagocytosis and antibody dependent cellular cytotoxicity. *P. falciparum* cell culture has been well established, whereas long-term *P. vivax* culture has been challenging. To overcome this, we are using a novel platform with the related *P. knowlesi* species expressing *P. vivax* MSPs, and short-term culture of *ex vivo* clinical *P. vivax* field isolates.

This work will increase our knowledge on the mechanisms of action of highly protective antibody responses against blood-stages of *P. falciparum* and *P. vivax* and identify important targets on merozoites than can be advanced for development of highly efficacious vaccines.

Mucosal delivery of self-assembling nanoparticle vaccines against SARS-CoV-2 using recombinant influenza virus vectors

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Background:

Despite successfully curtailing disease, vaccination against COVID-19 has been less efficacious against preventing the acquisition of SARS-CoV-2, particularly the Omicron variant. Recombinant influenza viruses are promising mucosal delivery vectors for boosting protection against acquiring or transmitting respiratory viruses by triggering robust secretory antibody, and tissue-resident memory T and B cell responses directly in the lung mucosa where viral replication occurs.

Methods:

The NS genomic segment of influenza A was engineered to express SARS-CoV-2 spike receptor binding domain (RBD) antigens, presented as monomeric, trimeric, or 24-mer self-assembling ferritin-based nanoparticle. Using an eight-plasmid reverse genetics system, we generated replication-competent PR8 and X31 recombinant viruses. C57BL/6 mice were intranasally immunised, and immunogenicity was assessed using serological assays and flow cytometric analysis of B cell responses in the lung and lung-draining mediastinal lymph nodes (mLN).

Results:

Immunised mice displayed potent serological and cellular immune responses against RBD, with the ferritin nanoparticle being significantly more immunogenic than monomeric or trimeric forms. While both parenteral and mucosal immunisation elicited robust anti-RBD IgG titres in serum, mucosal immunisation drove accumulation of anti-RBD IgA titres in both lung and nasal washes and seeded RBD-specific lung-resident memory and germinal centre B cells in the lung. RBD-specific B cells detected by confocal microscopy further demonstrated their localisation within regions of inducible bronchus-associated lymphoid tissue (iBALT) structures, present only in intranasally immunised animals. Interestingly, the immunogenicity of recombinant influenza-based vaccines was maintained in the face of pre-existing anti-vector immunity.

In the context of prior systemic immunity elicited by intramuscular spike vaccination, intranasal boosting with recombinant influenza vaccines (i.e., “prime-pull” strategies) could redirect IgG, IgA and memory B cells to the mucosa resulting in enhanced SARS-CoV-2 virus neutralisation in lung and nasal compartments as compared to parenteral only prime-boost immunisation. Moreover, intranasally boosted mice challenged with X31 expressing ferritin-RBD exhibited heightened frequencies of RBD-specific CD138+CD98+ plasma cells in mLN indicative of memory recall responses.

Conclusion:

Our findings demonstrate recombinant influenza-based vaccines can stably deliver SARS-CoV-2 RBD immunogens, including complex nanoparticle-arrays, generating robust lung-localised RBD-specific antibodies and mucosal B cells beyond that established by parenteral immunisation regimens. Thus, recombinant influenza viruses provide a versatile platform to augment protection against endemic and pandemic respiratory viruses.

TREML4 ablation in mice leads to the development of innate immune memory following polymicrobial sepsis

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Sepsis is a biphasic disease characterised by acute inflammation, leading to extensive immune suppression, exposing patients to secondary infections. Many clinical trials sought to treat sepsis by modulating inflammation, but this had no effect on patient mortality, primarily due to secondary infections. Recently, the focus has shifted to addressing the immunosuppressive phase of sepsis, aiming to return the host's immune system to a functional state and prevent further susceptibility to secondary infections. Previous work by Nedeva et al., (2020) identified TREML4, a triggering receptor expressed on myeloid cells (TREM), as the key regulator of immune cell death and inflammation, following polymicrobial sepsis. Genetic ablation of TREML4 in mice using CRISPR demonstrated its role in regulating many cellular responses, such as reducing apoptosis of innate immune cells in the absence of TREML4. TREML4 ablated mice had a higher rate of survival in acute sepsis and from secondary infection with *Pseudomonas aeruginosa* during chronic sepsis. This improved survival is due, in part, to long lasting epigenetic changes in innate immune progenitor cells of TREML4 ablated mice. Additionally, neutrophils, essential for innate immune cell pathogen defence, have been discovered to mediate this secondary infection survival advantage. Overall, epigenetic changes to neutrophil progenitor cells during the early stages of polymicrobial sepsis results in an innate immune memory that protects TREML4 ablated mice from secondary infections during the chronic phase of sepsis.

Gasdermin D mediates the severity of influenza a virus-induced lung disease

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Influenza virus is a highly infectious pathogen that has tormented humanity for years on end. Severe IAV lung disease is associated with hyperinflammation, including dysregulated cytokine levels and significant infiltration of immune cells into the lung, contributing to the progression of fatal lung damage. Gasdermin D (Gsdmd) is an executor of pyroptotic cell death, a pro-inflammatory and lytic form of cell death. Gsdmd is cleaved by caspase-1 within an activated NLRP3 inflammasome complex, allowing the insertion of N-terminal Gsdmd into the lipid membrane (1). The formation of a transmembrane pore is thought to facilitate the release of pro-inflammatory cytokines IL-1β and IL-18 (2). Cell lysis also results in the release of cellular contents including danger associated molecular patterns (DAMPs), which can feed forward to drive further NLRP3 activation. The role of Gsdmd in IAV has not been well described.

In this study, Gsdmd-deficient (*Gsdmd*$^{-/-}$) mice displayed better survival outcomes compared to wildtype controls, when infected with a high dose of IAV (10 4 plaque-forming units of HKx31 (H3N2)). Critically IAV infection induced the expression of cleaved GSDMD in lung epithelial cells. Further, bronchoalveolar lavage fluid from *Gsdmd*$^{-/-}$ mice revealed significantly reduced numbers of infiltrating neutrophils at day 3 and 5 post-infection, as well as pro-inflammatory cytokines including CCL2/MCP-1, IL-6, and TNFα. Interestingly, IL-1β and IL-18 responses, as well as IAV-induced alveolar macrophage cell death was not altered by Gsdmd deficiency. However, viral loads were reduced in *Gsdmd*$^{-/-}$ mice on day 3, with less severe pulmonary pathology, including decreased epithelial damage and cell death. Collectively, our studies uncover a role for Gsdmd in promoting damaging inflammation and the development of severe IAV disease.

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Understanding the protective role of goblet cells in Hirschsprung Disease

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Goblet cells line the gastrointestinal tract and are critical for the production and secretion of mucus. The mucus they secrete forms a physical barrier to protect the epithelium from pathogens and other sources of damage. A functional mucus layer is essential for intestinal homeostasis and disruption of this layer leaves the epithelium vulnerable, increasing the susceptibility to bacterial infection and inflammation.

Hirschsprung disease (HSCR) is a congenital enteric neuropathy that severely impairs muscular control in the colon. Corrective surgery is the only treatment option available. Hirschsprung associated enterocolitis (HAEC) is a life-threatening comorbidity of HSCR that causes extensive mucosal inflammation. Mucus secretion can be regulated through neural signalling and the absence of enteric innervation suggests that mucosal barrier defence mechanisms are compromised in HSCR. Although the pathogenesis of HAEC is poorly understood, disruption of mucosal barrier function may be a key contributor.

We hypothesise that goblet cells and the mucus layer are dysfunctional in HSCR patients and is predictive of HAEC susceptibility. We applied imaging techniques, including optical clearing and light sheet microscopy, and super resolution microscopy, to characterise the goblet cell profile along the length of the resected colon from 10 HSCR patients and 3 age-matched controls. To analyse the acquired images, we developed a novel analysis pipeline using ImageJ to measure the functional capacity of goblet cells to secrete mucus in response to neurotransmitters or electrical field stimulation. Differences in the location and distribution of goblet cells along the length of the HSCR colon was also assessed.

We have developed methods to investigate the function and distribution of goblet cells in the human colon. Applying these methods to archival tissue samples from HSCR patients will allows us to assess the relationship between goblet cells and the incidence of HAEC. Furthermore, understanding the differences in mucus barrier function may provide a link between HSCR and HAEC susceptibility, which can be used to inform and guide treatment approaches for patients.

Developing precision RNA therapeutics for Tuberculosis

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Over centuries, Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis (TB), has evolved alongside humans, developing sophisticated strategies to evade our immune response. Mtb primarily infects alveolar macrophages and creates a hidden replicative niche inside what is supposed to be our first defense against respiratory infections[1]. Antibiotics are currently our only treatment for TB, but toxic side effects and skyrocketing rates of antimicrobial resistance (AMR) urgently necessitate novel host-directed therapeutic approaches (HDT)[2]. Targeted delivery of HDTs that act at the host-pathogen interface to induce apoptosis of infected host cells holds great promise to clear the pathogen and promote adaptive immune responses without eliciting systemic side effects and limiting AMR[3].

Lipid nanoparticles (LNPs) have revolutionised the targeted delivery of RNA therapeutics. We have developed LNPs that enable improved RNA transfection of macrophages *in vitro* and preferentially target monocytes in mouse PBMCs *ex vivo*. These LNPs allow for direct RNA delivery to the lung *in vivo,* with luciferase mRNA expression in mouse lungs, 16h post intranasal instillation. Leveraging this versatile delivery platform, we were able to kill Mtb-infected primary human macrophages using siRNAs against key regulators of host cell apoptosis, cellular inhibitors of apoptosis (cIAP1/cIAP2). Additionally, we raised nanobodies against the major Mtb virulence factor, ESAT-6, which acts by limiting host cell apoptosis. LNP-encapsulated mRNA coding for these nanobodies has great potential to complement the host-directed siRNA approach to further boost apoptosis. We currently assess the potential of these LNP-delivered combination RNA therapeutics in a clinically relevant mouse model of TB infection.

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Enhancing thermal stability of live virus vaccine using metal organic frameworks

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Vaccines have proven to be one of the most effective strategies in controlling infectious diseases and are a key component towards the protection of our health and economy. However, the success of vaccination programs often face considerable challenges in resource-limited regions, primarily due to the necessity of maintaining cold chain throughout transportation and storage. The demand for temperature-stable vaccines has spurred research into innovative strategies for preserving vaccine efficacy under harsh environmental conditions for equitable distribution without the dependance of 'cold chain' logistics. Here, we report biomimetic-mineralization of a live viral vaccine using metal organic frameworks (MOFs) to enhance their storage stability. Using Newcastle disease virus vaccine NDV V4 as our candidate, we investigate the impact of different molar concentrations of MOF composites on encapsulation, reporting that an increase in molar concentrations influences recovery of virus. We describe the impacts of lyophilisation on MOF structural integrity, viral titre, and report on alternative techniques such as air drying or column drying, evaluating these strategies with a storage experiment at ambient temperatures. Additionally, we describe using a haemagglutination assay as a quick and easy method to measure MOF encapsulation of NDV, as well as report on a new cell line used in a tissue culture infectious dose assay (TCID50) that can accurately displays cytopathic effect of NDV without immunofluorescent staining.

Towards the development of non-antibiotic based strategies to clear Klebsiella pneumoniae from the gut

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Antimicrobial resistance and multidrug resistant organisms (MDROs) are a significant threat to global health. With few new antibiotics in the development pipeline, novel strategies to combat MDROs are urgently needed. Gut colonisation is a critical first step towards life-threatening infections caused by many MDROs such as carbapenem-resistant *Klebsiella pneumoniae* (CRE-Kp). Despite this, no therapeutics that can clear MDROs from the gut exist.

Modulation of the gut microbiota offers a promising strategy to remove MDROs from the gut, but significant knowledge gaps remain. This project will address these knowledge gaps by i) understanding genetic mediators in CRE-Kp using a colonisation model, ii) identify and characterise inhibitory bacterial species of the human gut microbiome against CRE-Kp using an integrative multi-omics approach and iii) understand mechanisms of bacterial species inhibition and potential therapeutic activity in vivo and in vitro.

We have developed miniaturised high throughput assays (co-culture, spot overlay and supernatant) to screen 1034 human gut commensals strains. From this, 49 commensal strains were found to inhibit *in vitro* growth of CRE-Kp and the mechanism of inhibition was further analysed. This project also uses a systems level approach to understand microbiome dynamics and community structure that correlate with CRE-Kp colonisation to guide the development of multi-species probiotics that act antagonistically to inhibit gut colonisation of CRE-Kp.

This will lay the foundation for the future development of microbiome-based diagnostics and precision biotherapeutics that will be useful new weapons in our ongoing fight against MDRO infections.

Assessment of monovalent and bivalent SMAC mimetics to both shock and kill the HIV reservoir

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Background:

Antiretroviral therapy (ART) can prevent the progression of human immunodeficiency virus (HIV) infection into acquired immunodeficiency syndrome (AIDS) however, it is not a cure[1]. This is due to the persistence of latently infected cells, CD4+T-cells that contain an integrated provirus. These cells are primed for survival due to the upregulation of pro-survival proteins such as inhibitors of apoptosis proteins (IAPs)[1]. Moreover, CD8+T-cells in people living with HIV (PWH) on ART, the key cells for controlling HIV, are exhausted. This may explain why the major HIV Cure approach (Shock and Kill) which seeks to reactivate (shock) the provirus to induce infected cell clearance by immune or viral mediated killing, has largely failed. SMAC mimetics (SMACm), a class of compounds originally developed for induction of apoptosis in tumour cells by inhibiting IAPs, have been shown to reverse HIV latency. Other studies indicate that SMACm may also reverse T-cell exhaustion. We hypothesised that SMACm could function to both “shock” and “kill” by reversing latency, inducing infected cell apoptosis, and stimulating anti-HIV immunity.

Methods: We assessed monovalent (GDC0152, GDC0197, LCL161 and xevinapant) and bivalent (AZD5582, BV6 and Birinapant) SMACm (100uM) in cells from HIV-negative donors and from PWH on ART. Latency reversal was assessed using a cell line (J-Lat clone 10.6) and a primary cell model using the Morpheus reporter virus[2]. Peripheral blood mononuclear cells (PBMC) from PWH on ART were cultured for 13 days in the presence of SMACm and HIV antigen loaded tetramers were used to analyse the effects of SMACm on HIV specific CD8-T+cell properties and functions.

Results: Bivalent SMACm showed higher levels of latency reactivation in the cell-line model and in the primary cell model (100nM; p=0.03 and p=0.05 respectively) while demonstrating similar toxicity in HIV-negative-CD4+T-cells. The monovalent SMACm GDC0197 enhanced HIV specific CD8+T-cell proliferation (mean FC over DMSO±SEM, 4.10±0.49, p=0.078) and reduced PD-1 expression (mean FC over DMSO±SEM, 0.5±0.0775, p=0.0076).

Conclusion: Bivalent SMACm induces greater latency reversal whilst the monovalent SMACm, GDC0197, improves HIV specific CD8+T-cell recall and may reduce T cell exhaustion. Further work is continuing on the impact of these compounds on elimination of infected cells, both in vitro and ex vivo.

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The polyphenol rich sugarcane extract (PRSE) has potential antiviral activity against influenza A virus *in vitro*

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Background: Influenza A virus (IAV) is one of the major global public health concerns. IAV is highly contagious and known for its high mutation rate which can generate pandemic strains with the potential for high mortality and morbidity. Therefore, IAV is now under annual surveillance at the global level. In addition to vaccination, antivirals are an option to combat IAV infection, and neuraminidase inhibitors have been the mostly used antivirals to treat IAV infection. However, the emerging resistance of IAV against neuraminidase inhibitors highlights the need for other potential alternative antivirals directed against IAV. Polyohenol rich sugarcane extract (PRSE) is an extract prepared from the molasses of a sugarcane plant that has anti-inflammatory, antioxidant and bactericidal activity. Thus, we aimed to evaluate it could also be used as an antiviral against IAV infection.

Methods/Results: Treatment of IAV-infected MDCK cells resulted in a dose-dependent virus inhibition when assessed by plaque assay and western blot. We could show that PRSE affected the early stages of viral replication including viral genome and mRNA transcription and viral protein expression. However, PRSE treatment of intact virions did not affect virus morphology, as assessed by electron microscopy, nor the ability of the virus to bind red blood cells. We extended our findings to show that PRSE had antiviral activity against a broad range of H3N2 and H1N1 IAV strains.

Conclusion: Overall, our findings show that PRSE has the potential to be used as an antiviral against IAV *in vitro*.

Redefining iNKT cells thymic development pathway in human

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Abstract

iNKT cells, a key subset of unconventional T cells, play an important role in the immune system. Understanding how these cells develop is crucial to harnessing them for therapeutic purposes. While many studies have examined iNKT cells from mice, relatively few have examined iNKT cells from the human thymus. The purpose of this study was to identify the thymic development pathway of iNKT cells in humans. The expression pattern of PLZF was chosen as a guide because PLZF plays a key role in mouse iNKT cell development. In addition to staining human thymus samples for PLZF, we also stained them for markers of T cell maturation and function. This pathway was then validated using a functional cytokine assay. As we expect the most mature cells in the blood, we checked the development pathway in donor-matched blood as well. We have identified a four-stage pathway of development for iNKT cells in the human thymus. stage 1 cells were low for PLZF expression, stage 2,3 iNKT cells were medium, and stage 4 were the highest. Also upon stimulation, iNKT cells started to produce TNFa from stage 2, IL-2 from stage 3, and IFg from stage 4. Blood samples only contained stage 3 and 4 cells. These data reveal distinct precursors of iNKT cells expressing PLZF and other maturation markers within thymic development. These findings may lead to new opportunities to manipulate iNKT cells to treat human diseases, including cancer, inflammation, and infection.

Understanding the role of rodent malaria *clag* genes in new permeation pathway formation

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Abstract:
Malaria is one of the leading infectious diseases in the world and is caused by protozoan parasites of the species *Plasmodium*. *Plasmodium* parasites infecting humans have developed resistance to all antimalarial drugs, and it is therefore critical for new therapeutics to be developed.
New permeation pathways (NPPs) have been validated as a crucial modification of the host erythrocyte, facilitating parasite acquisition of nutrients, and are therefore an attractive therapeutic target. Previous work has shown that NPP inhibition leads to parasite death, further validating the channel as a drug target (Pillai et al. 2010). Understanding the channel's structure is critical for targeted drug design. *RhopH1/clag* genes have previously been implicated in NPP formation, however, their contribution to the channels structure remains unclear (Nguitragool et al. 2011). Utilization of rodent malaria species *Plasmodium berghei* could be used to investigate *clag* gene role in NPPs if it can be shown gene functionality remains the same across the two species.
Activity of *P. falciparum* NPPs can effectively be determined by sorbitol mediated hypotonic lysis, however, there is currently no method available for assessing the functionality of NPPs in a rodent model. Development of a method capable of determining NPP functionality in *P. berghei,* such as that which exists in *P. falciparum,* would further aid the investigation of NPP structure.
This study therefore aimed to develop an osmotic lysis assay which would enable the determination of NPP functionality in infected rodent erythrocytes. A series of compounds were screened on infected rodent erythrocytes for their ability to selectively cause lysis. Guanidinium hydrochloride was found to effectively lyse infected erythrocytes with uninfected cells unaffected. Subsequently, NPP inhibitors and synchronous population assays showed lysis was mediated by the NPPs.
The study then looked to modify the *P. berghei rhopH1/clag* gene, such that it expressed *P. falciparum* c-terminal region to reveal if *clag* gene functionality is conserved across the two species. Transgenic parasites were generated with positive verification of genomic modification, proposing the conservation of *clag* gene function across species. This work shows that *clag* genes can be studied in rodent model *P. berghei* with a higher confidence of relatability to *P. falciparum* parasite function.

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Promoting Extrinsic Apoptosis to Diminish Latent HIV Reservoir in Vivo

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Human Immunodeficiency Virus (HIV) poses a significant global health threat [1]. The development of combination antiretroviral therapy (ART) has led to suppression of viremia in patients and a substantial improvement of the health condition of people living with HIV (PLWH) [2]. However, the existence of a latent HIV reservoir (cells harbor replication-competent HIV provirus) leads to HIV rebound following ART interruption [3]. This requires patients to take life-long medication and suffer from side-effects and chronic inflammation [4]. A functional cure for HIV is urgently needed.

Here we hypothesize that the HIV reservoir persists for a long time partly because they resist to cell death, which may serve as a therapeutic target to diminish the HIV reservoir. To study HIV infection *in vivo*, we injected human hemopoietic CD34+ stem cells in 48 hours-old immunodeficient (NSG) mouse pups to generate a humanized immune system mouse model (HIS mice) [5]. In this model, we used Xevinapant (Debio 1143), a SMAC-Mimetic that induces extrinsic apoptosis and has undergone multiple clinical trials as cancer treatment, to treat HIV-infected, viremia suppressed HIS mice. During the experiment, the percentage of human immune cells, CD4 T cell and CD8 T cell count was being monitored by flow cytometry, and HIV viral load was quantified by qPCR. We evaluated our treatment's efficacy by analytical treatment interruption (ATI), in which we interrupt all treatment including ART and compared the time to HIV rebound between groups.

Our results indicate that this HIS mice model can successfully recapitulated essential features of HIV infection in human. Notably, during ATI, we witnessed a delay in HIV rebound after 4 weeks of Xevinapant treatment compared to vehicle. These results suggest that exploiting cell death by extrinsic apoptosis may be an effective way to target the latent HIV reservoir.

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Exploring the Potential of *Plasmodium falciparum* Exportin-1 as a Target for 2- Aminobenzimidazoles through Nuclear Fractionation Coupled Proteomics

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The rapid emergence of artemisinin resistance highlights the urgent imperative for new antimalarials. A novel drug class 2-aminobenzimidazoles (ABIs) have exhibited remarkable potency against the erythrocytic stage of *Plasmodium falciparum*. Preliminary studies have identified *P. falciparum* exportin-1 (*Pf*XPO1), involved in nucleocytoplasmic export, as a potential ABI target. Notably, an ABI-resistant strain R1 revealed a H1061N point mutation within *Pf*XPO1. To validate *Pf*XPO1 as an ABI target, we developed a nuclear fractionation-coupled proteomics approach, probing nucleocytoplasmic transport between trophozoite stage parasites of the ABI-resistant line R1 and parent line DD2.

Analysis of nuclear fractions identified 85 significantly different proteins between R1 and the parent line DD2, whereas 69 proteins showed significant disparity in cytosolic fractions. Gene ontology analysis revealed perturbed proteins involved in DNA transcription, gene expression process, cellular oxidant detoxification and localization to cellular compartments such as RNA polymerase II. Notably, some perturbed proteins contained nuclear export signal (NES) binding regions for export through *Pf*XPO1.

These findings support the role of *Pf*XPO1 in nucleocytoplasmic transport of transcription-associated proteins. Further investigations aim to elucidate the mechanism of ABI resistance induced by H1061N mutation, by comparing nuclear and cytoplasmic proteome changes in R1 and DD2 parasite lines upon ABI treatment.