26–27 October 2023
Peter Doherty Institute
Parkville, Australia

Program

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Program at a Glance

Day 1: Thursday 26th

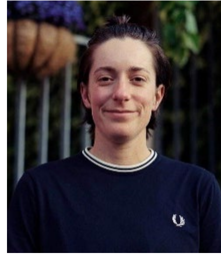
08:45 - 09:20	Registration
09:20 - 09:30	Welcome and Housekeeping
09:30 - 10:20	**Plenary Session 1: Dean Goodman**
10:20 - 10:45	**Short Talks I**
10:45 - 11:10	Morning tea
11:10 - 12:30	**Long Talks I: Techniques**
12:30 - 14:00	**Poster Session I** & Lunch
14:00 - 15:20	**Long Talks II: Host-Pathogen Interactions**
15:20 - 15:40	Afternoon tea
15:40 - 17:00	**Long Talks III: Immunology**
17:30	Conference Social Event at Bobbie Peels

Day 2: Friday 27th

10:00 - 11:05	**Long Talks IV: Drugs**
11:05 - 11:30	Morning tea
11:35 - 12:40	**Long Talks V: Immunology 2**
12:40 - 13:00	**Short Talks II**
13:00 - 14:30	**Poster Session II** & Lunch
14:30 - 15:50	**Long Talks VI: Molecular and Cellular Biology**
15:50 - 16:05	**Short Talks III**
16:05 - 16:30	Afternoon tea
16:30 - 16:50	Wrap up & Awards

Organising Committee

Kirsty McCann
Deakin University



Emma McHugh
The University of Melbourne

Carlo Giannangelo
Monash University

Mary-Lou Wilde
The University of Melbourne

Jerzy Dziekan
Walter Eliza Hall Institute

Charles Narh
Deakin University

Madeline Dans
Walter Eliza Hall Institute

Location

Peter Doherty Institute

Peter Doherty Institute Auditorium positioned near the main entrance of the building.

Address: 792 Elizabeth Street, Melbourne, 3000

09:20 - 09:30 Acknowledgment of Country
Welcome and Housekeeping

09:30 - 10:20 **Plenary Session 1: Dean Goodman**
"It's not in the blood!" New tools targeting malaria parasites during transmission
Sponsored by MIPS
Session chair: Emma McHugh

10:20 - 10:45 **Short Talks I**
Session chair: Coralie Boulet

ST1 **Geospatial mapping of malaria and anaemia among mothers and Infants in Papua New Guinea**
Catherine Ives - Burnet Institute, Australia & Monash University, Australia

ST2 **Developing mRNA vaccines targeting malaria blood stages**
Adam Thomas - Burnet Institute, Australia

ST3 **The Impact of Seasonal Malaria Chemoprevention (SMC) on *P. falciparum* Population diversity**
Zahra Razook - Centre for Innovation in Infectious Disease and Immunology Research (CIIDIR), Institute of Mental and Physical Health and Clinical Translation (IMPACT) and School of Medicine, Faculty of Health, Deakin University, Geelong, Victoria, Australia & Life Sciences Discipline, Burnet Institute, Melbourne, Victoria, Australia

ST4 **Can we target the mosquito stages of *Plasmodium* with 'drugs' to reduce transmission?**
Sarah Farrell - The University of Melbourne, Victoria, Australia

10:45 - 11:10 **Morning tea**

11:10 - 12:30 **Long Talks I: Techniques**
Session chairs: Carlo Giannangelo & Adedoyin Akinware

T1 **A comprehensive map of protein-protein interactions in the malaria parasite**
Christopher A. MacRaild - Monash Institute of Pharmaceutical Sciences, Monash University, Parkville.

T2 **Resistance Risk for the Antimalarial Drug Cabamiquine across Infection Models**
Eva Stadler - The Kirby Institute, UNSW Sydney, Kensington, NSW, Australia

T3 **Using multi-omics to unravel amino acid utilisation of blood stage *Plasmodium falciparum* malaria**
Ghizal Siddiqui - Drug Delivery Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia

T4 **Whole-genome CRISPR/Cas9 screen to identify host factors involved in *Plasmodium falciparum* liver infection**
Eva Hesping - Walter and Eliza Hall Institute of Medical Research, VIC, Australia & Department of Medical Biology, University of Melbourne, Parkville, VIC Australia

T5 **Quantitative 4D-imaging of cytoskeletal breakdown, tight junction formation and calcium signalling during *P. falciparum* invasion of erythrocytes**
Niall Geoghegan - Walter and Eliza Hall Institute of Medical Research, VIC, Australia

12:30 - 14:00 **Poster Session I** & Lunch

14:00 - 15:20 **Long Talks II: Host-Pathogen Interactions**
Sponsored by ACREME
Session chairs: Paolo Bareng & Mitchell Trickey

T6 **The PHIST protein PF3D7_0532300 interacts with the RBC membrane skeleton and modulates knob formation**
Mohini Shibu - Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC & Infectious Diseases and Immune Defence Division, Walter and Eliza Hall Institute, Melbourne, VIC

T7 **Cancelled**

T8 **Identification of novel malaria proteins involved in parasite-host cell interactions**
Christina Dizdarevic - Institute for Mental and Physical Health and Clinical Translation (IMPACT), School of Medicine, Deakin University, Waurn Ponds VIC

T9 **Nanobodies targeting malaria transmission-blocking candidate Pfs48/45**
Frankie M. T. Lyons - Infectious Diseases and Immune Defence Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, 3052, VIC, Australia

T10 **Blocking interactions of *Plasmodium falciparum* 6-cysteine proteins using inhibitory nanobodies**
Melanie H. Dietrich - The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia & Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia

15:20 - 15:40 Afternoon tea

15:40 - 17:00 **Long Talks III: Immunology**
Session chairs: Charles Narh & Damian Oyong

T11 **Defining targets and mechanism of action of immunity against *Plasmodium vivax* malaria.**
Rosy Cinzah - Burnet Institute, Melbourne, Australia & Department of Medicine, Microbiology and Immunology and Infectious Diseases, University of Melbourne, Melbourne, Australia

T12 **Functional antibodies to merozoite antigens are better maintained by adults than children in a longitudinal cohort study in Africa**
Joshua Waterhouse - Burnet Institute, Melbourne, Australia & Department of Microbiology, Monash University Melbourne, Australia

T13 **Association of novel potential IgG3 allotype with malaria in children from Sepik region of Papua New Guinea**
Maria Saeed - Department of Infectious Diseases, The Peter Doherty Institute for Infection and Immunity, University of Melbourne

T14 **Identification of monoclonal antibodies against *Plasmodium vivax* Apical Membrane Antigen 1 that promote functional responses**
Chiara Drago - Burnet Institute, Melbourne, Australia & Monash University, Clayton, Australia

T15 **Distinct neutrophil phenotypes in a population living in a malaria endemic area**
Sandra Chishimba - Burnet Institute, Life Sciences, Melbourne, VIC, Australia & Department of Medicine at Royal Melbourne Hospital, Melbourne Medical School, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, VIC, Australia

17:30 Conference Social Event at Bobbie Peels
Sponsored by Bio21 Institute

10:00 - 11:05	**Long Talks IV: Drugs** Session chairs: Madeline Dans & Peiyuan (Annie) Luo
T16	**Stopping malaria parasites before they StART: aryl-acetamide compound MMV006833 inhibits lipid transfer and ring development** Coralie Boulet - Burnet Institute, Melbourne, VIC 3004, Australia
T17	**MMV687794 impairs blood-stage *Plasmodium falciparum* invasion by perturbing lysophospholipids** Dawson B. Ling - Malaria Virulence and Drug Discovery Group, Burnet Institute, Melbourne, Victoria, Australia & Microbiology & Immunology, The University of Melbourne, Parkville, Victoria, Australia
T18	**Chemical-genetics using substrate peptidomimetics defines their on-target activity for the essential malaria aspartyl protease, plasmepsin V** Wenyin Su - Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC 3052, Australia
T19	**Assessing parasite clearance and parasite viability as measures of antimalarial drug activity in pre-clinical and early clinical trials** David S. Khoury - Kirby Institute, University of New South Wales, Kensington, New South Wales, Australia
11:05 - 11:30	Morning tea
11:35 - 12:40	**Long Talks V: Immunology 2** **Sponsored by the University of Melbourne** Session chairs: Charles Narh & Alessia Hysa
T20	**Investigating strategies to improve vaccine-induced antibody immunity** Jessica L. Horton **-** Burnet Institute, Melbourne & Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville
T21	**Vg1 gd T Cells regulate early CD8 T cell activation in response to Radiation Attenuated Sporozoite Vaccination** Declan Murphy - Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC
T22	**FcγRIIIA binding antibody on the surface of *Plasmodium falciparum* infected red blood cells is associated with protection** Elizabeth Aitken - Department of Infectious Diseases, Peter Doherty Institute, University of Melbourne, Australia
T23	**Investigation of *P. vixax* Elimination Using MDA** Md Nurul Anwar - School of Mathematics and Statistics, The University

of Melbourne, Parkville, Australia

12:40 - 13:00 **Short Talks II**
Session chair: Niall Geoghegan

ST5 **Enhancing Access to Radical Cure in Cambodia: Assessment of the Implementation of the STANDARD G6PD Test for *Plasmodium vivax* Case Management**
Sarah A. Cassidy-Seyoum - Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Australia

ST6 **Distinct immune responses associated with vaccination status and protection outcomes after malaria challenge**
Damian Oyong - Center for Global Infectious Disease Research (CGIDR), Seattle Children's Research Institute, Seattle, Washington, United States of America & Burnet Institute, Melbourne, Victoria, Australia

ST7 **The economic burden of zoonotic *Plasmodium knowlesi* malaria compared to human-only species on households in Sabah, Malaysia**
Patrick Abraham - Health Economics Unit, Centre for Health Policy, Melbourne School of Population and Global Health, The University of Melbourne & Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University

ST8 **Investigating the role of TRX2 in trafficking malaria virulence proteins**
Joyanta K. Modak - School of Medicine, Deakin University, Geelong, Australia & The Institute for Mental and Physical Health and Clinical Translation, Deakin University, Geelong, Australia

13:00 - 14:30 **Poster Session II** & Lunch

14:30 - 15:50 **Long Talks VI: Molecular and Cellular Biology**
Session chairs: Mary-Lou Wilde & Dawson Ling

T24 **Cytostome formation in artemisinin resistant *Plasmodium* parasites**
Long K. Huynh - Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville

T25 **Tracking inheritance of the apicoplast and mitochondrion in *P. berghei***
Sophie Collier - School of BioSciences, The University of Melbourne, Parkville

T26 **Serological markers predict *Plasmodium vivax* relapses in a returning Indonesian soldier cohort**
Lauren Smith - Walter and Eliza Hall Institute, Melbourne, Australia & Department of Medical Biology, University of Melbourne, Melbourne,

Victoria, Australia

T27 **Doxycycline inhibits both apicoplast and mitochondrial translation in *Plasmodium falciparum***
Michaela Bulloch - Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville

T28 **Functional characterisation of the GAPM proteins in asexual and sexual stage development**
Katrina Larcher - Department of Infectious Diseases, Doherty Institute, University of Melbourne, Victoria & Infectious Diseases and Immune Defence Division, Walter and Eliza Hall Institute, Victoria

15:50 - 16:05 **Short Talks III**
Session chairs: Lakvin Fernando

ST9 **Exploring the Potential of *Plasmodium falciparum* Exportin-1 as a Target for 2- Aminobenzimidazoles through Nuclear Fractionation Coupled Proteomics**
Yunyang (Eileen) Zhou - Monash Institute of Pharmaceutical Sciences, Drug delivery, disposition and dynamics, Global Health Therapeutic Program Area, Monash University, Parkville

ST10 **Host erythrocyte and reticulocyte cell signalling during infection with *Plasmodium* spp.**
Mohammad Jamiu Shuaib - School of Health and Biomedical Sciences, RMIT University, Bundoora VIC, Australia

16:05 - 16:30 Afternoon tea

16:30 - 16:50 Wrap up & Awards

Talk Abstracts

Day 1 — Thursday 26th October

Session chair: Coralie Boulet

ST1 Geospatial mapping of malaria and anaemia among mothers and infants in Papua New Guinea

Catherine Ives (1,2), Michelle J. L. Scoullar (1), Linda Reiling (1), Pele Melepia (1), Elizabeth Peach (1), Eliza M. Davidson (1,3), Ruth Fidelis (1), Hadlee Supsup (3), Priscah Hezeri (1), Wilson Philip (1), Dukduk Kabiu (1), Kerryanne Tokmun (1), Rose Suruka (1), Peter M Siba (5), William Pomat (6), Benishar Kombut (1,6), Brendan S. Crabb (1,2,4), Philippe Boeuf (1), Freya J.I Fowkes (1,2,4), Chris J. Morgan (1,4,7), James G. Beeson (1,2,4)

1. Burnet Institute, Australia.
2. Monash University, Australia.
3. East New Britain Provincial Health Authority, Kokopo, Papua New Guinea.
4. University of Melbourne, Australia.
5. Center for Health Research and Diagnostics, Divine Word University, Madang, Papua New Guinea.
6. Papua New Guinea Institute of Medical Research, Papua New Guinea.
7. Jhpiego, a Johns Hopkins University affiliate, Baltimore, USA.

Papua New Guinea (PNG) has the highest incidence of malaria in the Western Pacific. Pregnant women with malaria are at higher risk of mortality and poor outcomes for their newborns. Anaemia is also prevalent, partly due to malaria. This study describes the geospatial distribution of these conditions, and low birthweight, among women and their infants from pregnancy to the first 12-months post-partum, in East New Britain, PNG. 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 birthweight differences. Maternal malaria prevalence at ANC was 12.3%. This increased to 37.6% at 12-months. Maternal anaemia was highly prevalent. The peak was 83.2% at ANC while the lowest was 69.5% at 6-months. The greatest density of cases occurred in the central region, though major hotspots for each condition differed. In infants, anaemia was highly prevalent, 59.8% at 6 months and 57.4% at 12 months, and widespread. Low birthweight prevalence was 12.1% and evenly distributed, with one possible hotspot. There is a high burden of disease among women and infants and geospatial mapping provides insights into how future initiatives could target interventions in the highest burden areas.

ST2 Developing mRNA vaccines targeting malaria blood stages

Adam Thomas (1), James Beeson (1), Lee Yeoh (1), Timothy Ho (1)

1. Burnet Institute, 85 Commercial Rd, Melbourne VIC 3004.

The mRNA vaccine technology provides an efficient platform for multi-antigen formulations and unlike other platforms, it is suited for malaria vaccine development. Multiple antigens in single vaccine formulations are required to improve vaccine efficacy and overall protection, similar to the bivalent SARS-CoV-2 booster, and the multi-strain seasonal influenza vaccines. This allows for a rapid response to address emerging strains and produce a large number of doses. The antigens are expressed in vivo in host cells and displayed on the surface for immune system recognition. We have partnered with Monash Institute of Pharmaceutical Sciences and Moderna to explore the mRNA vaccine platform for malaria. This is a complex disease with different parasite life-cycle stages that are targets for vaccine development. We are using the blood-stage Merozoite Surface Protein 2 (MSP2) as a model antigen for this mRNA platform. This protein showed promise in early clinical trials whereby multiple alleles of recombinant MSP2 had induced antibody-dependant complement-mediated and cellular inhibition activity of the parasite. We designed MSP2 mRNA constructs and successfully expressed them in human HEK293 cells, following which we plan to package the mRNA in Lipid Nanoparticles (LNPs) delivery vehicles for animal model and vaccine efficacy studies. This platform will allow us to use blood-stage merozoite antigens that could protect against clinical illness and mortality, and combine them with antigens from other parasite stages, such as the sporozoite antigen CSP. The mRNA approach could surpass the current recombinant RTS,S Virus-Like Particle vaccine which has modest and short-lived efficacy.

ST3 The Impact of Seasonal Malaria Chemoprevention (SMC) on *P. falciparum* Population diversity

Zahra Razook (1,2), Kirsty McCann (1,2), Somya Mehra (1,2), Bourama Traore (3), Mahamoudou Touré (3), Daouda Sanogo (3), Fousseyni Kané (3), Drissa Konaté (3), Soumba Keita (3), Seydou Doumbia (3), Mahamadou Diakité (3), Alyssa E. Barry (1,2)

1. Centre for Innovation in Infectious Disease and Immunology Research (CIIDIR), Institute of Mental and Physical Health and Clinical Translation (IMPACT) and School of Medicine, Faculty of Health, Deakin University, Geelong, Victoria, Australia.
2. Life Sciences Discipline, Burnet Institute, Melbourne, Victoria, Australia.
3. West African International Center for Excellence in Malaria Research, University Clinical Research Center, University of Sciences, Techniques and Technologies of Bamako, Mali.

Seasonal Malaria Chemoprevention (SMC) has been proven to be effective in high transmission areas of seasonal malaria in sub-Saharan regions. We conducted a pilot study as part of the ICEMR programme to evaluate the impact of SMC intervention on parasite genetics by hypothesizing that SMC would lead to a reduction in parasite genetic diversity. We examined samples from cross-sectional surveys collected before and during the implementation of SMC from the Dangassa, Mali in 2015. Genotyping was done using SNP barcoding of 81 samples collected between 2013 – 2019. The barcodes included 176 biallelic SNP markers. We obtained 69 high quality genotypes and identified 80 polymorphic SNPs to measure patterns of population diversity and structure. We then compared parasite populations before and during SMC using nucleotide diversity statistics, Principal Co-ordinate Analysis and pairwise Identity-by-decent to observe population diversity and structure and parasite relatedness. Preliminary analyses revealed no clear genetic differentiation, reduced diversity or increased clustering patterns comparing the parasite populations collected before and during treatment. The study demonstrated a limited impact of the SMC intervention on the parasite population suggesting that further sustained control efforts will be needed to interrupt transmission. The study also demonstrates the utility of this SNP barcode for parasite genomic surveillance in West Africa.

ST4 Can we target the mosquito stages of *Plasmodium* with 'drugs' to reduce transmission?

Sarah Farrell (1), Anton Cozijnsen (1), Vanessa Mollard (1), Papireddy Kancharla (2), Rozalia A. Dodean (2), Jane Kelly (2), Christopher D. Goodman (1), Geoffrey I. McFadden (1).

1. The University of Melbourne, Victoria 3010 Australia.
2. Portland State University, Portland, Oregon, 97201 United States.

A decade-long decline in malaria cases has plateaued, primarily due to parasite drug resistance and mosquito resistance to insecticides used in bed nets and surface sprays. Here, we explore an innovative control strategy using anti-malarial compounds to target *Plasmodium* during the mosquito stages. This strategy has the potential to reduce the risk of drug resistance emerging due to the relatively small population of parasites within the mosquito. We screened a range of parasiticidal compounds by feeding them to mosquitoes. Multiple unique strategies were used to validate mosquito feeding behaviour. Different compounds were able to target specific stages of *P. berghei* development in the mosquito. Atovaquone-treated mosquitoes hosted fewer sporozoites, consistent with atovaquone blocking crucial mitochondrial electron transport in insect stages of the parasite. Borrelidin, a tRNA synthetase inhibitor, was able to significantly reduce sporozoite numbers. Azithromycin, an antibiotic targeting apicoplast protein synthesis, significantly lowered sporozoite infectivity in mice. Finally, a novel lead compound targeting the electron transport chain, T111, reduced sporozoite numbers of the human malaria parasite *P. falciparum*. Targeting mosquito staged parasites via baits or surfaces opens the option of using potent parasiticidal compounds that failed to meet the exacting standards required of human antimalarial drugs and would therefore improve malaria control for minimal cost.

Session chairs: Carlo Giannangelo & Adedoyin Akinware

T1 A comprehensive map of protein-protein interactions in the malaria parasite

Christopher A. MacRaild (1), Ghizal Siddiqui (1), Megan E. Zadow (2), Danny W. Wilson (2), Darren J. Creek

1. Monash Institute of Pharmaceutical Sciences, Monash University, Parkville.
2. Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide.

The parasites that cause malaria have been committed to an intracellular parasitic lifecycle for as much as a billion years, evolving a highly specialised and divergent biology to accommodate their complex lifecycle. Despite their place as one of the most important human pathogens, much of this biology remains poorly understood. We are applying new proteomic approaches including Protein Correlation Profiling (PCP) and Cross-Linking Mass Spectrometry (XLMS) to *P. falciparum*, to establish a comprehensive map of protein-protein interactions in this species. PCP and XLMS are highly complementary approaches to the study of protein interactions at proteome scale, but they have not been widely applied together, or outside of model organisms. We have developed new machine-learning protocols to enable the integration of our PCP and XLMS datasets with existing data, resulting in a complexome that currently covers almost half of the observable *P. falciparum* asexual blood- stage proteome. Our complexome confirms the presence of a range of eukaryotic protein complexes, many that have not been experimentally characterised in *Plasmodium*. We also identify conserved eukaryotic processes that have diverged in *Plasmodium*. For example, we identify highly divergent components of the CMG replicative helicase, illuminating the unusual mechanisms by which DNA replication is regulated during schizogony. Further, we identify protein complexes unique to *Plasmodium*, including novel complexes with diverse roles including host cell remodelling and translational and transcriptional control. This work significantly expands the known *P. falciparum* complexome, and provides insights that can be exploited to better understand parasite biology and to identify new therapeutic targets.

T2 Resistance Risk for the Antimalarial Drug Cabamiquine across Infection Models

Eva Stadler (1), Mohamed Maiga (2), Lukas Friedrich (3), Vandana Thathy (4,5), Claudia Demarta-Gatsi (6), Antoine Dara (2), Fanta Sogore (2), Josefine Striepen (4,10), Claude Oeuvray (6), Abdoulaye A. Djimdé (2), Marcus C.S. Lee (7,8), Laurent Dembélé (2), David A. Fidock (4,5,9), David S. Khoury (1), Thomas Spangenberg (6)

1. The Kirby Institute, UNSW Sydney, Kensington, NSW 2052, Australia.
2. Université des Sciences, des Techniques et des Technologies de Bamako (USTTB), Faculté de Pharmacie, Malaria Research and Training Center (MRTC), Point G, PB1805 Bamako, Mali.
3. Medicinal Chemistry & Drug Design, Global Research & Development, Discovery Technologies, Merck Healthcare, 64293 Darmstadt, Germany.
4. Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY 10032, USA.
5. Center for Malaria Therapeutics and Antimicrobial Resistance, Columbia University Irving Medical Center, New York, NY 10032, USA.
6. Global Health Institute of Merck, Ares Trading S.A., 1262 Eysins, Switzerland, affiliate of Merck KGaA, Darmstadt, Germany.
7. Wellcome Sanger Institute, Wellcome Genome Campus, CB10 1SA Hinxton, UK.
8. Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, DD1 4HN Scotland, UK.
9. Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, NY 10032, USA.
10. Present address: Weill Cornell Medical College, New York, NY 10021, USA.

Numerous pre-clinical tools exist for assessing how readily resistance may emerge to a given candidate antimalarial compound. However, it is not clear how well these pre-clinical measures predict resistance in a human treatment setting. Here, we explore data from in vitro studies of laboratory strains and clinical isolates, humanized mouse models, and volunteer infection studies of the candidate antimalarial compound cabamiquine, a *Plasmodium*-specific eukaryotic elongation factor 2 inhibitor. We examine the propensity of this agent to select for resistant *Plasmodium falciparum* parasites and show that the different infection models provide a wide range of frequencies of resistant mutants. We explored if this variability could be explained due to stochastic differences arising from experiment design choices, such as the number of parasites in an animal at the time of treatment. Using simulations of a stochastic mathematical model, we find that much of the observed variability in resistance potency across different preclinical models is predictable based on only the mutation rate, setup of the experiment, and fitness cost of resistant mutants. Overall, we show that pre-clinical infection models were highly predictive of resistance frequency in early human trials with cabamiquine. Thus, this work helps to understand the potential resistance risks for a given drug and translation of the resistance risk across different infection models.

T3 Using multi-omics to unravel amino acid utilisation of blood stage *Plasmodium falciparum* malaria

Ghizal Siddiqui (1), Christopher A MacRaild (1), Carlo Giannangelo (1), Katherine E Ellis (1), Amanda De Paoli (1), Peter Scammells (2), Sheena McGowan (3), Darren J Creek (1)

1. Drug Delivery Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia.
2. Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, 3052, Australia.
3. Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Victoria, 3800, Australia.

Plasmodium falciparum degrades large amount of host haemoglobin (Hb) during its red blood cell stage (RBC) and salvage the resulting amino acids (aas) for its own use. The parasite also actively takes up aas from its external environment, including isoleucine, which is absent from Hb and the parasite is unable to synthesise de novo. This requirement for aa scavenging, especially Hb digestion, is a point of critical vulnerability for the parasite, and is targeted by many antimalarial drugs. We used a multi-omics workflow consisting of metabolomics, fluxomics (using stable-isotope-labelled aas) and peptidomics to identify the source of aas (Hb or external) and their utilisation into critical and unique metabolic processes of the parasite. We demonstrated that related aas (charged, uncharged and hydrophobic) have similar uptake profiles within the parasite, suggesting the presence of previously uncharacterised aa transporters. Inhibition of Hb digestion with a range of protease inhibitors resulted in unique signatures of peptide intermediates, however, aa levels were unchanged. Metabolomics analysis of isotope incorporation from labelled aas revealed activity in essential parasite metabolic reactions, and also in unexpected metabolic pathways. This included the identification of several labelled metabolites in the methionine salvage pathway that was not known to be present. Together, our multi-omics approach provides detailed analysis of aa utilisation by the parasite.

T4 Whole-genome CRISPR/Cas9 screen to identify host factors involved in *Plasmodium falciparum* liver infection

Eva Hesping (1,2), Lisa Verzier (1,2), Marcel Doerflinger (1,2), Marco Herold (1,2), Justin Boddey (1,2)

1. Walter and Eliza Hall Institute of Medical Research, Brunswick East, VIC, Australia.
2. Department of Medical Biology, University of Melbourne, Parkville, VIC Australia.

The typical clinical symptoms of malaria arise when *Plasmodium* parasites infect red blood cells. However, the complex parasite journey begins much earlier in the infection.

The typical clinical symptoms of malaria arise when *Plasmodium* parasites infect red blood cells. However, the complex parasite journey begins much earlier in the infection process. After an infected mosquito bites a human, sporozoites are injected into the skin and first travel from the dermis to blood vessels and then to the liver. This initial step of malaria infection involves cell traversal, where the parasites migrate through host tissues by entering and egressing host cells, rupturing them upon exit. Interfering with this crucial step in *Plasmodium* infection could prevent malaria and the onward transmission of malaria parasites.

To shed light on the molecular interactions underlying this process, we developed a research pipeline combining a whole-genome CRISPR/Cas9 knock-out screen in human hepatocytes with a positive selection cell traversal assay to study host factors involved in *Plasmodium falciparum* cell traversal. Following positive selection, the pipeline employs gene sequencing around the sgRNA target site and bioinformatics analyses to identify significantly enriched genes post traversal. Encouragingly, several hit genes identified in the screen were already known to play roles in *Plasmodium* liver infection, validating the approach.

Hit genes were further validated using arrayed CRISPR/Cas9 driven gene disruption followed by fluorescence-based *P. falciparum* sporozoite traversal assays, which led to the identification of novel putative host traversal factors. Unravelling essential host factors in the first step of the malaria lifecycle by the most virulent human malaria parasite holds promise for advancing knowledge of sporozoite-hepatocyte interactions and migration patterns of related pathogens and may lead to interventions that limit malaria infection and onward transmission.

T5 Quantitative 4D-imaging of cytoskeletal breakdown, tight junction formation and calcium signalling during *P. falciparum* invasion of erythrocytes

Niall Geoghegan (1,2), Cindy Evelyn (1), Aurelie Dawson (1,2), Danushka Marapana (1,2), Alan Cowman (1,2), Kelly Rogers (1,2)

1. Walter and Eliza Hall Institute of Medical Research, VIC, Australia.
2. The University of Melbourne.

Understanding the complex and dynamic biology of Malaria at all stages of development is challenging for numerous reasons. The parasites are micron sized protozoans with complex machinery designed to efficiently infect a variety of host cell types. They are by their very nature inherently dynamic, as they need to traverse multiple host cells and tissues to efficiently replicate and propagate through their development. In this context, light microscopy offers the best solution to study the various life cycle stages in as close to physiological context as possible. This too, however, comes with limitations owing to the parasite's delicate and fragile nature when exposed to high light doses.

Invasion of red blood cells by is a dynamic event governed by numerous ligand receptor interactions and a delicate balance in host cell biophysical conditions. Understanding this process on its own terms is only possible by light microscopy. In this presentation I will outline the 4D analytical tool set, using lattice light-sheet microscopy, we have devised to understand how the parasite subverts the host membrane-cytoskeletal complex. Our tools shed new light on key steps in the invasion process such as: cytoskeletal breakdown, tight junction formation, membrane remodelling and calcium signalling.

Session chairs: Paolo Bareng & Mitchell Trickey

T6 The PHIST protein PF3D7_0532300 interacts with the RBC membrane skeleton and modulates knob formation

Mohini A. Shibu (1,3), Gerald J. Shami (1), Leann Tilley (1), Matthew W. A. Dixon (2,3)

1. Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC 3010.
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A key pathophysiology of malaria is the ability of infected RBC to adhere to the endothelial ligands of the host. A RBC infected by *P. falciparum* develops visible protrusions at the surface of the cell called knobs. The knob consists of a spiral protein scaffold of unknown composition that is closely associated with the RBC membrane skeleton and is capped by the knob-associated histidine-rich protein (KAHRP) which forms around the structure. Despite the importance of this structure to the presentation of the major virulence antigen PfEMP1 and severe disease states, little is known about its protein composition or the proteins required for its assembly. To investigate the composition of the knobs we performed immuno-precipitation experiments, using a cell line expressing a GFP tagged KAHRP. These experiments identified a number of candidate genes from the PHISTb family of exported proteins. In our work we show that PF3D7_0532300 (5323) localises to the RBC membrane skeleton. Knockout experiments show that deletion of this gene leads to large extended, lobed structures and smaller vesicle-like structures at the RBC membrane. Examination of these abnormal structures using super-resolution microscopy shows a reorganisation of RBC membrane skeleton components. We also show that 5323 interacts with host myosin and that this interaction is essential for normal knob formation. The data suggests a possible role for 5323 in facilitating knob organisation and formation.

T8 Identification of novel malaria proteins involved in parasite-host cell interactions

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Malaria is caused by protozoan parasites of the *Plasmodium* genus, which invade and remodel erythrocytes to grow and obtain essential nutrients. One organelle mediating invasion 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 roles of rhoptry proteins. Proximity labelling is a novel technique in proteomics that can identify proteins localising to a particular cellular region. In proximity labelling, an enzyme is fused to a gene to label nearby proteins. One such enzyme, TurboID, 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 rhoptry proteins. This research describes the development of TurboID-fused CERLI, which localises to the rhoptry cytoplasmic face. Mass spectrometry identified 129 proteins in CERLI-TurboID parasites, 14 of which are known rhoptry proteins. Enriched proteins localise to apical cellular compartments or to membranous structures, and include proteins involved in vesicular transport. Identification of rhoptry proteins provides the basis for understanding their trafficking through the secretory pathway and evaluation of their potential as novel therapeutic targets, which are desperately needed due to rising drug resistance of parasites.

T9 Nanobodies targeting malaria transmission-blocking candidate Pfs48/45

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Malaria transmission occurs when a female Anopheles mosquito takes a blood meal from an infected host, and *Plasmodium* gametocytes are taken up into the mosquito midgut. Here they undergo sexual reproduction to produce sporozoites that migrate to the mosquito's salivary glands, ready to infect another host. The mosquito stages are a major bottleneck in the *Plasmodium* life cycle when parasite numbers are low, representing a promising opportunity for intervention.

Malaria transmission can be blocked by inducing or administering antibodies that inhibit essential sexual stage antigens. A major target of transmission-blocking interventions is the 6-cysteine protein P48/45. P48/45 is expressed on the surface of gametocytes and gametes and is essential for male fertility, with knock-out males unable to attach to and fertilise female gametes. Recognition of P48/45 by human sera correlates with the ability of sera to block parasite transmission and antibodies against P48/45 have transmission-blocking activity. A recent trial of the most potent transmission-blocking antibody to date, TB31F, has demonstrated the potential of monoclonals as transmission-blocking prophylactics.

We have generated first collection of nanobodies against *Plasmodium falciparum* P48/45 and demonstrated specificity using western blotting, ELISA and bio-layer interferometry (BLI). We have isolated three high-affinity nanobodies that show transmission-reducing activity and are characterising the transmission-blocking potency of our nanobodies in standard membrane feeding assays. Competition BLI has confirmed our nanobodies bind a different epitope of P48/45 to TB31F and we are further defining this inhibitory epitope using X-ray crystallography to provide insights into the domains of P48/45 involved in parasite transmission.

T10 Blocking interactions of *Plasmodium falciparum* 6-cysteine proteins using inhibitory nanobodies

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The 6-cysteine protein family is one of the most abundant surface antigens of *Plasmodium* and expressed throughout the parasite life cycle. This protein family is conserved across *Plasmodium* species and play important roles in parasite fertilization and transmission, evasion of the host immune response and host cell invasion. Several 6-cysteine proteins are present on the parasite surface as hetero-complexes. To date it is not well understood how 6-cysteine proteins interact together or engage with host-cell proteins. We seek to understand the function and structural mechanisms of 6-cysteine proteins during the malaria parasite life cycle for development of novel anti-malarial interventions. We have solved numerous 6-cysteine protein crystal structures as single or tandem domain proteins, as well as in complex with their binding partners. Using an immunised nanobody platform, we have characterised the first collection of nanobodies to different 6-cysteine proteins. In particular, we are able to show that our nanobodies can block the transmission of the malaria parasite from mosquito to human. In our recent work we investigate interactions of large family members using a combination of advanced structural biology techniques and functional parasite assays. Together with our large panels of nanobodies we aim to further dissect the roles of the 6-cysteine proteins to contribute to a better understanding of this important family of proteins.

Session chairs: Isobel Walker & Damian Oyong

T11 Defining targets and mechanism of action of immunity against *Plasmodium vivax* malaria.

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The *P. vixax* circumsporozoite protein (PvCSP) is essential for establishing a successful infection and therefore a promising vaccine target. Antibodies play a key role in immunity against malaria and have been shown in *Plasmodium falciparum* to act through different mechanisms including complement-fixation and engaging Fc-receptors (FcyR) on immune cells. However, knowledge is limited on the functions of antibodies targeting *P. vixax* and PvCSP specifically. Therefore, we measured antibody magnitude (IgG) and function (complement-fixation and binding of FcyRI, FcyRIIa, FcyRIIIa/b) to full-length PvCSP, the central repeat region (CRR) of the two major allelic variants (PvCSP-VK210 and PvCSP-VK247), and 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 PvCSP alleles. We observed significant antibody responses against both variants of PvCSP, including the CRRs, the N- and C-terminal regions. And, antibodies showed a degree of allele-specificity. FcyRI binding was consistently high across all PvCSP regions tested, followed by FcyRIIIa/b binding. Whereas FcyRIIa binding, and complement-fixation were significantly lower. These results provide new insights into the important roles of different regions and variants of PvCSP as targets of *P. vixax* antibody responses including mechanisms of action involved. This knowledge will inform future PvCSP-based vaccine design.

T12 Functional antibodies to merozoite antigens are better maintained by adults than children in a longitudinal cohort study in Africa

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A highly efficacious malaria vaccine could greatly enhance our capacity to combat the disease, but the sole licensed vaccine (RTS,S) only provides modest, short-lived protection. A major hurdle in malaria vaccine development is our poor understanding of malaria antibody dynamics over time. Antibodies play a central role in naturally-acquired and vaccine-induced immunity, but there is limited understanding as to which targets and functions are better sustained over time by people living in malaria endemic regions. To address this knowledge gap, we defined functional antibody responses against two merozoite antigens (MSP2 & AMA1) from a 2-year longitudinal cohort in Malawi, where children and adults were sampled monthly and during any febrile episodes. Consistent with previous studies, adults displayed better protection from malaria illness, lower parasite levels during infection, and higher *Plasmodium*-targeting IgG than children. Excitingly, we make the novel observation that, in the absence of infection, adults are better than children at maintaining functional IgG that engage Fcγ-Receptors Ia and IIIa, which are key mediators of monocyte phagocytosis activity. Better maintenance of these functional antibodies may be an important factor in explaining why adults in endemic areas are less at risk of malaria illness. Understanding this higher maintenance may inform vaccine strategies to achieve longer-lasting vaccines, potentially including induction that engages Fcγ-Receptors.

T13 Association of novel potential IgG3 allotype with malaria in children from Sepik region of Papua New Guinea

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Malaria causes death and severe illness in children <5 years. Recent work has established the importance of malaria-specific IgG3 in malaria immunity. Antibody allotypes due to single nucleotide polymorphisms (SNPs) of IgG3-Fc regions can modulate IgG3 Fc-mediated functions. A novel IgG3 potential allotype, G3m29, was recently reported in pregnant women from the Sepik, Papua New Guinea, and shown to have enhanced affinity to FcγRIIa. We hypothesized that the presence of G3m29 protects from malaria in children. We used Sanger sequencing to study the sequences of CH2 and CH3 domains of IgG3, in a cohort of children in the the Sepik aged 1-3 years (N=203) whose malaria episodes were recorded over 18 months. Associations between exposure and outcomes were examined using linear regression. We identified SNPs and compared sequences to the references in immunogenetics (IMGT) database. 78% of the cohort were either heterozygous (n=82, 40%) or homozygous (n=77, 38%) for the potential allotype G3m29. We found a decrease in total number of *Plasmodium* infections in children with potential G3m29 allotype compared to non-G3m29 allotype carriers (β= -1.736, 95% CI [-3.39-0.079], p <0.05). This effect was most pronounced for *P. vixax* asymptomatic infections (β=-1.06, 95% CI [-2.01-0.12], p <0.05). G3m29 carriers had significantly lower levels of total IgG to *P. vixax* vaccine candidate proteins than non-G3m29 carriers. The potential G3m29 appears to protect against *P. vixax* infections.

T14 Identification of monoclonal antibodies against *Plasmodium vivax* Apical Membrane Antigen 1 that promote functional responses

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Monoclonal antibodies (mAbs) are a novel approach to malaria prevention and control. We generated mAbs specific to *P. vixax* (Pv) Apical Membrane Antigen 1 (PvAMA1), assessed their ability to initiate protective immune functions and quantified reactivity against AMA1 from other *Plasmodium* strains and species. Specific mAbs were identified as promising leads for further investigation.

Parasites expressing PvAMA1 were unable to invade erythrocytes in the presence of mAbs. These mAbs also exhibited high reactivity with various markers of humoral immunity, such as C1q complement protein and Fc-receptors, suggesting stimulation of downstream functional effects and immune activation. mAb #838839 was the strongest at binding to C1q and FcγRIIa, also binding considerably to FcγRI and FcγRIIIa. It was the best performer overall. Several species-transcending mAbs were identified by testing mAb binding to different recombinant AMA1 proteins. Two mAbs exhibited significant binding to *P. knowlesi* and *P. falciparum* AMA1. These mAbs may potentially confer cross-species protection, which would be beneficial in endemic areas where coinfections are especially prevalent.

To the best of our knowledge, this research is the first to illustrate that anti-PvAMA1 mAbs can mediate antibody effector mechanisms against Pv, suggesting that they may induce protective immune responses. These results provide in vitro proof-of-concept that mAbs may be an effective therapeutic to Pv.

T15 Distinct neutrophil phenotypes in a population living in a malaria endemic area

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Emerging data suggests that neutrophils may play a significant role in malaria immunity through antibody-mediated parasite clearance. However, limited data exists on their phenotypes relevant to immunity or functional changes during malaria. We characterized neutrophil phenotypes among residents of a malaria endemic area in Papua New Guinea by examining Fcγ-receptors (FcγR) expression which mediate opsonic phagocytosis, and other functional surface molecules. Participants included infected children (*P. falciparum* and *P. vixax*), and uninfected children and adults. Comparisons were also made with malaria-naïve adults. Neutrophils from *P. vixax* infected children had increased CD66b expression compared to uninfected children. FcγRI expression on neutrophils from infected children was increased compared to uninfected children and adults. Neutrophil proportions co-expressing FcγRIIa and FcγRI, and FcγRIIa and CD54 were higher in uninfected children compared to adults. Neutrophils from uninfected residents (adults and children) had increased FcγRI, CD54, and CD66b, decreased FcγRIIa and FcγRIII compared to malaria-naïve donors. Differences were also observed between males and females infected with *P. falciparum* but not among *P. vixax* infected. These results reveal potentially important phenotypic changes in neutrophils relevant to functional immunity to malaria and could help inform development of efficacious vaccines that harness neutrophil functions.

Talk Abstracts

Day 2 — Friday 27th October

Session chairs: Madeline Dans & Peiyuan (Annie) Luo

T16 Stopping malaria parasites before they StART: aryl-acetamide compound MMV006833 inhibits lipid transfer and ring development

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Efforts to eradicate malaria have stalled partly due to parasites developing resistance to antimalarials. Hence, drugs with novel modes of action are urgently needed. We recently identified an aryl-acetamide compound MMV006833 (M833) that stops *P. falciparum* parasites from growing inside erythrocytes after invasion. M833-resistant parasites contained mutations in the StART lipid transfer protein (PF3D7_0104200). Engineering these mutations into drug-sensitive parasites reproduced resistance to M833. Structure activity relationship studies improved the potency of the compound >30-fold, with the most potent analogue, W991, having a subnanomolar EC50. We demonstrated direct binding of W991 to StART using three different techniques, and demonstrated an absence of drug binding to mutant StART. Overall, our data confirms that StART is the biological target of M833 and analogues. To understand the mode of action of these compounds, invading merozoites were filmed by lattice light sheet microscopy. In the presence of StART inhibitors, merozoites were unable to transform into amoeboid rings. Exposing invading parasites to StART inhibitors for only 4 hours was sufficient to block ring development for at least 3 days. W991 also inhibited the development of oocysts in mosquitoes. Overall, we hypothesise that StART is involved in the establishment of the parasitophorous vacuole membrane and reorganisation of internal membranes needed for the growth of merozoites into rings.

T17 MMV687794 impairs blood-stage *Plasmodium falciparum* invasion by perturbing lysophospholipids

Dawson B. Ling (1,2), Madeline G. Dans (3), Greta E. Weiss (1), Zahra Razook (4,5), Somya Mehra (4), Christopher A. MacRaild (6), Darren J. Creek (6), Alyssa E. Barry (4,5), Brendan S. Crabb (1,2), Hayley E. Bullen (1,2), Paul R. Gilson (1,2)

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Responsible for parasite proliferation and symptomatic malaria, parasite invasion of red blood cells (RBCs) represents an attractive novel drug target. Our group discovered a compound that specifically blocked invasion from schizonts, MMV687794. Genomic analysis on MMV687794-resistant parasites unveiled mutations in an alpha/beta hydrolase enzyme containing a lysophospholipase (LysoPL) motif we termed ABH-83. To validate ABH-83 as the drug target, these mutations were inserted into wild-type parasites using CRISPR/Cas9, which recapitulated the MMV687794-resistant phenotype. An epitope tag and a GlmS riboswitch were also introduced into the parasites, enabling closer examination of the role(s) of ABH-83. By conducting a time-course western blot series on the transgenic parasites, the LysoPL ABH-83 is most highly expressed in schizonts, concordant with a role in invasion. ABH-83 has also been visualised by microscopy at the rhoptry surface, organelles that secrete important invasion-related proteins during RBC invasion. Using a western blot-based assay, reduced ABH-83 expression led to decrease rhoptry protein (Rh5) processing. Lipidomics data indicate MMV687794-treated schizonts have elevated lysophospholipids, an effect less pronounced in parasites with mutant ABH-83. These results suggest that ABH-83 is involved in rhoptry lipid metabolism vital for its functioning and, subsequently, efficient merozoite invasion of RBCs. Further lipidomic investigations aim to examine this.

T18 Chemical-genetics using substrate peptidomimetics defines their on-target activity for the essential malaria aspartyl protease, plasmepsin V

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The prevalence of resistance of malaria parasites against drugs in the field leads to a need for novel antimalarial discovery and development. Plasmepsin V is an aspartyl protease which is essential for the export of proteins from the parasite to the host erythrocyte during the asexual stage of parasite, making it an ideal target for novel antimalarial development. Peptidomimetics that mimic the substrate of plasmpesin V have been designed and shown to elicit parasite death via blocking protein export. While the peptidomimetics have been used to validate plasmepsin V as a bone fide antimalarial target, disparities between biochemical and parasite activity have questioned their on-target activity. Here, we generated a parasite line with reduced sensitivity to the peptidomimetics. A single nucleotide polymorphism (SNP) located in the plasmepsin V gene was identified through whole genome sequencing. Reverse genetics and biochemical assays using immunoprecipitated plasmepsin V were used to validate the resistance-causing SNP. This data was supported by methods such as cellular thermal shift assays that showed target engagement with plasmepsin V in parasites. This data supports the previous evidence that the peptidomimetics kill the malaria parasite by targeting plasmepsin V and further establishes plasmepsin V as a promising antimalarial drug target.

T19 Assessing parasite clearance and parasite viability as measures of antimalarial drug activity in pre-clinical and early clinical trials

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The rate of parasite clearance in a host after drug treatment is a common surrogate measure of antimalarial drug activity - particularly in preclinical and early clinical trials. However, it has been observed for short-acting drugs, such as artesunate, that parasite clearance occurs after the drug becomes undetectable in the host - suggesting a disconnect between when a drug is acting and when parasites are cleared. We recently developed an ex vivo regrowth assay to measure whether circulating parasites are viable. This revealed that parasites are rendered non-viable much faster than they are cleared from circulation. Thus, measuring parasite clearance after drug treatment in humans can underestimate the speed of drug activity. We have recently extended this work to assess parasite clearance and viability as measures of drug activity in pre-clinical mouse models and human studies, and further validate our viability assay. We demonstrate that after artesunate treatment in humanised mice, parasite viability declined much faster than total parasitemia, mimicking the results observed in humans. However, modelling revealed some differences in the PK-PD relationship in mice and humans, which are not explained by viability. Direct analysis of parasite viability using limiting dilution confirmed the validity of our ex vivo regrowth assay. This work highlights the value of parasite viability for assessing drug activity in pre-clinical and early clinical drug development.

Session chairs: Charles Narh & Alessia Hysa

T20 Investigating strategies to improve vaccine-induced antibody immunity

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A vaccine capable of eliciting sustained immunity to malaria is critical to protect at-risk individuals from severe disease and death. However, inducing long-lasting protection remains an elusive goal as the most advanced malaria vaccines do not show high efficacy beyond the first year. Antibodies are vital to malaria immunity, and short-lived vaccine efficacy is associated with rapidly waning antibody levels. We therefore explored how the magnitude and durability of antibody responses may be improved by modifying vaccine design features and host characteristics. Given the prevalence of childhood undernutrition in many regions with a high malaria burden, we first investigated the immune effects of dietary micronutrient deficiencies. We found that mice fed a zinc deficient diet showed impaired induction of IgG following malaria vaccination and that this effect worsened with specific adjuvants, suggesting zinc availability may affect the immunogenicity of malaria vaccines and clinically relevant adjuvants. Further, antigen properties and the type of adjuvant impacted the induction and maintenance of antibody responses in mice fed standard diets, with some variation driven by the availability of T helper cell epitopes. Our studies highlight the complex interactions between vaccine features and host properties which contribute to the induction and longevity of antibody responses, and propose future avenues to explore in order to achieve sustained immunity to malaria.

T21 Vg1 gd T Cells regulate early CD8 T cell activation in response to Radiation Attenuated Sporozoite Vaccination

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Radiation Attenuated Sporozoite (RAS) vaccination in mice, induces sterilising immune responses predominantly through the generation of CD8+ liver tissue resident memory T cells. gd T cells are essential in this response, as a lack of gd T cells inhibits malaria-specific CD8+ memory T cell development, however, how gd T cells exert this function is poorly understood. Flow cytometry and confocal microscopy on spleens from RAS vaccinated mice was used to characterise CD8+ and gd T cell activation in the first 48 hours after vaccination. Upregulation of CD69 and CD25 among CD8+ T Cells and Vg1+ gd T Cells was observed by flow cytometry, suggesting antigen-specific engagement of the T cell receptor of both populations in response to RAS vaccination. This activation phenotype was also observed via confocal microscopy 24 hours after vaccination whereby adoptively transferred *Plasmodium* berghei-specific CD8+ T cells clustered with other CD8+ T cells and gd T cells in the spleen after vaccination. Alternatively, vaccination using Heat Killed Sporozoites (HKS) retained the CD69+CD25+ activated phenotype among Vg1+ gd T cells, suggesting that gd T cells are not responding to the damage caused by RAS during vaccination, but that they are possibly recognizing a conserved sporozoite antigen. Finally, inhibition of Vg1 gd T cells through administration of a Vg1.1 monoclonal antibody 48 hours before vaccination, blocked *Plasmodium* berghei-specific CD8+ T cell expansion. Vg1+ gd T cells are therefore the gd T cell population responding to RAS vaccination, facilitating the generation of protective CD8+ T cell memory responses.

T22 FcγRIIIA binding antibody on the surface of *Plasmodium falciparum* infected red blood cells is associated with protection

Elizabeth Aitken (1), Natasha Sharma (1), Wina Hasang (1), Kwok Zi Rou (1,2), P. Mark Hogarth (3), Bruce Wines (3), Morten Nielsen (4), Maria Ome-Kaius (5), Mwayiwawo Mandanitsa (6), Victor Mwapasa(7), Kamija Phiri (7), Ken Maleta (7), Feiko ter Kuile (9), Holger Unger (1,8,9), Stephen Rogerson (1)

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As interactions between antibody and Fcy receptors (FcyR) are vital for leukocyte activation and may be dependent on antigen arrangement we developed an assay to measure binding of FcyRI, IIA and IIIA to opsonised protein in its native form, on the surface of the parasite. We then used this assay to assess the relationship between FcyR binding antibodies on placental-binding infected red blood cells (iRBC) and exposure and protection from disease using plasma from infected and uninfected, primi- and multigravidae from Malawi (n=10/group) and plasma from PNG women infected in mid-pregnancy who did (n=50) or did not have (n=27) placental malaria at delivery. We also correlated levels of FcyR binding of opsonised iRBC with related antibody measures; IgG towards the iRBC and FcyR binding of antibodies on recombinant placental-binding PfEMP1. FcyRI and FcyRIIIA binding antibodies on iRBC were higher in multigravidae and both FcyRI (P=0.02) and FcyRIIIA (P<0.001) binding antibodies on iRBC were associated with protection from placental malaria. FcyRI, IIA and IIIA binding antibody on the iRBC were weakly to moderately correlated with IgG levels to the iRBC, and only FcyRI binding antibody on the iRBC was correlated with FcyRI, IIA or IIIA binding antibodies on recombinant placental binding PfEMP1. This novel assay allows measurement of specific FcyR binding antibodies on the parasite and suggests a clear role for FcyRIIIA in clearance of opsonised iRBC.

T23 Investigation of *P. vixax* Elimination Using MDA

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Plasmodium vivax is one of the most geographically widespread malaria parasites and is mainly found in South-East Asia, Latin America, and some parts of Africa. *P. vixax* is unique compared to most other *Plasmodium* parasites due to its ability to remain dormant in the human liver as hypnozoites and subsequently reactivate after the initial infection (i.e. relapse infections). More than 80\% of *P. vixax* infections are due to hypnozoite reactivation. Hence, it is crucial to target the hypnozoite reservoir in order to eliminate *P. vixax*. In this study, we use a stochastic multiscale model to study the impact of multiple mass drug administration (MDA) rounds with a radical cure on *P. vixax* elimination. We explicitly model the impact of the radical cure drug on each of the hypnozoites and infections. We derive the optimal timings of MDA rounds (under a deterministic framework) and obtain the probability of *P. vixax* elimination. Our model indicates that the more rounds of MDA, the better the chance of *P. vixax* elimination and up to two MDA rounds will have a very minimal effect on the probability of elimination (this depends on other model parameters as well). To achieve a higher probability of elimination, MDA with a very high-efficacy drug should be considered. Furthermore, a simplified approach to MDA timings can provide similar results compared to the optimal approach.

Session chair: Niall Geoghegan

ST5 Enhancing Access to Radical Cure in Cambodia: Assessment of the Implementation of the STANDARD G6PD Test for *Plasmodium vivax* Case Management

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Plasmodium vivax forms dormant liver stages (hypnozoites) that can reactivate weeks or months after the initial infection causing relapses. Primaquine and tafenoquine are the only drugs which can kill hypnozoites but can cause haemolysis in individuals with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. The STANDARD G6PD (SD Biosensor, ROK; “Biosensor”) is a novel point-of-care diagnostic that can facilitate safer treatment. In 2021, Cambodia rolled out the Biosensor to health centres. To assess the implementation, 19 focus group discussions, 67 interviews, and 32 observations were conducted with 39 patients, 99 healthcare workers, 12 policymakers and officials, and 3 implementing partners. A thematic analysis was done. We found that in a research-saturated setting, health centre staff have adopted the Biosensor well despite complex test procedures. Key factors enabling the adoption are a sense of safety with the test’s numeric output, an understanding of the rationale for testing, and observation of successful treatment outcomes. A main implementation challenge includes patients not reaching health centres after diagnosis by community health workers. This may be caused by the opportunity cost for patients, patients’ perception of vivax severity, understanding of the benefits of primaquine treatment, and fears of side effects. Our data offers insights for other vivax endemic countries on key factors affecting the implementation and wider rollout of this diagnostic test.

ST6 Distinct immune responses associated with vaccination status and protection outcomes after malaria challenge

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Understanding mechanisms of sterilizing immunity to malaria is needed to improve vaccine efficacy. Vaccination with irradiated *Plasmodium falciparum* sporozoites (PfRAS) induces high level of sterilizing immunity in clinical trials, and historically been utilized as a tool to study protective immune mechanisms to malaria. To identify protection-associated immune responses to malaria, we performed transcriptome profiling of whole blood and in-depth cellular profiling of peripheral blood from volunteers receiving either PfRAS or non-infectious mosquito bites (Mock), followed by *P. falciparum* controlled human malaria infection (CHMI) to assess protection outcomes. scRNAseq showed cell responses to CHMI in Mock group were predominantly inflammatory. Blood transcriptome profiling revealed that responses between non-protected (NP) and Mock were largely similar after CHMI, characterized by innate cell and inflammatory signatures. In contrast, transcriptome profile in protected (P) group was associated with increase in type I and II IFN and NK cell pre-CHMI, followed by T and B cell signatures as early as one day post-CHMI. Surface protein phenotyping showed different induction profiles in vδ2+ γδ T cells, CD56+ CD8+ Tem cells, and non-classical monocytes between P and infected groups (NP and Mock) following treatment and parasite clearance. Our data demonstrate that PfRAS protective responses are associated with early and rapid changes in IFN, NK cell, and adaptive immune response.

ST7 The economic burden of zoonotic *Plasmodium knowlesi* malaria compared to human-only species on households in Sabah, Malaysia

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Introduction: The rise of the zoonotic monkey parasite *Plasmodium knowlesi* as the dominant cause of malaria in Malaysia challenges WHO elimination goals. Malaysia has free universal access to malaria care; however, out-of-pocket costs are unknown. This study estimated the household costs of illness from malaria cases across different *Plasmodium* species infections in Sabah, Malaysia. Methods: Household costs were estimated from patient-level surveys in four hospitals between 2013 and 2016. Direct medical costs and indirect costs (travel, food, and productivity losses) were included. Results: 152 malaria cases were enrolled: *P. knowlesi* (n=108), *P. falciparum* (n=16), *P. vixax* (n=22), and *P. malariae* (n=6). Across all cases, the mean total costs were US$122 (SD=104), with productivity losses accounting for most (56%) costs. While the mean direct costs for healthcare were $53 (SD=65), the mean indirect costs while seeking care were $68 (SD=72). *P. vixax* had the highest total household cost at US$177, followed by *P. falciparum* (US$116), *P. knowlesi* (US$114), and *P. malariae* (US$93). Conclusion: Household out-of-pocket costs were driven by productivity losses; primarily attributed to infections in working-aged males in rural, agricultural-based occupations. Costs for *P. knowlesi* were similar to *P. falciparum* and lower than *P. vixax*. The high costs for *P. vixax* were due to medical costs. Despite Malaysia's free malaria medical care, patients and families face substantial costs.

ST8 Investigating the role of TRX2 in trafficking malaria virulence proteins

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Background: Malaria is caused by infection with *Plasmodium* parasites, with ~400,000 deaths and >200 million cases each year. Due to major problems with resistance to current antimalarials, new strategies to combat *Plasmodium* are desperately needed. This project aims to identify the role of thioredoxin (TRX2) in *P. falciparum*, a component of the parasite's protein export machinery (PTEX), in trafficking cysteine-rich proteins such as PfEMP1 to the host cell surface and its contribution to parasite survival. Methods: To determine the role of TRX2 in parasite survival, HA-tagged transgenic parasites were created using CRISPR/Cas9, enabling regulated knockdown of HA-tagged TRX2 with glucosamine. PCR was used to confirm correct integration, and TRX2 knockdown after glucosamine treatment was monitored by western blot. Phenotypic analysis of knockdown was performed by monitoring parasite growth and export of PfEMP1 by immunofluorescence. Results: Diagnostic PCR results indicated successful creation of PfTRX2-HAglmS transgenic parasites. Western blot analysis confirmed that glucosamine treatment reduced TRX2 protein expression by >85%. Knockdown of TRX2 protein resulted in a significant reduction of parasite growth (50%) and a defect in PfEMP1 protein export to the erythrocyte membrane. Conclusion: Using molecular and biochemical techniques, it has been shown that TRX2 is important for optimal growth of *P. falciparum* and export of PfEMP1 protein to the host cell surface.

Session chairs: Mary-Lou Wilde & Dawson Ling

T24 Cytostome formation in artemisinin resistant *Plasmodium* parasites

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Resistance against the frontline antimalarial, artemisinin, is mediated by mutations in the Kelch 13 (K13) gene. We demonstrated that the K13 protein is required for normal formation of the cytostome; the apparatus which brings haemoglobin from the host red blood cell into the *Plasmodium* parasite. Haemoglobin digestion supplies essential amino acids for parasite growth and release free haem which is required for the activation of artemisinin. Parasites expressing mutant K13 have a slowed rate of parasite feeding which is characterised by a reduction of haem biosynthesis and delayed growth. This reduced level of haem leads to less artemisinin activation, resulting in less parasite death. However, the mechanism by which mutation of K13 cases this slowed feeding phenotype remains unclear. We hypothesised that mutations in K13 reduce its stability and abundance, affecting the rate at which new cytostomes are formed and thus parasite feeding. Using expansion microscopy, we resolved K13 as ring-shaped structures that localise to the periphery of the parasite. We performed expansion microscopy at timepoints throughout the asexual life cycle and the compared the morphology and number of K13 rings present in the mutant vs WT. We found that during ring stages, K13 mutant parasites formed new K13 rings at a slower rate than the WT. Some K13 mutants also appeared to form incomplete rings. These data provide a potential mechanism linking the artemisinin resistance mutations to reduced rate of haemoglobin uptake.

T25 Tracking inheritance of the apicoplast and mitochondrion in *P. berghei*

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Plasmodium parasites harbour a single mitochondrion and a single relic plastid (apicoplast) throughout their life cycle. Both organelles are essential and are used as drug targets. Previous genetic cross studies indicated that both organelles are maternally inherited during sexual reproduction, but the mechanisms underpinning such uniparental inheritance were unknown. To investigate organellar inheritance in *Plasmodium*, we observed *P. berghei* lines with fluorescently tagged apicoplasts and mitochondria using static and live-cell microscopy techniques. We tracked the fate of both organelles in male and female gametocytes undergoing gametogenesis by lattice light-sheet microscopy. The mitochondrion and apicoplast are excluded from newly formed male microgametes during exflagellation. By contrast, in activated female gametocytes an elongated, perinuclear positioned apicoplast and an expanded mesh-like mitochondrial network encapsulate or cradle the nucleus throughout.
To explore whether the organellar genome is degraded prior to elimination, we used digital droplet PCR to show that there is a substantial decrease in the relative copy number of the apicoplast and mitochondrial genomes in male gametocytes compared to females.
Alongside this, we are testing for paternal leakage of mitochondrial DNA by force crossing *P. berghei* lines with selectable polymorphisms in the mitochondrial genome. After screening 1.4 million sporozoites across five crosses, we have identified a single male leakage event.
Overall, this work helps to better inform future therapeutic strategies targeting these organelles, and will improve our understanding of how organelle encoded resistance mutations are transmitted and how this might impact malaria treatment.

T26 Serological markers predict *Plasmodium vivax* relapses in a returning Indonesian soldier cohort

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Relapses from *Plasmodium* vivax hypnozoites (latent liver-stage parasites) are a major obstacle for elimination. Detecting hypnozoites remains a challenge, yet *P. vixax* blood-stage infections induce strong and enduring antibody responses. Antibodies serve as indicators of recent and ongoing infections, enabling identification of individuals recently exposed to *P. vixax* and susceptible to carrying hypnozoites. This study assessed antibody signatures towards a panel of eight validated antigens to predict relapse risk in two cohorts of soldiers (n = 570) who returned to a malaria-free area from a nine-month deployment in a malaria-endemic area (2018 and 2022). Soldiers underwent Luminex serology and light microscopy on recruitment day and were then actively follow-up every two weeks for up to six months, as well as at time of febrile symptoms, until first recurrent *P. vixax* parasitaemia. We used a Random Forest classification algorithm to identify soldiers exposed during the previous nine months.127 soldiers (22%) experienced relapse during follow-up (Cohort 1 = 24 (8%) and Cohort 2 = 103 (36%)). Our diagnostic tool achieved an AUC of 0.9, with 83% sensitivity and 84% specificity in identifying future relapses using blood samples from recruitment. We will present the results and discuss their implications for the development of a novel public health intervention *P. vixax* serological testing and treatment (PvSeroTAT) for relapse prevention.

T27 Doxycycline inhibits both apicoplast and mitochondrial translation in *Plasmodium falciparum*

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Doxycycline is a tetracycline-class antibiotic used for malarial prophylaxis and as a partner drug. Doxycycline's antimalarial mechanism of action has widely been accepted, though not demonstrated, as a translation inhibitor specifically blocking the prokaryotic 70S ribosomes of the *Plasmodium* apicoplast. At low concentrations (<5 μM) doxycycline exhibits a delayed death phenotype, typical of inhibitors of apicoplast housekeeping processes. At higher concentrations (>10 μM) doxycycline has rapid schizonticidal activity via an unknown and apicoplast-independent mechanism. In other eukaryotes, and plausibly in *Plasmodium*, doxycycline inhibits mitochondrial 70S ribosomes.
To measure changes in protein abundance we used a mass spectrometry approach to assess the steady state protein levels and protein turnover using isotope-labelled amino acids. We directly detected apicoplast encoded proteins for the first time and showed that these decrease following treatment with doxycycline and the proposed apicoplast translation inhibitor clindamycin. Furthermore, only high concentrations of doxycycline reduced the abundance of mitochondrial encoded proteins, which we show affects mitochondrial activity. Mitochondrial encoded proteins are required for the formation of complexes in the electron transport chain. Perturbations to oxidative phosphorylation were detected in both *Plasmodium* and the related parasite *Toxoplasma gondii* following high doxycycline treatments. Our data demonstrates the mechanism of action of apicoplast translation inhibitors for the first time and reveal doxycycline as the first described mitochondrial translation inhibitor of *Plasmodium*.

T28 Functional characterisation of the GAPM proteins in asexual and sexual stage development

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Plasmodium falciparum has a complex lifecycle with stages in the human host and mosquito vector. Transition between different cellular niches during the lifecycle relies on cell invasion, cellular motility, and shape change. The structure underpinning this biology is the inner membrane complex (IMC). The IMC is a double membrane structure, that acts as a scaffold for cell shape and anchors an actin myosin motor used in RBC invasion. Despite being well studied little is known about the organisation, assembly of the structure and the function of many of its resident proteins. In this work we characterise the three Glideosome Associated Protein(s) with Multiple membrane spans (GAPM). Using a gene tagging and conditional knockout (cKO) approach, combined with super-resolution microscopy and proteomic analyses the function of these proteins across blood stage development was elucidated. In the absence of each GAPM protein, segmented schizonts are formed however they are unable to invade RBCs. Examination of invasion using Lattice light sheet microscopy reveals a novel defect in invasion. In addition, deletion of each GAPM in gametocytes led to an inability of parasites to mature beyond stage III of development. Airyscan microscopy of cKO parasites suggests an incorrect coupling of the IMC to the microtubule network and a reduction in other IMC proteins is controlling this arrested development. This work sheds light on IMC function in invasion and gametocyte development.

Session chairs: Lakvin Fernando

ST9 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 (PfXPO1), involved in nucleocytoplasmic export, as a potential ABI target. Notably, an ABI-resistant strain R1 revealed a H1061N point mutation within PfXPO1. To validate PfXPO1 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 PfXPO1.These findings support the role of PfXPO1 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.

ST10 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.

Poster Abstracts

P1 **Defining the fine specificity of RTS,S-induced antibodies to polymorphic and conserved epitopes of the *Plasmodium falciparum* circumsporozoite protein**

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RTS,S is the only malaria vaccine recommended for at-risk African children but confers modest short-lived protection against disease. RTS,S is based on the *Plasmodium falciparum* circumsporozoite protein (CSP) of the 3D7 reference strain. The vaccine construct includes the CSP central repeat region composed of NANP and NVDP sequences, which is highly immunogenic. However, antibody magnitude to the repeat region is weakly associated with protection in children. RTS,S also includes the CSP C-terminal region, which is highly polymorphic and the 3D7 allele represents less than 10% of African parasite isolates. Here, we investigated the specific epitopes within the central repeat region and C-terminal region (3D7 and non-3D7 alleles) targeted by RTS,S- antibodies in children. These findings will inform strategies to modify RTS,S and enhance the induction of protective antibodies. We evaluated antibody responses in Mozambican children (n=737) vaccinated with RTS,S in a phase IIb clinical trial. We quantified IgG magnitude to 26 peptides representing epitopes within the central repeat and C-terminal (3D7 and non-3D7 alleles) regions of CSP. We found that: 1) Short NANP sequences may better represent protective epitopes than longer NANP sequences. 2) RTS,S antibodies had reduced binding to non-3D7 alleles of the C-terminal region than the vaccine strain. Our findings elucidate the specificity of protective RTS,S antibody responses and the impacts of polymorphisms on vaccine responses.

P2 Defining functional antibody epitopes of merozoite surface protein 2 to inform next-generation vaccine design

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Better malaria vaccines are needed as RTS,S only showed moderate and short-lived efficacy in endemic settings. Merozoite surface proteins (MSPs) aid erythrocyte invasion, leading to clinical symptoms. Therefore, vaccines against MSPs may prevent malaria illness. MSP2 is especially suitable for development as it is abundant and is a target of acquired immunity. Antibodies with specific functions target malaria parasites effectively and are associated with protection. These antibodies bind serum complement proteins or engage Fc-receptors (FcRs) on immune cells. Downstream processes then kill parasites, such as via pore formation or opsonic phagocytosis. The epitopes of these antibodies are undefined but are needed for optimal vaccine design. We examined MSP2 for regions and epitopes targeted by complement-fixing and FcR-binding antibodies. Peptides based on MSP2 regions were tested with sera from an endemic cohort. The cohort displayed high IgG reactivity against all peptides. We then identified peptides that potently bound complement-fixing antibodies. Regions and epitopes will also be measured for FcR engagement via opsonic phagocytosis by innate cells. Promising regions will be further evaluated in vaccine designs using animal studies. A vaccine construct enriched in epitopes targeted by functional antibodies may outperform full-length protein vaccines. The fine-tuning of protective epitopes can also be applied to other MSPs to design an effective multi-antigen vaccine.

P3 **Investigating the MMV GHPB and COVID box for inhibitors of *Plasmodium falciparum* egress or invasion**

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The development of novel antimalarials is imperative to combat the deadly disease malaria, especially due to emerging drug resistance against current antimalarials. The asexual blood stages of egress and invasion have been identified as appealing targets as their inhibition would prevent progression to the next replication cycle. Invasion is a particularly attractive target as merozoites unable to invade and trapped outside erythrocytes are susceptible to immune clearance. Phenotypic assays of compounds from the Medicines for Malaria Venture COVID and Global Health Priority boxes have been conducted to find inhibitors of *Plasmodium falciparum* egress or invasion. These assays were conducted using bioluminescent reporter parasites that allow the identification of compounds that inhibit egress and invasion. This project has identified four compounds that inhibited invasion by >50% at less than 1μM. To understand how the compounds worked, further investigation was undertaken to identify the specific egress and invasion steps that were inhibited.

P4 Bis-1,2,4-triazines, a novel class of potent and irresistible antimalarial, likely target the parasite nucleus

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Bis-1,2,4-triazines are a class of fast-acting antimalarial candidates with low-nanomolar potency against asexual and early sexual blood stage *Plasmodium falciparum*. Whilst their peak activities are observed against the trophozoites, the lead bis-1,2,4-triazine can kill rings within 5 hours with IC50 below 100 nM. Their mode of action is elusive to date, however, appears to be novel, as no cross-resistance has been observed to a wide range of drug-resistant parasites. In order to investigate their mode of action, we have attempted to select for bis-1,2,4-triazine-resistant parasites in vitro. No resistance could be generated in the hypermutable Dd2-Polδ parasites or subjecting a step-wise drug challenge to Pf3D7 and PfDd2 over 24 months. This indicates that bis-1,2,4-triazines have a very low propensity for resistance emergence. We also employed complementary untargeted proteomics methods to identify the potential target(s) of bis-1,2,4-triazines, one of which is live cell thermal stability proteomics. When we applied this method to representative bis-1,2,4-triazines, and the lead compounds stabilised 21 nuclear proteins at 65°C with a fold-change >1.5 ($p < 0.05$). Some of these proteins play important roles in DNA repair, DNA replication and gene expression. In order to validate these results, we are currently performing an orthogonal chemoproteomic approach, limited proteolysis-MS, to more precisely identify which of these represent the primary target of bis-1,2,4-triazines.

P5 Comparison of molecular surveillance methods to assess changes in the population genetics of *Plasmodium falciparum* in high transmission

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A major motivation for developing molecular methods for malaria surveillance is to measure the impact of control interventions on the population genetics of *Plasmodium falciparum* as a potential marker of progress towards elimination. We assess 3 established methods (i) single nucleotide polymorphism (SNP) barcoding (panel of 24-biallelic loci), (ii) microsatellite genotyping (panel of 12-multiallelic loci), & (iii) varcoding (var gene diversity, akin to microhaplotyping) to identify changes in parasite population genetics in response to a short-term indoor residual spraying (IRS) intervention. Typical of high seasonal transmission in Africa, multiclonal infections were found in 82.3% (median 3; range 1-18) & 57.8% (median 2; range 1-12) of asymptomatic individuals pre- & post-IRS, respectively, in Bongo District, Ghana. As directly phasing multilocus haplotypes for population genetic analysis is not possible for biallelic SNPs & microsatellites, we chose ~200 low-complexity infections biased to single & double clone infections for analysis. Each genotyping method presented a different pattern of change in diversity & population structure as a consequence of variability in usable data & the relative polymorphism of the molecular markers (SNPs < microsatellites < var). Varcoding & microsatellite genotyping showed the overall failure of the IRS to significantly change the population structure from pre-IRS. The 24-SNP barcode provided limited information for analysis, largely due to the biallelic nature of SNPs leading to a high proportion of double-allele calls & isolate relatedness compared to microsatellites & varcoding. Relative performance, suitability, cost of methods relevant to sample size & local malaria elimination in high-transmission endemic areas are discussed.

P6 Association of *Plasmodium falciparum* specific afucosylated IgG with immune protective function activation

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Immunoglobulin G (IgG) antibodies serve a critical role in the activation of immune-protective function against *Plasmodium falciparum* via the Fc gamma receptors (FcγR). Recent focus on IgG fucosylation highlighted the heightened binding affinity of afucosylated IgG to FcγRIIIa compared to fucosylated IgG, resulting in enhanced antibody dependent cellular cytotoxicity (ADCC). In this study, we utilized the Fucose-sensitive Enzyme-linked immunosorbent assay (ELISA) for Antigen-Specific IgG (FEASI), an immunoassay that is capable of quantifying Fc fucosylation of antigen-specific IgG antibodies. FEASI consists of two ELISA assays; the first is to measure the levels of antigen-specific IgG independent of fucosylation using total IgG or N162A mutant FcγRIIIa receptor, while the second gives FcγRIIIa specific binding readouts which is highly sensitive to IgG fucosylation. The output of both ELISAs is converted into a ratio that represents the level of fucosylation in a given sample. Here we examined the plasma from N=167 *P. falciparum* infected pregnant women of varying gravidities using FEASI. Our results showed varying levels of IgG fucosylation within the cohort and further experiments that explore the association between the levels of afucosylated IgG with placental malaria during delivery, neutrophil phagocytosis and NK cell activation are planned. These results have important implications in the understanding of naturally acquired protection to malaria in pregnant women.

P7 **Acceptability and operational feasibility of malaria reactive surveillance and response strategies in north-eastern Cambodia: a qualitative study**

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Cambodia aims to eliminate malaria by 2025 and adopted the 137 reactive surveillance and response (RASR) strategy that entails notification, investigation, classification, and reporting of malaria cases within 1 day, reactive case detection within 3 days, and foci investigation and response activities within 7 days. However, the acceptability and operational feasibility of the Cambodia 137 strategy is yet to be assessed.
Focus group discussions and in-depth interviews with malaria program stakeholders and field staff, village malaria workers, mobile and migrant populations and forest goers were conducted in two provinces of north-eastern Cambodia between August 2022 and March 2023. The study participants were recruited purposively to explore their opinions on implementation of RASR strategy. Deductive followed by inductive thematic analysis was applied. The current RASR strategy in Cambodia was acceptable to the malaria program stakeholders, village malaria workers, and mobile and migrant people. Field implementation of RASR activities was considered to be successful due to strong commitment and support from the government, enthusiastic participation and interests of service providers, effective inter-sectoral collaboration between local authorities and health sector, and adequate human resource and commodities. However, this study highlighted some operational barriers to effective implementation of RASR strategy such as limited access to mobile network and internet, poor community participation, transportation difficulties, frequent migration of people, and heavy workload of health centre staff. Optimisation of RASR strategy by taking these barriers into consideration could have facilitated in achieving Cambodia's malaria elimination goal by 2025.

P8 The role of the human kinase B-Raf in *Plasmodium falciparum* blood-stage infection

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Malaria, an infection caused by parasites from the genus *Plasmodium falciparum*, remains a primary global health concern. The selection of mutations under drug pressure have resulted in emergence of strains resistant to treatment, contributing to 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 these B-Raf inhibitors except the PLX8394.We now intend to investigate the role of the B-Raf kinase during the erythrocytic stage of infection, and the mechanism for PLX8394-resistance.

P9 Understanding the role of rodent malaria clag genes in new permeation pathway formation

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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 RBC, facilitating nutrient acquisition, and are an attractive therapeutic target. Clag genes have previously been implicated in NPP formation, however, their role in the channel is unclear. Rodent malaria species *P. berghei* could be used to investigate clag's contribution to NPPs. Unfortunately, there is currently no method available for assessing NPP functionality in a rodent model. This study therefore aimed to develop an osmotic lysis assay to determine NPP functionality. Compounds were screened for their ability to cause lysis, guanidinium-hydrochloride was found to exclusively lyse infected RBCs. Subsequently, NPP inhibitors and synchronous population assays showed lysis was mediated by the NPPs. Next, the *P. berghei* clag gene was modified 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, proposing the conservation of clag gene across species. This work shows that clag genes can be studied in rodent model with a higher confidence of relatability to *P. falciparum* function.

P10 Investigating immunity to non-pregnancy specific *Plasmodium falciparum* antigens and protection against placental malaria

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Placental malaria (PM) is caused by *Plasmodium falciparum*-infected erythrocytes (IEs) sequestration in placenta via CSA. Antibody (Ab) response to *P. falciparum* antigens has been reported to reduce parasitemia in the placenta and possibly contribute to protection. We investigated if non-pregnancy-specific Abs contributed to protection against PM. We used plasma from pregnant Malawian women with parasitemia at antenatal booking, who either had PM (n=75) or no PM (n=88) at delivery. Total IgG, IgG1-4, IgA1-2, IgM, Ab engaging FcγRIIA, FcγRIIIA, FcγRIIB, FcγRIIIB and C1q levels to 13 *P. falciparum* recombinant antigens which comprised 8 merozoites (PfRh5, PfRh2a1, EBA175, MSP1-p19, MSP2, MSP3, MSP9, AMA1), antigens active in the schizont stage (PfSEA1-1A, PfGARP), vaccine candidate (PfCSP) and pregnancy-specific antigens (VAR2CSA, DBL1-ID2a) were quantified by Luminex multiplex assay. Levels of Ab features were compared using Welch’s t-test and a volcano plot was generated to visualize distribution of Ab features between the two groups. In univariate analyses, 27 Ab features were more abundant in women with PM with 19 being Ab features to merozoites. Among 5 Ab features higher in pregnant women with no PM were IgA1 antibodies to MSP2, MSP9, PfRh2a1, PfRh5 and complement binding Abs to PfRh5. Abs to merozoite antigens are primarily markers of exposure to malaria. Further analysis will include machine learning techniques to identify Ab features contributing to protection.

P11 Determination of glycophorin C genotypes prevalence and association with *Plasmodium falciparum* density and diversity in malaria patients in Ghana

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Genetic diversity poses a barrier to success of vaccine development targeting *Plasmodium* species as evolutionarily favourable genes that have been chosen have significantly been impacted by it. Several malaria-protective polymorphisms have been linked to genes in the red blood cells that modify or impair their structure or activity. Identifying the genes involved and their impact on malaria risk is a potentially useful technique of examining the host-parasite relationship. The study investigated the genotypes of Glycophorin C (GYPC) protein and their effect on *Plasmodium falciparum* density and diversity in symptomatic malaria patients across Ghana. A total of 214 dry blood spot archived samples collected in 2021 from ten randomly chosen health facilities across Ghana's sixteen regions were used to characterize GYPC genotypes in the Ghanaian population into GYPC homozygous wild type, heterozygous and homozygous GYPC exon-3 deletion using PCR and agarose gel electrophoresis and determine its association with *P. falciparum* density, diversity using PET-PCR and nested PCR respectively. Out of the 214 samples with a history of febrile illness, 201(94%) had the GYPC heterozygote with 13 (6%) of these being with the homozygous GYPC wild type and no record of the homozygous GYPC exon-3 deletion wild type in the Ghanaian population. There was however no significant association between the GYPC genotypes and *P. falciparum* density (p=0.285) and diversity (p=0.805). This study serves as a baseline study to provide functional data on the impact of GYPC exon-3 deletion on *Plasmodium falciparum* infection in Ghana thus adding up to our current understanding of vector-host interactions.

P12 Mapping the landscape of sex determination in *P. falciparum*

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Transmission of the malaria parasite *Plasmodium falciparum* from the human host to the mosquito requires a specialised cell called the gametocyte. Male and female gametocytes are produced and both sexes are required for fertilisation and sexual development within the mosquito midgut. The production of gametocytes and their fertilisation in the mosquito midgut represent a significant bottleneck in the parasite's lifecycle. Moreover, male and female gametocytes have been found to exhibit different susceptibility profiles toward current frontline antimalarial drugs. Despite the importance of these lifecycle stages to disease transmission, a deep understanding of the biology underpinning sex differentiation and development is lacking. Malaria parasites do not have sex chromosomes, so the mechanisms underpinning sex determination are likely driven by differential transcriptional regulation and protein expression. Using an in-silico approach we identified several genes that are exclusively transcribed in early gametocytes and show more than 10-fold enrichment in males and females respectively. Utilizing a selection-linked integration approach, we have created gene knockouts of one female PF3D7_1115100 (FD5) and one male PF3D7_0809700 (MD6) specific genes. The project aims to characterise the defects, sex ratio, and transmission ability of the FD5 and MD6 gene knockouts and shed more light on the genes driving sex differentiation and determination in *P. falciparum*.

P13 Lactate Dehydrogenase antigen detection tool for symptomatic and cryptic infections of *Plasmodium* vivax

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Malaria remains a major global health issue despite ongoing control efforts. Australia's neighbouring countries in the Asia-Pacific region are burdened with a high prevalence of *Plasmodium vivax* infections. Distinct features of *P. vixax*, such as the i) presence of an arrested liver stage that can cause relapses and ii) a high prevalence of asymptomatic individuals with low-density infections, makes diagnosis and surveillance challenging. Addressing these concerns, the Mueller laboratory developed and validated *P. vixax* specific antibodies to serve as serological exposure markers (SEMs) that can identify individuals with recent exposure (up to nine months) to *P. vixax*. A key limitation of the SEM assay is the false-negative misclassification of infected individuals (with a low and developing antibody response). We developed a *P. vixax*-specific Lactate Dehydrogenase (PvLDH) antigen assay, identifying 84% of the PCR-positive current infections and correlating (r=0.68) with parasite density. The assay is currently unable to detect PvLDH in individuals with low-density asymptomatic infections indicating that whilst the assay is a good diagnostic measure, it is not yet optimised for use as a surveillance tool. Interestingly, the assay identified PvLDH in a relapsing individual with PCR negative timepoints. This highlights the assay's potential to be a valuable research tool for understanding hidden parasite dynamics that are missed by conventional detection tools.

P14 ROLE OF IgG ANTIBODIES IN PROTECTION FROM PLACENTAL MALARIA BIRTH OUTCOMES

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Placental malaria (PM) is a public health issue linked to poor pregnancy outcomes. Antibodies against VAR2CSA, a variant surface protein found on infected erythrocytes, protect against *Plasmodium falciparum* infections in pregnant women. This study aims to associate VAR2CSA antibody levels with poor pregnancy outcomes: Low Birthweight (LBW), preterm delivery, Small for Gestational Age (SGA) and maternal anaemia. Pregnant Malawian women (n=466) infected with malaria were recruited at 16-28 gestation weeks. Total IgG levels to recombinant VAR2CSA (DBL1X-ID2a) domains were measured at enrolment and delivery via indirect ELISA. Results show significantly higher IgG levels in multigravid women (3.50 ± 0.10 AU) than primigravid women (3.38 ±0.10 AU). High antibody levels were observed at enrolment (3.45 ±1.09 AU) compared to delivery (2.89 ± 0.97 AU). However, IgG antibody levels measured at enrolment were not associated with reduced LBW (aOR=1.16, 95%CI 0.81-1.67, p= 0.4), preterm delivery (aOR=1.24, 95%CI 0.81-1.88, p=0.32), SGA (aOR=0.96, 95%CI 0.74-1.25, p=0.75) and maternal anaemia (aOR=1.08, 95%CI 0.84-1.40, p=0.55). This shows no significant associations between the antibody levels at enrolment with protection from poor pregnancy outcomes. The antibody responses to VAR2CSA are likely markers of PM rather than protection from infection.

P15 Combination of redox modifiers with artemisinin results in increased parasite susceptibility to artemisinins.

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Resistance has been recorded 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. Using a combination of drug inhibition assays, redox assays and multi-omics-based approaches, 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) and increased oxidative burden within parasites after 1 h incubation. The addition of SFN to existing antimalarial therapies would re-sensitise resistant parasites to existing antimalarials thereby extending their life span. Ongoing studies will elucidate the mechanism responsible for this synergistic activity and determine the safety and efficacy of this approach in drug-resistant in vivo models of malaria.

P16 The Effect of N6-methyladenosine (m6A) Knockdown on mRNA Export and Stability in Intraerythrocytic *Plasmodium falciparum*.

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mRNA abundance studies have revealed the dynamic regulation of gene expression during the erythrocytic replication cycles of *Plasmodium falciparum*. Although this phenomenon is well known, much of the biology of mRNA in *Plasmodium* remains unstudied. RNA binding proteins and RNA base modifications are known to facilitate the export of mRNA to the cytoplasm and control the stability of mRNA in the cytoplasm in better studied eukaryotes. These mechanisms allow the cell to better regulate the expression of genes. Base modifications such as N6-methyladenosine (m6A) and some components of the nucleocytoplasmic transport system are conserved between humans and P *falciparum*. m6A has previously been reported to affect translational efficiency in *P. falciparum*, and it destabilises specific mRNA through an unknown mechanism. The effect of m6A on mRNA export nor any other mechanism of sequence specific regulation of mRNA export have been described in *P. falciparum*. The aim of this study was to study the effect of m6A on mRNA stability and mRNA export. The methylation of adenosine to form m6A is catalysed by the m6A writer. We used knock-sideways to create a functional knockdown of the catalytic protein in the m6A writer. The stability of mRNA was determined by measuring the abundance of specific mRNA in the absence of transcription. mRNA export was studied using mRNA-FISH with an oligo-dT50 probe. Our results indicate that there is some effect on both mRNA stability and mRNA export that is m6A dependent.

P17 Establishing genomic surveillance for early warning of antimalarial drug resistance in Bangladesh.

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Bangladesh has achieved an 80% reduction in malaria cases over the last decade, but these gains are threatened by the rapid spread of *Plasmodium falciparum* resistance to the frontline artemisinin and partner drugs in the Greater Mekong Subregion (GMS). Over 90% of malaria cases occur in the Chittagong Hill Tracts (CHT) districts, bordering Myanmar. To date, there is no evidence of artemisinin resistance in the CHT; however, a large influx of refugees fleeing Myanmar into Bangladesh warrants dedicated surveillance. The National Malarial Elimination Programme (NMEP) currently receives data on antimalarial drug efficacy from clinical surveys in select locations; however, the high financial cost and logistical complexities constrain their frequency and ability to detect emerging resistance in other locations. My proposed research program aligns closely with and extends the activities of the NMEP and leading malaria genomics researchers to establish amplicon sequencing as a sustainable, high-throughput molecular surveillance platform to monitor antimalarial drug resistance and transmission dynamics. The resulting data will provide early warning signals to prevent widespread resistance to artemisinin and partner drugs in the CHT. The study will generate genomic data on antimalarial drug resistance and parasite genetic relatedness in the CHT and establish the capacity for processing the data that leverages existing informatics pipelines. The statistical outputs will inform the NMEP with clear intelligence on where to upscale antimalarial interventions, conduct clinical surveys of drug efficacy, and change drug policy if high levels of resistance are detected.

P18 A novel mechanism of clindamycin resistance in *Plasmodium*

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The antibiotic clindamycin kills malaria parasites by targeting the prokaryotic translational machinery of the apicoplast. Currently, clindamycin is used in combination with quinine to treat malaria in pregnant women, however its resistance mechanisms in *Plasmodium* remain largely unknown. We selected for clindamycin resistance in *P. falciparum* and in *P. berghei*. Clindamycin resistant *P. falciparum* had acquired a point mutation in the apicoplast 23S ribosomal RNA—the canonical mechanism of resistance in bacteria. However, two independently generated, clindamycin resistant lines of *P. berghei* exhibited no mutations in the apicoplast translational machinery. Rather, whole genome sequencing revealed apparent loss-of-function mutations in two genes (folD and fmt) that encode enzymes responsible for formylation of methionine on initiator tRNA. Translation of apicoplast-encoded proteins likely commences with a formyl methionine, but evidence is lacking. To test if disruption of initiator methionine formylation confers clindamycin resistance, we deleted folD and fmt genes in *P. berghei* and confirmed resistance. But how does abrogation of this pathway make parasites less sensitive to clindamycin and do they start translation with an unformylated methionine? We set out to answer these questions using various proteomic and biochemical assays. Clindamycin resistance is more complex than originally thought and further investigation is imperative to dissect this new resistance mechanism.

P19 Approximately optimal surveillance of *Plasmodium knowlesi* malaria

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Plasmodium knowlesi malaria is increasingly identified throughout Southeast Asia, maintained through a wildlife transmission cycle involving macaque hosts and mosquito vectors. While the disease is likely linked to the habitats of non-human host and vector species, spatial information on the disease's distribution is sparse and biased; the selection of sites for future surveillance should be carefully planned. An optimal spatial design for *P. knowlesi* malaria surveillance in western Indonesia will balance competing criteria. Sites proximal to land use and land cover types of interest, including forest and oil palm plantation, are often in areas that are difficult to access. We draw on the predictions of a geospatial model of relative *P. knowlesi* malaria risk, to prioritise sites where it is likely a case will be detected. We aim to select a network of sites, balancing epidemiological factors including risk model outputs with logistical factors such as site accessibility and a site network's overall "shadow". To avoid evaluating the utility of all possible surveillance designs, we apply methods of simulation-based optimal design, to explore design space and find approximately optimal designs for *P. knowlesi* malaria surveillance.

P20 **Fc-dependent functional antibody responses in immunity to severe *Plasmodium falciparum* malaria in children**

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Young children are particularly susceptible to severe and fatal forms of malaria caused by *Plasmodium falciparum*. Vaccines are a key intervention strategy to help reduce the global burden of malaria, however, there are currently no vaccines that target *P. falciparum* merozoites. 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 against *P. falciparum* merozoites likely function by directly blocking invasion and mediating Fc-dependent functional antibody responses. These Fc-dependent responses include complement activation and cross-linking with Fc-receptors on immune cells leading to antibody-dependent opsonic phagocytosis, cellular cytotoxicity or 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 will evaluate Fc-functional antibody responses 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.

P21 Zooming in on the *Plasmodium* dynamins

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Artemisinin is the world's primary antimalarial, meaning the biggest threat to global treatment is rising artemisinin resistance in the field. Due to its short half-life, artemisinin is commonly administered in combination with a longer-acting partner drug. Therefore, a rise in artemisinin resistance allows malaria time to develop extra resistance mutations against its partner too. We are exploring the cellular mechanisms behind artemisinin resistance in *Plasmodium falciparum* – the deadliest malaria species. We want to determine whether the three *Plasmodium* dynamin-like proteins are involved in artemisinin resistance. Since artemisinin is known to be activated by *P. falciparum*'s haemoglobin breakdown pathway, resistance is commonly associated with the way that *P. falciparum* feeds on red blood cells. However, no one has been able to accurately describe exactly how this feeding occurs. We believe that dynamin-like proteins likely play a role in feeding, and that a characterisation of this protein family will consequently contribute towards better understanding of artemisinin resistance. In a related eukaryotic parasite, dynamin-like proteins have been shown to form the trademark oligomeric rings usually associated with the 'pinching off' of cellular membranes, and these rings were even localised to feeding structures. Therefore, we believe it is important to determine whether this is also true of *P. falciparum*, since it will likely confirm a role in artemisinin resistance.

P22 **How to catch a parasite red-handed? Looking for *Plasmodium falciparum* exported proteins in the infected hepatocyte**

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Liver stages of *Plasmodium falciparum* infection are important targets for interventions, yet there is much still to learn about the molecular mechanisms that allow the parasite to establish the infection and not be eliminated by their host cell. It is likely that, as in other apicomplexan parasites, there are proteins exported from the parasite into the host cell that interfere with cellular immunity and allow the infection to progress. We aim to identify parasite proteins exported into the hepatocyte using proximity ligation approaches that have already been successfully established in blood-stage *P. falciparum*. We use two different hepatocyte cell lines, one expressing TurboID with the addition of a Nuclear Export Signal (NES) thus retaining it in the cytoplasm, and the other cell line expressing TurboID with a Nuclear Localisation Signal (NLS), both from Hepatitis D virus. Turbo-ID biotinylates proteins in its vicinity with a short pulse of biotin. Following *P. falciparum* sporozoite infection using a GFP fluorescent parasite line, infected hepatocytes are sorted and concentrated by flow cytometry to overcome the common low infection rate. The biotinylated proteins can be extracted using streptavidin beads, and then analysed by mass spectrometry. The analysis will focus on identifying both parasite proteins in the host cell and differences in host protein expression during infection.

P23 Identifying Merozoite Surface Proteins as Targets of Protective Functional Antibody Responses against *Plasmodium falciparum* and *P. vixax* Malaria

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Highly effective vaccines are needed for *Plasmodium falciparum* (Pf) and *P. vixax* (Pv) elimination. RTS,S is the only approved vaccine for Pf; efficacy is modest and short-lived in target populations. There are currently no *P. vixax* vaccines, with limited candidates in development. Antibodies play an important role in malaria immunity, and better understanding of targets and mechanisms of action of protective antibodies is needed to advance the development of Pf and Pv vaccines. Here, we aim to explore merozoite surface proteins (MSPs) of Pf and Pv as targets of protective functional antibody responses. We will evaluate antibody samples from naturally exposed individuals in malaria endemic regions and human and animal vaccines studies. Samples will be analysed in novel immunoassays identifying functions of antibodies targeting MSPs, including: antibody-mediated complement activation against merozoites and invasion inhibition, antibody binding to Fcγ-receptors and merozoite opsonic phagocytosis, and antibody dependent cellular cytotoxicity. Pf cell culture is well established, whereas long-term Pv culture is challenging. To overcome this, we will use gene-edited *P. knowlesi* parasites expressing Pv MSPs, and short-term culture of ex vivo clinical *P. vixax* field isolates. This work will increase our knowledge on the functional capacity of protective antibody responses against Pf and Pv and identify targets on merozoites to advance vaccine development.

P24 Molecular docking studies on the interaction of *Plasmodium falciparum* PfRH5 protein with selected antimalarial drugs

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PfRH5 is a 63-kDa protein of the reticulocyte-binding homolog (RH) family of the human malaria parasite, *Plasmodium falciparum*. These proteins are localized in the apical region of the merozoite and are involved in the invasion of erythrocytes. PfRH5 interacts with basigin (BSG), the receptor on the surface of RBCs. This interaction is essential in invasion, making PfRH5 a major blood stage malaria vaccine candidate. PfRH5 has ~500 amino acids and lacks a transmembrane domain. It is smaller than other RH proteins (ave. ~300kDa). The RBC-binding domain is at the cysteine-rich N-terminus. Here, the potential interactions of the PfRH5 protein with 10 antimalarials were studied using molecular docking. Docking results showed binding energies from –9.2 to -5.4 kcal/mole. PfRH5 and atovaquone were predicted to have the most stable interaction (-9.2 kcal/mole), followed by mefloquine (-8.8 kcal/mole) and lumefantrine (-7.6 kcal/mole). Atovaquone, mefloquine and lumefantrine were predicted to bind along the same region at residues 242 - 510. This region of PfRH5 is currently not described as a target of antimalarials. The interactions of PfRH5 with the antimalarials may include hydrophobic, van der Waals, pi-alkyl interactions and hydrogen bonds. These docking results predict that the selected antimalarials may interact with the PfRh5 protein. This may imply a possible mode of action of these drugs on the malaria parasite. Further studies may verify if these molecular interactions occur in vitro or in vivo. This may be helpful in drug design and in studying parasite resistance to current antimalarials.

P25 A screen for identifying inhibitors of *Plasmodium falciparum* protein export identifies dual inhibitors of protein trafficking and parasite invasion

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Due to emerging parasite resistance to front-line drugs, the discovery and characterisation of new anti-malarial drugs with novel modes of action is urgent. A potential source of novel druggable targets is the parasite's protein export pathway that delivers essential parasite proteins into the red blood cell (RBC). Exported proteins share a common essential export pathway, thus it is an attractive drug target.

We screened compound libraries for inhibitors of *P. falciparum* protein export, measuring the fraction of protein trafficked into the parasitophorous vacuole (PV) and RBC compartments after drug treatment. MMV396797 was identified as an inhibitor of protein secretion into the PV. MMV396797-resistant parasites revealed that this pyrazolopyrimidine inhibits parasite Phosphatidylinositol 4-kinase (PI4KIIIb), thus blocking protein transport at the Golgi.

Since parasites also traffic proteins to organelles specialised for invasion into RBCs, we screened compound libraries for invasion inhibitors. We found the pyridyl-furan compound, OGHL250, that was further engineered to produce a series of inhibitors with a low nanomolar potency. Due to structural relation to MMV396797, these compounds were tested against MMV396797-resistant parasites and were resisted. We found that these compounds block secretion and inhibit PI4KIIIb. The pyridyl-furan series appear to block invasion and/or the conversion of merozoites into ring-stage parasites through inhibition of protein trafficking.

P26 Sequence elements within the PEXEL motif and its downstream region modulate PTEX-dependent protein export in *Plasmodium falciparum*.

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In order to transform their host erythrocyte into a hospitable niche and to evade the human immune system, blood-stage *Plasmodium falciparum* parasites must export hundreds of proteins into this host cell. Most exported proteins contain a conserved N-terminal *Plasmodium* export element (PEXEL) with the consensus sequence RxLxE/Q/D. Proteolytic cleavage of the PEXEL in the parasite's endoplasmic reticulum licences the protein to be trafficked to the parasitophorous vacuole space that surrounds the parasite, putatively bound to the HSP101 chaperone. HSP101 then docks with the remaining components of the *Plasmodium* translocon for exported proteins (PTEX) at the vacuolar membrane. PTEX recognises the xE/Q/D-capped N-terminus of the cargo protein and translocates it into the host cell. Here, we show that charge reversal mutation of the PEXEL's conserved E/Q/D residue can reduce the efficiency of a) cleavage by the plasmepsin V protease in the endoplasmic reticulum and b) PTEX-mediated export across the vacuolar membrane, suggesting a dual role for this residue. We also present evidence that both the length and the amino acid composition of the unstructured 'spacer' region between the PEXEL and the folded functional region of the exported protein are important for cargo interaction with PTEX, with truncation of the 'spacer' or mutation of its hydrophobic amino acids resulting in markedly reduced export.

P27 Interleukin-2, not chemokine receptors CXCR3, CXCR5 or CXCR6, controls Th1/Tfh fate bifurcation during blood-stage malaria

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CD4+ T cell immunity to blood-stage malaria is suboptimal in humans and experimental mice. Two subsets, T-helper 1 (Th1) and follicular helper T cells (Tfh), partially control *Plasmodium* parasites via IFNγ or by supporting high-affinity antibody production [1]. Discovery of mechanisms controlling Th1/Tfh fate may offer new opportunities for accelerating the onset of immunity to malaria. Previously, we observed early co-expression of chemokine receptors CXCR3 and CXCR5 by *Plasmodium*¬-specific TCR transgenic PbTII cells in P.chabaudi¬-¬infected mice, prior to their bifurcation towards either Th1 or Tfh fates [2]. We hypothesized here 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 and examined for effects on 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, played a critical role in supporting Th1 differentiation, but not clonal expansion. Further experiments suggested IL-2-signalling also controls CD4+ T cell differentiation during experimental vaccination. Hence, the balance between humoral and cellular immunity during malaria infection or vaccination can be modulated by Interleukin-2.

P28 Transmission dynamics and population structure of *P. falciparum* and *P. vixax* in Mondulkiri Province, Cambodia

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A cross-sectional survey conducted in Kaev Seima District, Mondulkiri Province in Cambodia by the Asia Pacific ICEMR demonstrated that living and working in forested areas is a risk factor for both *P. falciparum* and *P. vixax* malaria. It is not known whether infections outside the forest are driven by infections acquired inside the forest. Population genetic analyses can reveal 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. vixax* 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 for elimination.

P29 Expanding molecular detection of malaria utilising Rapid Diagnostics Tests from surveillance programs in the Greater Mekong Subregion

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Clinical malaria cases in the Greater Mekong Subregion (GMS) have been declining due to the deployment of several interventions, including the use of Rapid Diagnostic Tests (RDT). RDT is widely used for screening symptomatic patients, but subclinical and asymptomatic malaria may not be diagnosed due to their low-density infections below the RDT detection level (200 parasites/µL). To strengthen the surveillance in elimination programs, we aimed to optimise a protocol to improve the detection of residual malaria that is being missed by conventional approaches. We tested four DNA extraction protocols in RDTs that were spiked with laboratory-cultured parasites and optimised a protocol to process samples in a high-throughput manner. Our protocol uses a small portion of the RDT for molecular assays, allowing its remains to be stored for serosurveillance. Multiplex real-time PCR was performed to detect *Plasmodium* spp. infections, lowering the limit of detection to <2 parasites/µL. To validate and evaluate the effectiveness of the use of tested RDTs for molecular assays, and to augment surveillance of residual malaria in the GMS, we will process RDTs collected by community health workers in 488 villages across Lao PDR and Cambodia between July 2021 and February 2023 (n=12,137). This study will determine the utility and feasibility of performing molecular and serological surveillance in used RDTs to improve the sensitivity of current programs, and thus accelerate malaria elimination.