30-31 October 2025
Bio21 Molecular Science & Biotechnology Institute
Parkville, Australia

Melbourne Malaria2025

Program

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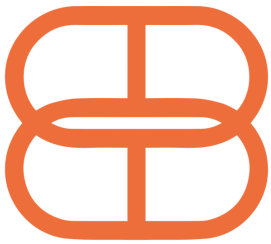

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Program at a glance

Day 1: Thursday 30th October

08:30 - 09:20	Registration
09:20 - 09:30	Welcome and Housekeeping
09:30 - 10:20	**Plenary I: Steven Kho**
10:20 - 10:45	**Short Talks I**
10:45 - 11:10	Morning tea
11:10 - 12:30	**Long Talks I: Molecular Epidemiology & Public Health**
12:30 - 14:00	**Poster Session I**
13:00 - 14:00	Lunch
14:00 - 15:20	**Long Talks II: Drugs**
15:20 - 15:45	**Short talks II**
15:45 - 16:10	Afternoon tea
16:10 - 17:30	**Long Talks III: Novel Techniques**

Day 2: Friday 31st October

09:30 - 10:20	**Plenary II: Adele Lehane**
10:20 - 10:45	**Short Talks III**
10:45 - 11:10	Morning tea
11:10 - 12:30	**Long Talks IV: Immunology**
12:30 - 14:00	**Poster Session II**
13:00 - 14:00	Lunch
14:00 - 15:20	**Long Talks V: Molecular & Cellular Biology**
15:20 - 15:45	**Short talks IV**
15:45 - 16:10	Afternoon tea
16:10 - 17:00	Wrap up & Awards
18:00	Social Event at the Queensberry Hotel

Organising Committee

Carlo Giannangelo
Monash University

Mary-Lou Wilde
The University of Melbourne

Alessia Hysa
Burnet Institute

Katelyn Stanhope
Burnet Institute

Mohini Anjna Shibu
The University of Melbourne

Mun Hua Tan
The University of Melbourne

Sophie Collier
The University of Melbourne

Location

Bio21 Institute of Molecular Science & Biotechnology

Address: 30 Flemington Rd, Parkville, 3052

Accessibility: Bio21 is a fully accessible venue, offering lift and step-free access to ensure all delegates can navigate the space comfortably.

Bio21 Map

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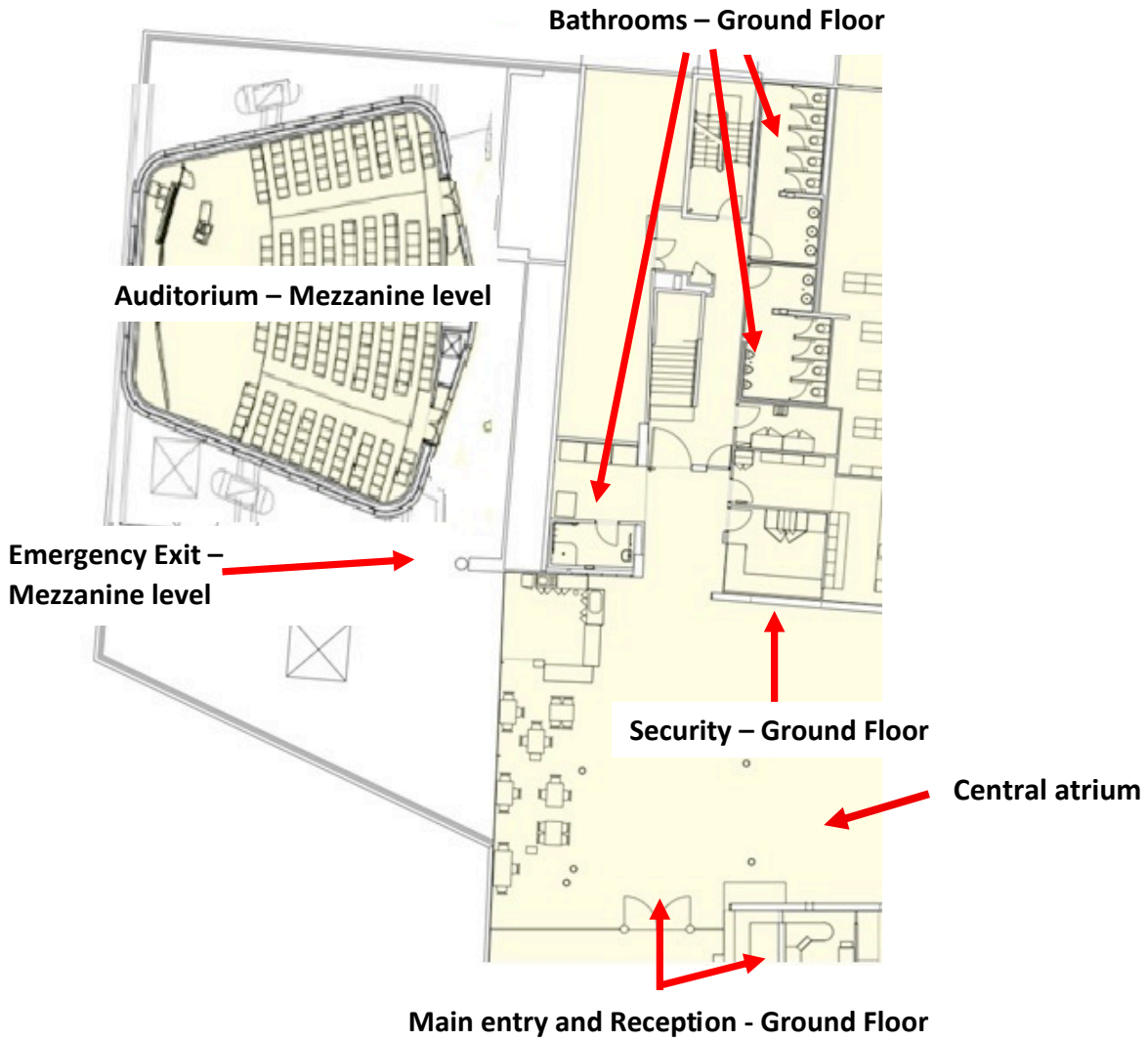

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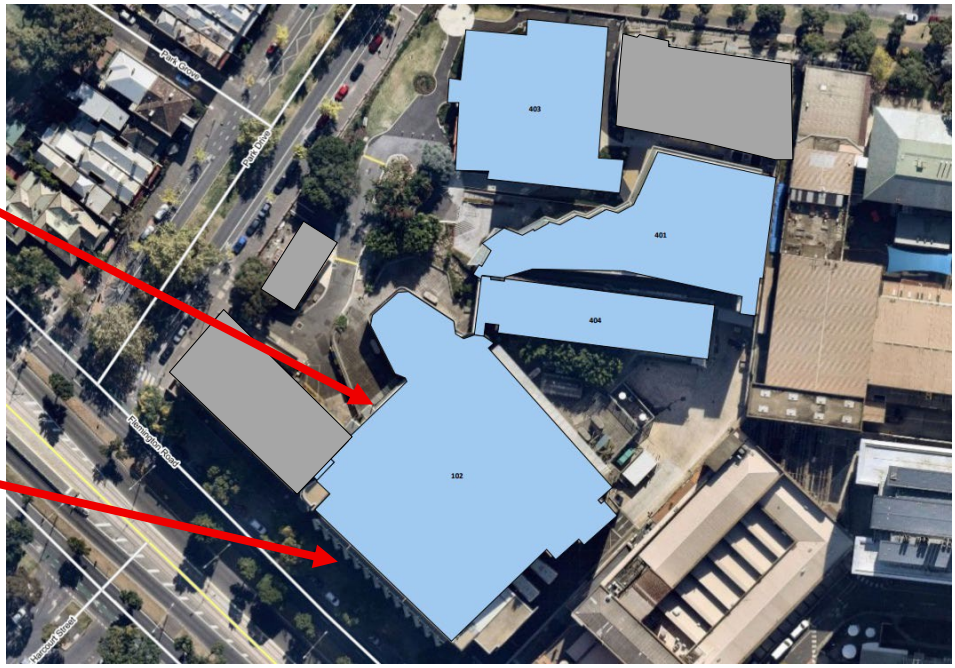

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08:30 - 09:20 Registration

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

09:30 - 10:20 Plenary session I
Session Chair: Mun Hua Tan

Hidden Splenic Reservoirs of *P. vivax* and *P. falciparum*
Steven Kho - Menzies School of Health Research and Charles Darwin University

Sponsored by The University of Melbourne School of BioSciences

10:20 - 10:45 Short Talks I
Session Chairs: Edith Spiers & Dionne Argyropoulos

ST1 **Applying tensor-structured decomposition to identify patterns of protective immunity against malaria**
Isobel Walker – Peter Doherty Institute, University of Melbourne,

ST2 **Antibody-dependent neutrophil phagocytosis of *Plasmodium falciparum* infected erythrocytes is mediated by FcγRIIa**
Maria Saeed – The Peter Doherty Institute, University of Melbourne

ST3 **Dysregulated germinal centre: Increased splenic regulatory Tfh and atypical memory B cells in chronic malaria**
Damian Oyong – Burnet Institute

ST4 **Investigating the role of Kelch 13 protein in *Plasmodium falciparum* gametocytes**
Haowen Deng – Department of Biochemistry and Pharmacology, University of Melbourne

10:45 - 11:10 Morning tea

11:10 - 12:30 **Long Talks I: Molecular Epidemiology & Public Health**
Session Chairs: Katelyn Stanhope & Ellen Kearney
Sponsored by the Burnet Institute

LT1 **Dynamics of *Plasmodium malariae* infections in Papua New Guinea**
Myo Naung – Burnet Institute & Centre for Innovation in Infectious Diseases and Immunology Research (CIIDIR), Institute of Mental and Physical Health and Clinical Translation (IMPACT) and School of Medicine, Deakin University

LT2 **Understanding the distributions of African malaria vectors**
Gerry Ryan – The Kids Research Institute Australia & The University of Melbourne

LT3 **The cost-effectiveness of novel strategies for the radical cure of vivax malaria in Ethiopia**
Noah Savarirayan – Melbourne School of Population and Global Health, The University of Melbourne

LT4 **Molecular epidemiology of clinical and asymptomatic *Plasmodium falciparum* malaria at low transmission, and sources of resurgent infection**
Sonakshi Madan – Centre for Innovation in Infectious Disease and Immunology Research, IMPACT and School of Medicine, Deakin University

LT5 **Population genetic signatures of *Plasmodium falciparum* transmission decline and rebound in a hyperendemic area of Papua New Guinea**
Kirsty M. McCann – Centre for Innovation in Infectious Disease and Immunology Research (CIIDIR), Institute for Mental and Physical Health and Clinical Treatment (IMPACT), School of Medicine, Deakin University & Burnet Institute

12:30 - 14:00 **Poster Session I**
13:00 - 14:00 Lunch (Concurrent with Poster Session I)

14:00 - 15:20 **Long Talks II: Drugs**

Session Chairs: Carlo Giannangelo & Eileen Zhou

Sponsored by Molecular and Biochemical Parasitology

LT6 **PfK13-associated artemisinin resistance slows drug activation and enhances antioxidant defence, which can be overcome with sulforaphane.**

Ghizal Siddiqui – Monash Institute of Pharmaceutical Sciences, Monash University

LT7 **Repurposing human medicines as novel antimalarials against *P. falciparum* malaria.**

Khoi Nguyen – Burnet Institute

LT8 **Combination of redox modifiers with artemisinin results in increased parasite susceptibility to artemisinins**

Annie Roys – Monash University

LT9 **Dual inhibition of Plasmepsins IX and X in *Plasmodium falciparum* sporozoites inhibits development within *Anopheles stephensi* mosquitoes**

Elena Lantero-Escolar – WEHI

LT10 **Unfolding malaria parasite biology: targeting protein disulphide isomerases to block *Plasmodium* invasion and transmission**

Senna Steen – Burnet Institute

15:20 - 15:45 **Short talks II**

Session Chairs: Carlo Giannangelo & Eileen Zhou

ST5 **Identifying adaptive immune signatures of protection against *P. vivax* malaria**

Pinkan Kariodimedjo – Burnet Institute

ST6 **Unravelling clindamycin resistance in *Plasmodium***

Jessica Home – School of BioSciences, The University of Melbourne

ST7 **Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance**

Hanqing Zhao - WEHI

ST8 **The voltage dependent ion channel is an essential mitochondrial protein in *P. falciparum***
Mitchell Trickey – Institute for Mental and Physical Health and Clinical Translation (IMPACT), Deakin University & Monash Institute of Pharmaceutical Sciences, Monash University

15:45 - 16:10 Afternoon tea

16:10 - 17:30 **Long Talks III: Novel Techniques**
Session Chairs: Sophie Collier & Jessica Home

LT11 **PvSeroTaT: Optimisation and standardisation of a machine learning method to identify individuals recently infected with *Plasmodium vivax***
Dionne Argyropoulos – WEHI

LT12 **Expansion microscopy reveals how Kelch 13 mutations impair feeding in artemisinin resistant *Plasmodium* parasites**
Long Kim Huynh – Department of Biochemistry and Pharmacology, The University of Melbourne

LT13 **Validation of Solvent Proteome Profiling for Antimalarial Drug Target Deconvolution**
Jessica Ji – Monash Institute of Pharmaceutical Sciences, Monash University

LT14 **Feeding antimalarials to mosquitoes disrupts malaria parasite transmission**
Sarah N. Farrell – School of Biosciences, The University of Melbourne

09:30 - 10:20 **Plenary session II**
Session Chair: Mary-Lou Wilde

Transporters as drug targets and resistance determinants
Adele Lehane – Australian National University

Sponsored by the Monash Institute of Pharmaceutical Sciences Global Health Therapeutic Program Area

10:20 - 10:45 **Short Talks III**
Session Chairs: Mary-Lou Wilde & XueXin Xia

ST9 **Novel approaches to identifying key targets of immunity to *Plasmodium vivax***
Kaitlin Pekin – Burnet Institute

ST10 **Distinct and divergent neutrophil profiles in human infections with *P. falciparum* and *P. vivax***
Sandra Chishimba – Burnet Institute

ST11 **Determining *P. falciparum* virulence antigens that elicit protective immunity against severe malaria**
Prerna Prashanth – Bio21 Institute, The University of Melbourne

10:45 - 11:10 Morning tea

11:10 - 12:30 **Long Talks IV: Immunology**
Session chairs: Alessia Hysa & Alexander Harris
Sponsored by the Peter Doherty Institute for Infection and Immunity

LT15 **Malaria Drives the Emergence of Cytotoxic Memory from Phenotypically Diverse Innate Vδ2 T Cells**
Nicholas Dooley – Burnet Institute

LT16 **Anti-malarial drugs impose quantitative not qualitative effects on B-cell immunity in experimental malaria**
Oliver Skinner – Peter Doherty Institute, University of Melbourne

LT17 **Multi-Antigen mRNA Vaccine Development for Blood-Stage Malaria**
Timothy Ho – Burnet Institute

LT18 **Can JAK/STAT inhibition improve humoral immunity in malaria?**
Julianne Hamelink – Burnet Institute, The University of Queensland & Queensland Institute of Medical Research

LT19 **Repeated *Plasmodium* infection in mice progressively modulates CD4+ T cell recall**
Brooke Wanrooy – Peter Doherty Institute, University of Melbourne

12:30 - 14:00 **Poster Session II**
13:00 - 14:00 Lunch (Concurrent with Poster Session II)

14:00 - 15:20 **Long Talks V: Molecular & Cellular Biology**
Session chairs: Mohini Shibu & Elena Lantero-Escolar

LT20 **Characterising the role of glideosome-associated proteins throughout *P. falciparum* development**
Katrina Larcher – Doherty Institute, University of Melbourne & WEHI

LT21 **The Bromodomain Protein PfBDP2 Regulates Chromatin Structure and Gene Expression in *P. falciparum***
Nicholas C. Lauw – Faculty of Science, The University of Melbourne & Bio21 Institute

LT22 **PfPTRAMP-CSS as a multi-stage malaria vaccine target – Part 1**
Pailene S. Lim – WEHI

LT23 **PfPTRAMP-CSS as a multi-stage malaria vaccine target – Part 2**
Nicolai Jung – WEHI,

LT24 **Improving the capacity of vaccine-induced antibodies to arrest the growth of *Plasmodium falciparum***
Alysha Literski – Burnet Institute

15:20 - 15:45 **Short talks IV**
Session Chairs: Chiara Drago & Patience Nayebare

ST12 **The GID E3 ligase complex controls cell fate programs for sexual development of *Plasmodium falciparum***
Danushka Marapana – WEHI

ST13 **Developing a Human Lymphoid Organ Model to Decode Immune Response to Malaria**
Rama Dhenni – Burnet Institute

ST14 **Validating antibody prevalence to PkSERA3 antigen 2, a *Plasmodium knowlesi*-specific biomarker of exposure.**
Zi Kang Ooi – WEHI

15:45 - 16:10 Afternoon tea

16:10 - 17:00 Wrap up & Awards

18:00 Social Event at the Queensberry Hotel
593 Swanston St, Carlton VIC 3053

Talk abstracts

Day 1: Thursday 30th October

Plenary I — Thursday (09:30am – 10:20am)

Session chair: Mun Hua Tan

Hidden Splenic Reservoirs of *P. vivax* and *P. falciparum*

Steven Kho[1]
[1]Menzies School of Health Research and Charles Darwin University, Australia

Global malaria elimination is complicated by the existence of hidden infectious reservoirs. In a paradigm-shifting discovery, we have revealed that the human spleen is the major parasite reservoir in individuals naturally-infected with the two human malaria species responsible for most global morbidity and mortality, *Plasmodium falciparum* and *P. vivax*. The magnitude of splenic parasite concealment was substantial, reshaping the vision of malaria as an infection developing predominantly in the spleen rather than exclusively in the blood, sustained by a novel intrasplenic parasite life cycle accentuated by pooling of target cells. These findings have transformed malaria biology and supports the need for a shift in malaria research to focus on the spleen. Parasite populations in the spleen represent additional obstacles in malaria elimination and likely arises from novel mechanisms in *Plasmodium* biology and human malaria that could be targeted to reduce global malaria burden. The overarching goal of the Menzies spleen program is to elucidate the fundamental biology of the newly-discovered splenic *Plasmodium* population in humans, and identify novel approaches to detect and overcome splenic parasite survival. This seminar will discuss our recent discoveries in splenic malaria and ongoing research including in the field of pathophysiology, genetics, diagnostics and modelling.

Session chairs: Dionne Argyropoulos & Edith Spiers

ST1 Applying tensor-structured decomposition to identify patterns of protective immunity against malaria

Isobel Walker[1], Oscar Lloyd Williams[1], Aaron Meyer[2], Yuchi Ji[1], Wina Hasang[1], James Kazura[3], Sidney Ogalla[4], David Midem[5], Laurens Manning[6], Timothy Davis[6], Ivo Mueller[7], Moses Laman[8], Elizabeth Aitken[1], Stephen Rogerson[1]

[1]Peter Doherty Institute for Infection and Immunity, University of Melbourne, Australia; [2]Samueli School of Engineering, University of California, Los Angeles, USA; [3]Case Western Reserve University, USA; [4]Kenya Medical Research Institute, Centre for Global Health Research, Kenya; [5]Chulaimbo Sub-County Hospital, Kisumu, Kenya; [6]Universtiy of Western Australia, Australia; [7]Walter and Eliza Hall Institute of Medical Research, Australia; [8]Papua New Guinea Institute of Medical Research, Papua New Guinea;

Both the quantity and quality of antibodies are important for immunity to malaria. 'Systems serology' involves measuring antibodies to multiple antigens, as well as multiple antibody Fc features, like isotype, subclass and leukocyte receptor binding. Such studies generate large, high-dimensional data that is challenging to interpret.

We have applied a technique called 'Tensor-structured decomposition' to simplify systems serology data into 'components' that summarize unique points of variation. Importantly, the contribution of the original variables to each component can be visualized across multiple dimensions (for example, across subjects, times, antigens and Fc features), offering a key advantage over other dimension reduction techniques. We have applied tensor-structured decomposition to two systems serology datasets, to uncover patterns of protective immunity against severe malaria in Papua New Guinean children and Kenyan children.

In Papua New Guinean children, we determined that overall cytophilic antibodies to all variant surface antigens measured were associated with protection from severe malaria. In Kenyan children, we identified a subset of variant surface antigen targets of antibodies that are associated with protection from cerebral malaria across three time points. Tensor-structured decomposition provides novel insights into naturally acquired protective immunity to severe malaria and can be applied to other multi-dimensional datasets.

ST2 Antibody-dependent neutrophil phagocytosis of Plasmodium falciparum infected erythrocytes is mediated by FcγRIIa

Maria Saeed[1], Elizabeth Aitken[1,2], Bruce Wines[3,4], Stephen Rogerson[1,5]

[1]Department of Infectious Diseases,The Peter Doherty Institute, University of Melbourne, Australia; [2]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia; [3]Immune Therapies Group, Burnet Institute for Medical Research and Public Health, Melbourne, VIC, Australia; [4]Department of Immunology, School of Translational Medicine, Monash University, Melbourne, Australia; [5]Department of Medicine, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia.

FcγRIIIb, a glycosylphosphatidylinositol (GPI) linked receptor, is the most abundant FcγR on neutrophils followed by FcγRIIa. There is evidence that antibody dependent neutrophil phagocytosis (ADNP) protects against malaria, but the role of specific FcγRs involved is not clear. To probe the relative importance of FcγRIIIb and FcγRIIa in ADNP of *Plasmodium falciparum* infected erythrocytes (IEs), purified neutrophils from healthy donors were treated with tumor necrosis factor (TNF) to mobilize the intracellular FcγRIIIb to the surface followed by phosphatidylinositol phospholipase C (PIPLC) treatment to cleave FcγRIIIb. In TNF/PIPLC treated neutrophils, relative gMFI of FcγRIII decreased by 79% while FcγRIIa detection increased by 82%, compared to untreated neutrophils (relative gMFI = 100%). When opsonised IEs were incubated with TNF/PIPLC treated neutrophils, relative ADNP by FcγRIIIb-depleted neutrophils increased (585% ± 108%) compared to untreated neutrophils (100%, $p = 0.042$). Using FcγR blocking we show that compared to no-blocker (relative phagocytosis = 100%), relative ADNP was reduced over five-fold by FcγRIIa blocker alone (~17% ± 1.5%, $p<0.05$) and to the same degree by combined FcγRIIa and FcγRIII blockers (~24% ± 5.5%, $p <0.05$). We found that FcγRIIa is the main phagocytic receptor mediating ADNP of IEs and FcγRIIIb acts as a decoy receptor.

ST3 Dysregulated germinal centre: Increased splenic regulatory Tfh and atypical memory B cells in chronic malaria

Damian Oyong[1,13], Jessica Loughland[1], Putu Wardani[3], Hasrini Rini[2], Noy Kambuaya[2], King Alexander[3], Theo Situmorang[2], Leo Leonardo[2], Agatha Puspitasari[4], Ristya Amalia[4], Aisah Amelia[4], Maulina Aini[4], Dewi Margayani[2], Desandra Rahmayenti[2], Prayoga Prayoga[2], Leily Trianty[5], Enny Kenangalem[2,3], Nurjati Siregar[6], Pierre Buffet[7,8], Stuart Tangye[9,10], Jeanne Rini Poespoprodjo[2,3,11], Rintis Noviyanti[5], Nicholas Anstey[12], Steven Kho[2,12], Michelle Boyle[1,13]

[1]Burnet Institute, Australia; [2]Timika Malaria Research Program, Papuan Health and Community Development Foundation, Timika, Papua, Indonesia; [3]Rumah Sakit Umum Daerah Kabupaten Mimika, Timika, Papua, Indonesia; [4]Exeins Health Initiative, Jakarta, Indonesia; [5]Eijkman Research Center for Molecular Biology-BRIN, Cibinong, Indonesia; [6]Rumah Sakit Cipto Mangunkusumo and University of Indonesia, Jakarta, Indonesia; [7]UMR_S1134, BIGR, Inserm, Université de F-75015 Paris, Paris, France; [8]Laboratory of Excellence GR-Ex, Paris, France; [9]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; [10]School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, Sydney, NSW, Australia; [11]Department of Paediatrics, University of Gadjah Mada, Yogyakarta, Indonesia; [12]Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, NT, Australia; [13]Monash University, Central Clinical School, Melbourne, VIC, Australia.

Immunity to malaria develops slowly, and sterilising protection is rarely achieved despite repeated infections. This may reflect malaria-driven disruption of adaptive immunity, leading to impaired memory responses and reduced vaccine efficacy. A key site where adaptive immunity develops is the germinal centre (GC), particularly within spleen for clearing blood-borne pathogens. GC reactions rely on coordinated interactions between T follicular helper (Tfh) cells and B cells to generate high-affinity antibodies. However, recent studies show that the spleen is also a major reservoir for *Plasmodium* parasites. While splenic architecture is disrupted during chronic malaria, impact on GC responses, particularly on GC Tfh and B cells, remains poorly defined. We examined 58 human spleens collected during splenectomy in Timika, Indonesia, including individuals with *P. falciparum*, *P. vivax*, and endemic controls. Chronic malaria was associated with GC expansion, characterised by increased GC Tfh ($CD4+CXCR5^{hi}PD1^{hi}$) and plasmablasts. While *P. falciparum* malaria was marked by Tfh1-dominated response, *P. vivax* infection showed skewing towards Tfh2 cells. Single-cell transcriptomics identified GC Tfh with expression of *IFNG* and *LAG3* in *P. falciparum*, suggesting a regulatory phenotype. Concurrently, atypical memory B cells were expanded during infection. Chronic malaria drives dysregulated GC responses, highlighting parasite-mediated immunomodulation with implications for vaccine strategies.

ST4 Investigating the role of Kelch 13 protein in *Plasmodium falciparum* gametocytes

Haowen Deng, Sophie Collier, Stuart Ralph
Department of Biochemistry and Pharmacology, University of Melbourne, Australia.

Resistance *of Plasmodium falciparum* to artemisinin, the current frontline anti-malarial, greatly threatens global malaria control. This resistance is mainly driven by mutations in the parasites' Kelch 13 (K13) protein. During the intraerythrocytic cycle, K13 is likely involved in stabilising the parasites' cytostome, a double membrane invagination responsible for haemoglobin uptake from the host red blood cell, by forming a ring around the cytostomal neck. Activation of artemisinin requires reaction with haem released from digested haemoglobin. The K13 mutation is thought to cause drug resistance through decreasing cytostomal uptake of haemoglobin, leading to reduced activation of artemisinin. How K13 modulates this process is undetermined. K13's role in gametocyte stages is unknown, and the role of K13 and K13 mutations on parasite transmission is also unresolved. Using an endogenously tagged version of K13, we imaged K13 throughout gametocyte development, using standard fixes as well as expansion microscopy. In early gametocytes (stages I-III), K13 was resolved to form a ring structure, similar to its asexual counterpart. However, in late-stage gametocytes, K13 formed a hollow tubular structure, likely serving a function distinct from nutrient uptake. We will investigate the essentiality of K13 in gametocytes and its potential role in differential transmission of artemisinin-resistant parasites.

Session Chairs: Katelyn Stanhope & Ellen Kearney

LT1 Dynamics of *Plasmodium malariae* infections in Papua New Guinea

Andreas Wangdahl[1,2], **Myo Naung[1,3],** Zahra Razook[1,3], Maria Ome-Kaius[4], Moses Laman[4], Ivo Mueller[5], Alyssa Barry[1,3], Leanne Robinson[1,5]

[1]Life Sciences, Burnet Institute, Melbourne, Victoria, Australia; [2]Karolinska Institutet, Stockholm, Sweden; [3]Centre for Innovation in Infectious Diseases and Immunology Research (CIIDIR), Institute of Mental and Physical Health and Clinical Translation (IMPACT) and School of Medicine, Deakin University, Geelong, Victoria, Australia; [4]Vector Borne Diseases Unit, Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea; [5]Infection and Global Health Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Plasmodium malariae, remains one of the most neglected among human-infecting *Plasmodium* species, with limited data on its epidemiology, genetic diversity, and infection dynamics. Here, we present the first comprehensive genetic surveillance of *P. malariae* in Papua New Guinea (PNG), characterising infection patterns and population diversity using microsatellite markers. From two longitudinal cohorts including 768 children aged 0.5-11 years, a total of 374 samples qPCR-positive for *P. malariae* from 239 different individuals were genotyped. Samples from the same individuals at multiple timepoints were available allowing analysis of within-host infection dynamics. *P. falciparu*m co-infection increases the odds of *P. malariae* infection (aOR 9.0, 95% CI 5.6–14.6) while age, sex, and bed-net use were not significant. Analysis of six microsatellite loci revealed substantial genetic diversity, with expected heterozygosity (He) ranging from 0.18 to 0.77. Allele frequencies varied significantly across markers, with some markers dominated by single alleles while others showed balanced diversity. Availability of multiple longitudinal samples from the same individuals enables within-host analyses to distinguish among persistent infection, recrudescence, and new infections, providing insight into *P. malariae* dynamics over time. This study establishes a foundational framework for understanding the genetic diversity, molecular epidemiology, and infection dynamics of P. malariae in PNG.

LT2 Understanding the distributions of African malaria vectors

Gerry Ryan[1,2], Marianne Sinka[3], Nick Golding[1,4]

[1]The Kids Research Institute Australia, Australia; [2]University of Melbourne; [3]University of Oxford; [4]University of Western Australia.

Malaria control programs are eager to understand the spatial distributions of the mosquitoes that transmit malaria in order to efficiently target activities like vector control and bed net distribution. Here we present new distribution maps dominant and secondary vectors in Africa, including the first such maps for several species. Our work is based on the Vector Atlas, a new online data hub with a comprehensive collection of malaria vector occurrence records. Although previous maps exist for several dominant vector species, we update these with new data, and develop new methods that allow us to account for the sampling biases inherent in collection data, and physiological limits on occurrence. Our maps are a key resource for informing malaria control activities in Africa. With linkage to the Vector Atlas, they can be systematically updated as data collection continues, and in collaboration with in-country researchers and national malaria programmes, are augmented to answer specific questions in malaria control with additional country-level data.

LT3 The cost-effectiveness of novel strategies for the radical cure of vivax malaria in Ethiopia

Noah Savarirayan[1], Tamiru Shibiru Degaga[2,3], Megha Rajasekhar[1], Eyerusalem Beyene Erjabo[3], Samuel Alemu Bamboro[3], Tedla Teferri Tego[4], Hellen Mnjala[2], Grant Lee[2], Rodas Temesgen[3], Fitsum Getahun Kiros[3], Kamala Thriemer[2], Angela Devine[1,2]

[1]Melbourne School of Population and Global Health, University of Melbourne; [2]Division of Global and Tropical Health, Menzies School of Health Research, Charles Darwin University; [3]College of Medicine & Health Sciences, Arba Minch University, Arba Minch, Ethiopia; [4]Arba Minch General Hospital, Arba Minch, Ethiopia.

Background

Radical cure of vivax malaria eliminates liver hypnozoites but risks haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This study examined the cost-effectiveness of G6PD screening before prescribing 7-day-high-dose primaquine or single-dose tafenoquine compared to standard care in Ethiopia (14-day-low-dose primaquine without G6PD screening).

Methods

Data were derived from the EFFORT trial (NCT04411836) and supplemented through a literature review. A decision tree model was constructed from the provider perspective. Incremental cost-effectiveness ratios were compared against a threshold of $927 per disability-adjusted life-year (DALY) averted. One-way and probabilistic sensitivity analyses were performed. Costs were reported in 2024 United States Dollars ($).

Results

Tafenoquine was cost-effective compared to standard care, with a cost of $408 per DALY averted from the provider's perspective, decreasing to $274 from the societal perspective. Tafenoquine costs less and averts DALYs when compared to 7-day-high-dose primaquine. The results were most sensitive to the rate of symptomatic recurrence and drug prices in the one-way sensitivity analysis. Tafenoquine had an 80% probability of being cost-effective compared to standard care at the threshold of $927 per DALY averted.

Conclusions

Tafenoquine is cost-saving and averts DALYs compared to 7-day-high-dose primaquine, and is likely cost-effective compared to standard care in Ethiopia.

LT4 Molecular epidemiology of clinical and asymptomatic *Plasmodium falciparum* malaria at low transmission, and sources of resurgent infection

Sonakshi Madan[1], Kirsty McCann[1,2], Zahra Razook[1,2], Dulcie Lautu-Gumal[1,2,3,4], Shazia Ruybal-Pesántez[3,4,5], Benson Kiniboro[6], Peter M. Siba[6], Stephan Karl[7], Maria Ome-Kaius[6], Moses Laman[6], James W. Kazura[8], Ivo Mueller[3,4], Leanne J. Robinson[2,3,6], Alyssa E. Barry[1,2,3]

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Molecular epidemiology of malaria at low transmission can inform control efforts by monitoring parasite populations and identifying transmission dynamics over space, time and other epidemiological variables. Low-transmission regions report high proportions of sub-microscopic, asymptomatic *P. falciparum* infections. Without active-surveillance, these infections persist as transmission reservoirs and may form isolated, genetically distinct parasite populations. Limited exposure to local strains may heighten susceptibility to severe clinical malaria through foreign genotypes, associated with their epidemic-like spread through the population. We hypothesise that asymptomatic infections in low-transmission regions arise from locally circulating strains whereas clinical infections are associated with genetically-distinct foreign strains. We compared *P. falciparum* isolates using genetic markers designed to capture population structure, geographic origin (SNP barcode) and strain diversity (varcode). Samples from Papua New Guinea – clinical and asymptomatic from 2012 (low-transmission), and asymptomatic from 2016 (resurgence) were genotyped to measure associations with symptoms and resurgence. At low transmission, clinical infections are genetically distinct to residual asymptomatic infections.Some asymptomatic lineages persisted across years, possibly contributing to resurgence. These results provide insights into malaria dynamics during low transmission and will help identify sources of resurgent infections for better planning and efficient resource distribution to track, control and eliminate malaria in endemic regions.

LT5 Population genetic signatures of *Plasmodium falciparum* transmission decline and rebound in a hyperendemic area of Papua New Guinea

Kirsty M. McCann[1,2], Dulcie Lautu-Gumal[2,3,4], Zahra Razook[1,2], Rasha K. Elias[1], Shazia Ruybal-Pesantez[5], Somya Mehra[2], Elma Nate[6], Maria Ome-Kaius[6], Moses Laman[6], Ivo Mueller[4], Leanne J. Robinson[2,7], Alyssa E. Barry[1,2,3,4]

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Malaria genomic surveillance is key to understanding how control strategies drive elimination. Classifying transmission dynamics and zone boundaries helps assess control impact and guide targeted efforts. In Papua New Guinea, extensive measures since 2006 initially reduced parasite prevalence, but recent rebounds and artemisinin resistance have emerged. We used SNP barcoding to genotype 624 *P. falciparum* isolates collected from Madang and East Sepik Provinces at nine time points between 2005-2020. We conducted population genetic analyses to investigate genomic signatures of transmission decline and rebound, and features that may explain the resurgence. Increasing proportions of related parasites were observed during decline consistent with a population bottleneck and more focal transmission, followed by an increase in related pairs in East Sepik in 2016 when prevalence rebounded which were maintained in more recent timepoints, i.e., 2019. These patterns suggest dynamic shifts in transmission, with resurgence allowing for both persistence and diversification of parasite lineages. Relatedness networks reveal the presence of several clonal lineages after transmission reduced, with expansion and recombination of distinct lineages as transmission rebounded. This highlights the need for sustained control to reduce the parasite population and advance malaria elimination in PNG.

Session Chairs: Carlo Giannangelo & Eileen Zhou

LT6 PfK13-associated artemisinin resistance slows drug activation and enhances antioxidant defence, which can be overcome with sulforaphane.

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Artemisinin resistance is increasingly prevalent in Africa, raising concerns and highlighting the need to better understand the cellular mechanisms behind this resistance. In P*lasmodium falciparum*, artemisinin resistance is primarily attributed to mutations in the PfKelch13 gene. In this study, we performed proteomics analysis on a range of artemisinin-resistant (both laboratory-generated and field isolates) and sensitive *P. falciparum* parasites at 3-6h ring- and 22-24h trophozoite-stage, revealing dysregulation of only PfKelch13 protein abundance. Reduced PfKelch13 levels were linked to impaired hemoglobin digestion, decreased free heme levels, and consequently, decreased artemisinin activation. Resistant parasites also exhibited elevated thiol levels, indicating a more reduced state. Targeting the parasite redox capacity with sulforaphane potentiated artemisinin activity *in vitro* and *in vivo*, offering a potential strategy to overcome resistance. Our findings provide critical insights into the molecular mechanisms of artemisinin resistance and suggest novel therapeutic interventions to restore drug sensitivity.

LT7 Repurposing human medicines as novel antimalarials against *P. falciparum* malaria.

Khoi Nguyen[1,2], Brendan Crabb[1,2], Hayley Bullen[1,2], Paul Gilson[1,2]

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Plasmodium falciparum has developed drug resistance to nearly all available antimalarials, highlighting the critical need for novel therapeutics to eliminate malaria. However, *de novo* drug discovery is often challenged with lengthy pre-clinical and clinical testing, and requires considerable financial investments before the therapies can reach the clinic. Therefore, to accelerate drug development and reduce costs, we screened a library of regulatory-approved small-molecule drugs for other human indications that could be repurposed as novel antimalarials against *P. falciparum*. A library of 634 compounds was screened using bioluminescent reporter parasites to quantify growth inhibition and we found 48 candidates that inhibited >80% parasite growth when used at 2μM. We further triaged this list down to 9 compounds, eliminating the rest due to undesirable properties. Through stage-specific growth inhibition assays, we discovered two antivirals and one antibacterial that inhibited all key stages of *P. falciparum*'s asexual life cycle. To identify the targets of our anti-infective compounds, we next selected for mutations that conferred drug resistance but were unsuccessful, indicating that our compounds may be refractory to resistance and supporting their clinical viability. We are now employing alternative methods to identify the leads' functional targets to prioritise their development for clinical malaria.

LT8 Combination of redox modifiers with artemisinin results in increased parasite susceptibility to artemisinins

Annie Roys, Carlo Giannangeloab, Darren Creek

Monash University, Australia.

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.

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

LT9 Dual inhibition of Plasmepsins IX and X in *Plasmodium falciparum* sporozoites inhibits development within *Anopheles stephensi* mosquitoes

Elena Lantero-Escolar[1,2], John A. McCauley[3], David B. Olsen[3], Alan F. Cowman[1,2], Justin A. Boddey[3]

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Plasmepsin IX (PMIX) and X (PMX) are essential aspartyl proteases in all *Plasmodium* spp. that cleave many proteins required for egress and invasion of host cells across the lifecycle. The dual PMIX and PMX inhibitor WM382 prevents merozoites egress and invasion of erythrocytes, blocks transmission to mosquitoes and completely attenuates the egress of liver merozoites and erythrocytic infection. PMIX/PMX are expressed in mosquito stages, however their function in this host remains unknown. When treating mice with WM382 4-hours prior injection with *P. berghei* sporozoites, parasites developed normally. Then, are PMIX and PMX important in the sporozoite development before transmission? We tested this hypothesis by dosing *An. stephensi* mosquitoes with WM382 beginning at oocyst stages and this significantly reduced the number of sporozoites located within the salivary glands. This suggests that PMIX and PMX processes substrates required for sporozoite egress from oocysts and/or invasion of salivary glands, such as AMA1 that contains a putative PMIX/X cleavage site. We will present our up-to-date understanding of the function of these aspartyl proteases in *P. falciparum* sporozoites as they develop within the mosquito host.

LT10 Unfolding malaria parasite biology: targeting protein disulphide isomerases to block *Plasmodium* invasion and transmission

Senna Steen[1], Molly Schneider[1], Claudia Barnes[1], Trent Ashton[2], Will Nguyen[2], Maria Gancheva[3], Emma Yuxin Mao[3], Amelia Ford[4], Ghizal Siddiqui[5], Darren Creek[5], Andrew Blagborough[4], Danny Wilson[3], Brad Sleebs[2], Paul Gilson[1], Hayley Bullen*[1], Fiona Angrisano*[1]

[1]Burnet Institute, Australia; [2]Walter and Eliza Hall Institute, Australia; [3]University of Adelaide, Australia; [4]University of Cambridge, UK; [5]Monash Institute of Pharmaceutical Sciences, Australia.

Antimalarial resistance threatens malaria control, necessitating new therapeutics with novel targets. *Plasmodium* parasite egress from human erythrocytes and invasion of new host cells instigates clinical manifestations of malaria, while transmission to the mosquito vector drives ongoing disease burden.

All essential biological processes within the parasite critically rely on the correct manufacturing of protein machinery to complete the lifecycle across both hosts. Egress, invasion, and transmission are underpinned by a repertoire of highly folded and disulphide-bonded proteins.

Protein disulphide isomerases (PDIs) are established eukaryotic protein folding chaperones and disulphide-bonding mediators. The primary malaria parasite, *P. falciparum,* possesses four highly conserved PDIs. Previous work has demonstrated essentiality of PDI-Trans in transmission and its vulnerability to chemical inhibition.

We now show that PDI-Trans knockdown also perturbs folding of essential egress and invasion proteins, and treatment with repurposed PDI inhibitors recapitulates this effect. We have generated compounds that prevent *Plasmodium* invasion/egress, transmission, and growth across multiple species with single digit nanomolar potency and irresistibility in asexual stages.

With an established relationship between highly conserved PDIs and integral processes of egress, invasion, and transmission in *Plasmodium*, repurposing existing PDI inhibitors may offer an expedited and novel means of eliminating malaria parasites through dual-stage pan-species activity.

Session Chairs: Carlo Giannangelo & Eileen Zhou

ST5 Identifying adaptive immune signatures of protection against *P. vivax* malaria

Pinkan Kariodimedjo[1], Jo-Anne Chan[1], Rintis Noviyanti[2], Diana Hansen[3], Herbert Opi[1], James Beeson[1]

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The WHO has recommended development of highly efficacious malaria vaccines. Unfortunately, there are currently no vaccines available for *P. vivax*, the leading cause of malaria in the SE Asian region. *P. vivax* vaccine research is limited due to a lack of knowledge on the mechanisms and targets of protective *P. vivax* immunity. Previous studies have shown important roles for antibodies and cellular immune responses in *P. falciparum*; however, their role, including how they interact in *P. vivax* protective immunity is poorly understood.

Using a cohort of healthy, asymptomatic and symptomatic *P. vivax* infected individuals from Timika, Papua, Indonesia, we aimed to identify signatures of protective immunity, including interactions between antibodies and cellular responses associated with protection against symptomatic *P. vivax* malaria. We characterized antibody responses (IgG, IgG subclasses, IgM, FcγRs and C1q) to 33 *P. vivax* antigens using multiplex bead array assays and carried out association studies between signatures of protective antibody and cellular responses. We have identified antigen-specific antibody responses associated with protection from symptomatic *P. vivax* malaria and analysis of their correlation with distinct T and B cell populations is underway.

Understanding of protective immune signatures against *P. vivax* will be crucial to development of *P. vivax* vaccines.

ST6 Unravelling clindamycin resistance in *Plasmodium*

Jessica Home[1], Hayley Buchanan[1], Lee Yeoh[2], Michael Duffy[3], James Beeson[2], Geoffrey McFadden[1], Dean Goodman[1]

[1]School of BioSciences, The University of Melbourne, Australia; [2]Burnet Institute, Melbourne, Victoria, Australia; [3]Department of Microbiology and Immunology, The University of Melbourne, Australia.

Clindamycin is a well-tolerated antibiotic that kills malaria parasites by targeting the apicoplast prokaryotic translational machinery, but mechanisms of resistance in *Plasmodium* remain largely unknown.

We selected for clindamycin resistance in *P. falciparum* and in *P. berghei*. Clindamycin resistant *P. falciparum* had point mutations in the peptidyl transferase site of apicoplast-encoded 23S ribosomal RNA—the same mechanism of clindamycin resistance in bacteria. These mutants exhibited impaired development in mosquitoes, suggesting a fitness cost associated with resistance that should restrict spread of such resistance. In contrast, two independently generated clindamycin resistant *P. berghei* lines had loss-of-function mutations in different nucleus-encoded genes responsible for formylating the initiator methionine used for apicoplast translation. Changes to this machinery have never been connected to clindamycin resistance in any organism.

To validate this novel resistance mechanism, we disrupted initiator methionine formylation genes in both *P. berghei* and *P. falciparum* and confirmed clindamycin resistance. Interestingly, methionine formylation mutants conferred lower resistance levels than 23S rRNA mutants but had no mosquito transmission impairment, suggesting weaker resistance but more facile spread. Clindamycin resistance in *Plasmodium* is thus more complex than anticipated, and further investigation of the methionine formylation pathway is imperative to dissect this new resistance mechanism.

ST7 Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance

Hanqing Zhao[1,2,3], Lauren Smith[1,2], Rhea Longley[1,2], Ivo Mueller[1,2,3]

[1]Infection and Global Health, Walter and Elisa Hall Institute of Medical Research (WEHI), Australia; [2]Department of Medical Biology, University of Melbourne, Australia; [3]School of Global Health, Shanghai Jiao Tong University School of Medicine, China.

Malaria remains a major global public health burden. *Plasmodium vivax* poses a challenge to malaria surveillance due to its unique life cycle. Recently, serology has emerged as a promising surveillance tool. It is based on antibody detection of serological exposure markers. Our previous work identified a panel of eight *P. vivax* antigen markers that demonstrated good sensitivity and specificity in detecting recent *P. vivax* exposure in endemic cohorts. However, difficulties in protein expression and purification have limited the translational potential of the serology tools. In this study, we focus on B cell epitopes, the special regions of antigen markers that bind antibodies, which can potentially overcome these challenges and may provide improved performance over the antigens.

Using computational immunology tools, we predicted both conformational and linear B cell epitopes with potentially high antigenicity. In the follow-up epitope screening experiments, we selected the best-performing epitope peptides as candidates that have superior seroprevalence in IgG responses to sera from current *P. vivax* infections in endemic regions. In comparison to the recombinant protein markers, the combined use of several epitope candidates achieved higher sensitivity and specificity. These epitope candidates were further validated in observational cohort samples.

ST8 The voltage dependent ion channel is an essential mitochondrial protein in *P. falciparum*

Mitchell Trickey[1,2,3], Joyanta Modak[1,2], Mrittika Chowdury[1,2], Tania de Koning-Ward[1,2]

[1]School of Medicine, Deakin University, Australia; [2]Institute for Mental and Physical Health and Clinical Translation (IMPACT), Deakin University, Australia; [3]Monash Institute of Pharmaceutical Sciences (MIPS), Australia.

Despite global gains combating malaria, the increasing incidence of antimalarial drug resistance to front line therapeutics demands new drugs with novel targets be developed. Here the essentiality of the voltage dependent anion channel (VDAC) was investigated, via the generation of a transgenic *P. falciparum* line to permit conditional knockdown of *vdac*. Additionally, epitope tagging of *vdac* facilitated both its localisation and affinity purification. Knockdown of *vdac* led to a survival defect in the asexual red blood cell stages, although incomplete knockdown did not inhibit parasite perpetuation. The failure to generate conventional *vdac* knockouts in both *P. falciparum* and *P. berghei* indicates VDAC is essential for parasite survival. Immunofluorescent microscopy localised VDAC to the mitochondria, whilst mass-spectrometry and proteomic analysis of immunoprecipitated VDAC and its associated proteins validated a mitochondrial localisation. VDAC knockdowns were shown to sensitise parasites to mitochondrial target drug Atovaquone as determined by half maximal growth assays. This provided further indication for a role at the outer mitochondrial membrane, possibly involving the transport of intermediates in the pyrimidine biosynthesis pathway. Whilst the precise role of VDAC at the mitochondria requires further investigation, this channel is an essential and unique target for the future design of novel antimalarial therapeutics.

Long Talks III: Novel Techniques Thursday (04:10pm – 05:30pm)

Session Chairs: Sophie Collier & Jessica Home

LT11 PvSeroTaT: Optimisation and standardisation of a machine learning method to identify individuals recently infected with *Plasmodium vivax*

Dionne Argyropoulos[1,2], Lauren Smith[1,2], Nick Walker[1], Janise Lin[1], Macie Lamont[1], Anju Abraham[1], Pailene Lim[1,2], Eamon Conway[1,2], Jetsummon Sattabongkot[3], Marcus Lacerda[4,5], Ventis Vahi[6], Michael White[7], Ramin Mazhari[1,2], Ivo Mueller[1,2], Rhea Longley[1,2]

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The unprecedented persistence of *Plasmodium vivax* is largely driven by the hidden reservoir of hypnozoite liver-stage parasites, presenting a key obstacle to elimination. Here we present a machine learning algorithm that classifies recent *P. vivax* infections using serological biomarkers to identify likely hypnozoite carriers. Antibody levels persist even after infections are cleared, and thus can act as indicators of current and recent *P. vivax* infections. This algorithm underpins the *P. vivax* serological testing and treatment (PvSeroTaT) strategy, an alternative to mass drug administration. Serological assays were performed on samples from year-long observational cohort studies conducted from low-transmission settings in Thailand, Brazil and the Solomon Islands, as well as negative controls (*N*=2,635). An eight-antigen combination was used to train a random forest classification algorithm which achieved an area under the receiver operated characteristic curve (AUC) of 0.874. We then compared the final algorithm against multiple machine learning classifiers to validate its performance. An online R Shiny application (PvSeroApp) was developed to automate serological data processing, quality control and serostatus classification, and facilitated PvSeroTaT implementation in multiple countries. Ultimately, this sero-surveillance strategy enables targeted anti-hypnozoite therapy and strengthens the toolkit for *P. vivax* elimination.

LT12 Expansion microscopy reveals how Kelch 13 mutations impair feeding in artemisinin resistant *Plasmodium* parasites

Long Kim Huynh[1], Ben Liffner[2], Niall Geoghegan[3], Sabrina Absalon[2], Stuart Ralph[1]

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Resistance to the frontline antimalarial, artemisinin, is predominantly driven by mutations in Kelch 13 (K13). K13 is required for regulating cytostomes; the apparatus which facilitates haemoglobin uptake from host red blood cells into the *Plasmodium* parasite. Haemoglobin digestion supplies the parasite with essential amino acids while releasing haem-iron required for artemisinin activation. K13 mutants exhibit slower feeding rates and reduced haem levels, leading to decreased artemisinin activation and reduced parasite death. However, the exact mechanism by which K13 mutations impair parasite feeding remains unclear.

We propose that K13 mutations reduce protein stability, impacting cytostome formation and maintenance. Using ultrastructure expansion microscopy coupled with super-resolution microscopy, we resolved K13 as distinctive ring structures (~160 nm diameter) localising to cytostome necks. We also performed live-cell lattice light-sheet microscopy to investigate K13 dynamics throughout the asexual blood-stage cycle. Quantitative analyses using an automated machine learning pipeline found that K13 mutant parasites formed 18% fewer K13 rings than wild-type controls despite producing similar merozoite numbers, and exhibited a 4-hour developmental delay. Additionally, some K13 mutants displayed aberrant cytostome morphologies correlating with impaired haemoglobin uptake. These findings establish mechanistic links between K13 mutations and slowed parasite feeding underlying artemisinin resistance, informing strategies to manage resistance.

LT13 Validation of Solvent Proteome Profiling for Antimalarial Drug Target Deconvolution

Jessica Ji[1], Joshua Morrow[1], Christopher MacRaild[1], Haijian Zhang[2], Carlo Giannangelo[1], Ralf Schittenhelm[2], Darren Creek[1], Ghizal Siddiqui[1]

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As a global health threat, the rising drug resistance in malaria accelerates the urgent need for new therapeutics. Target elucidation is a critical step in antimalarial drug discovery, enabling a deeper understanding of the molecular mechanisms of action of both existing and novel compounds. This study validates solvent-induced proteome profiling (SPP) as a proteomics-based approach for identifying drug-protein interactions in *Plasmodium falciparum*. SPP detects ligand-induced protein stability shift, identifying drug target/s without the need for compound modifications. Here, we successfully generated solvent denaturation curves for the *P. falciparum* proteome, and demonstrated the utility of SPP with five antimalarial compounds: pyrimethamine, atovaquone, cipargamin, MMV1557817 and OSM-S-106. Notably, we propose live-cell treatment SPP as a novel approach to identify the validated target of pyrimethamine, *Pf*DHFR, with high specificity. We also introduced the novel one-pot mixed-drug SPP to evaluate multiple drugs within a single experimental setup. This simplifies the workflow and includes positive controls to affirm experimental performance. Overall, this study demonstrates that SPP can be successfully applied in both lysate and live-cell treatment conditions to elucidate drug targets in *P. falciparum,* and provide insights of the mechanisms of drug action, aiding the optimisation of existing antimalarials and the development of novel therapies.

LT14 Feeding antimalarials to mosquitoes disrupts malaria parasite transmission

Sarah N. Farrell[1], Anton Cozijnsen[1], Vanessa Mollard[1], Papireddy Kancharla[2], Rozalia A. Dodean[3], Jane X. Kelly[2,3], Geoffrey I. McFadden[1], Christopher D. Goodman[1]

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A decade-long decline in malaria cases has plateaued, primarily due to parasite drug resistance and mosquito resistance to insecticides. Here, we explore an innovative control strategy that targets *Plasmodium* with antimalarials during the mosquito stages. This strategy could reduce the risk of resistance emerging because a small population of parasites within the mosquito is subject to selection. We screened a range of parasiticidal compounds by feeding them to mosquitoes already infected with mouse malaria (*P. berghei*). T111, a next generation compound targeting the parasite electron transport chain, reduced sporozoite numbers in *P. berghei* at equivalent concentrations to the gold standard electron transport chain inhibitor, atovaquone. T111 also prevented sporozoite production in mosquitoes infected with human malaria, *P. falciparum,* even after very short exposure times. Encouragingly, T111 remained efficacious after being freeze-dried onto a substrate and later reconstituted with water, suggesting this compound would be effective in easy-to-distribute-and-deploy transmission control devices. Our findings suggest that targeting mosquito-stage parasites via sugar baits should limit malaria transmission. Importantly, this control strategy vastly increases the range of potentially useful parasiticidal compounds to include those failing to meet the exacting standards required for human antimalarial drugs, potentially improving malaria control for minimal cost.

Talk abstracts

Day 2: Friday 31st October

Session chair: Mary-Lou Wilde

Transporters as drug targets and resistance determinants

Adele Lehane

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Recent studies revealed that membrane transport proteins, which move solutes across a membrane bilayer or lipids from one membrane leaflet to the other, are overrepresented as drug targets and resistance mediators in *Plasmodium falciparum*. This finding comes from the testing of numerous compounds with activity against blood-stage parasites in 'in vitro evolution' experiments, wherein parasites are exposed to the compounds over a prolonged period of time to select for resistant parasites. Mutations or copy number variations in genes encoding transporters were frequently associated with resistance, with some of these transporters yet to be characterised. Some transporters confer resistance by translocating drugs away from their site of action, while others perform essential functions that are inhibitable by small molecules (i.e. serve as drug targets). It has been suggested that some transporters may double as drug transporters and drug targets. This talk presents our work on determining the functions of *P. falciparum* plasma membrane transporters and the roles of different transporters as drug targets and resistance determinants.

Session chairs: Mary-Lou Wilde & XueXin Xia

ST9 Novel approaches to identifying key targets of immunity to *Plasmodium vivax*

Kaitlin Pekin[1,2], Liriye Kurtovic[1,3,4], Gaoqian Feng[1,5], Jill Chmielewski[2], Isabelle Henshall[2], Daisy Mantila[6], Benishar Kombut[6], Maria Ome-Kaius[6], Moses Laman[6], Ivo Mueller[7], Leanne Robinson[1,7,8], Danny Wilson[2], D. Herbert Opi[1,3,9], James Beeson[1,3,9,10]

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There is need for highly efficacious malaria vaccines targeting *Plasmodium falciparum* and *P. vivax*, but there are no licensed *P. vivax* vaccines, and few candidates are under development. Developing *P. vivax* vaccines is challenging due to the absence of continuous parasite culture. Merozoite surface proteins (MSPs) are promising vaccine candidates as they are exposed to the immune system, particularly antibodies. Investigating the functions of *P. vivax* MSP antibodies is essential to understanding key immune mechanisms and identifying promising vaccine candidates.
In a study of *P. vivax* exposed Papua New Guinean children, I evaluated the ability of antibodies to induce multiple antibody functional activities to *P. vivax* MSPs (MSP4, MSP5, MSP7 and MSP3), and to whole merozoites using the closely related and culture adapted *P. knowlesi*. *P. knowlesi* merozoites were targets of multiple *P. vivax* functional antibody activities. Antibodies to *P. vivax* MSPs engaged Fcγ-receptors and promoted complement fixation with activity varying between antigens. Opsonic phagocytosis was seen for all antigens and strongly correlated with FcγR-binding while ADCC activity varied between antigens. These results provide novel insights into *P. vivax* whole merozoite immunity and identify *P. vivax* MSPs that are targets of potent protective functional antibody responses and potential vaccine candidates.

ST10 Distinct and divergent neutrophil profiles in human infections with *P. falciparum* and *P. vivax*

Sandra Chishimba[1,2], Daisy Mantila[3], Henson Dima[3], Jessica Loughland[1,4], Dean Andrew[4], Lee Yeoh[1,2], Gaoqian Feng[1,2], Shirley Lu[1], Nicholas Dooley[1,4], Ivo Mueller[6], Shazia Ruybal[1,6], Leanne Robinson[1], Moses Laman[3], Rebecca Webster[4], Bridget Barber[4], Raffi Gugasyan[1], Stephen Rogerson[5], Michelle Boyle[1,4], James Beeson[1,2,5]

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Emerging data suggest that neutrophils play a significant role in antibody-mediated naturally acquired and vaccine-induced immunity to human malaria. However, much of their profiles and functions in malaria infection remains unknown. We profiled neutrophil expression of Fcγ-receptors and adhesion molecules involved in parasite killing and clearance, in malaria-exposed residents (infected- and uninfected-children and uninfected adults) in Papua New Guinea, and in a clinical trial of controlled human malaria infection in malaria-naïve adults. There were significant differences in phenotypes from children infected with P. vivax or P. falciparum malaria, and uninfected-children and -adults. Neutrophils differed between uninfected-children and -adults. There were marked differences between neutrophil phenotypes in males and females infected with P. falciparum. In controlled human experimental P. falciparum infections, neutrophil phenotypic changes occurred early, prior to substantial blood-stage parasitemia and some changes persisted weeks post-treatment. Early phenotypic changes suggest the influence of immune system communication rather than direct parasite interaction. Neutrophil transcriptional changes associated with defence showed enhanced function relevant for parasite clearance. Overall, our findings reveal novel neutrophil phenotypic and functional changes during malaria infection. These findings advance our understanding of neutrophil functions during infection and in immunity which are relevant to inducing protective functions in vaccine design.

ST11 Determining *P. falciparum* virulence antigens that elicit protective immunity against severe malaria

Prerna Prashanth[1], Gerry Tonkin-Hill[2], Michael Duffy[1], Anna Bachmann[3]

[1]Bio21 Institute, Melbourne, Australia; [2]Peter MacCallum Cancer Centre, Melbourne, Australia; [3]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Severe *Plasmodium falciparum* malaria remains a major cause of mortality, particularly in children. Protective immunity develops after repeated infections and is associated with antibodies that target PfEMP1, a variant surface antigen encoded by the diverse *var* gene family. While specific PfEMP1 subsets, such as those linked to EPCR and ICAM-1 binding, have been implicated in severe disease, virulence-associated variants and protective antibody responses remain incompletely defined.

To address this, we are applying RNA sequencing analysis to profile *var* gene expression in 934 clinical isolates from severe and asymptomatic malaria cases in Ghanaian children. By de novo assembling transcripts and conducting differential expression analyses, we capture the repertoire of expressed PfEMP1s across clinical phenotypes. Preliminary results suggest distinct expression biases, with severe cases showing higher representation of domain cassettes such as DC5, DC8, and DC11, which have been previously associated with severe malaria.

These findings provide a framework for downstream serological studies, in which patient-derived antibody binding can be characterised against differentially expressed PfEMP1s. Together, this integrative approach will help determine which antigens drive pathogenicity and which antibody responses are protective, informing rational therapeutic and vaccine design.

ST12

Long Talks IV: Immunology Friday (11:10am – 12:30pm)

Session chairs: Alessia Hysa & Alexander Harris

LT15 Malaria Drives the Emergence of Cytotoxic Memory from Phenotypically Diverse Innate Vδ2 T Cells

Nicholas Dooley[1,3], Dean Andrew[2], Teija Frame[2], Zuleima Pava[1], Jessica Loughland[1], Damian Oyong[1], Fabian Rivera[2], Reena Mukhiya[1], Julianne Hamelink[1], Luzia Bukali[2], Jessica Engel[2], Rebecca Webster[2], James McCarthy[2], Bridget Barber[2], J. Alejandro Lopez[3], Chris Engwerda[2], Michelle Boyle[1]

[1]Life Sciences Department, Burnet Institute, Melbourne, Australia; [2]Infection and Inflammation Department, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia; [3]School of Environment and Science, Griffith University, Brisbane, QLD, Australia.

γδ T cells play critical roles in malaria, yet their heterogeneity during infection remain incompletely defined. Using longitudinal single-cell RNA sequencing of γδ T cells from malaria-naive volunteers undergoing controlled human malaria infection (CHMI), we identified 14 transcriptionally distinct clusters with subset-specific activation trajectories and functional diversification. During primary infection, Vγ9Vδ2 T cells dominated peripheral responses, expanding into cytotoxic, inflammatory, antigen-presenting-like, and proliferating subsets marked by GZMB, PRF1, HLA-DRA, CD74, MKI67, and CDK1. Flow cytometry confirmed activation, proliferation, and increased cytotoxic protein expression. Functional assays demonstrated innate cytotoxicity against blood-stage parasites independent of antibody-mediated activation. Despite induction of APC-like Vδ2 T cells both in vivo and in vitro, they did not enhance naïve CD4+ T cell activation or promote parasite-specific antibody responses. Secondary CHMI elicited terminal effector memory (TEMRA) Vδ2 T cells enriched for cytotoxic markers, correlated with monocyte-derived inflammatory mediators (CXCL9, S100A9) and systemic inflammation (CRP, ART, CX3CL1) but not parasite clearance. JAK1/2 inhibition (ruxolitinib) during primary infection transiently suppressed cytotoxic activation yet preserved memory responses. Enhanced re-activation was not driven by epigenetic changes but reflected heightened cytokine responsiveness to increased serum cytokines during reinfection.This high-resolution atlas delineates γδ T cell adaptation in malaria, informing immunotherapy and vaccine design.

LT16 Anti-malarial drugs impose quantitative not qualitative effects on B-cell immunity in experimental malaria

Oliver Skinner[1], Saba Asad[1], Marcela Moreira[1], Zheng Ruan[1], Jorene Lim[1], Hyun Jae Lee[1], Cameron Williams[1], Shihan Li[1], Takahiro Asatsuma[1], Megan Soon[2], Jessica Engel[3], Wang Jin[4], David Khoury[4], Adam Uldrich[1], Kelvin Tuong[2], Hamish King[5,6], Ashraful Haque[1]

[1]Peter Doherty Institute, University of Melbourne, Victoria, Australia; [2]Ian Frazer Centre for Children's Immunotherapy, Child Health Research Centre, Faculty of Medicine, University of Queensland, Australia; [3]QIMR Berghofer Medical Research Institute, Herston, Brisbane, Queensland, Australia; [4]Kirby Institute, University of New South Wales, Kensington, New South Wales, Australia; [5]The Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia; [6]Epigenetics and Developmental Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Anti-malarial drugs are used to prevent and treat malaria. Studies in malaria-endemic regions suggest cessation of anti-malarial drug treatment increases subsequent susceptibility to parasitic infection, and impairs development of parasite-specific antibodies. We hypothesised here that anti-malarial drugs impair splenic B-cell differentiation during blood-stage *Plasmodium* infection. To test this, we mapped splenic B-cell responses over time in experimental mice infected with blood-stage *Plasmodium chabaudi chabaudi* AS parasites, and treated with antimalarial drugs from the peak of infection. Using paired scRNA-seq and BCR-seq, we found that germinal centre (GC) B-cells accrued BCR mutations at comparable rates regardless of drug treatment. Moreover, GC composition, with respect to light, dark and grey zone GC B cells, remained qualitatively unaltered. Instead, drug treatment quantitatively reduced splenic GC B-cells, pre-plasma cells and pre-memory B-cells, which associated with reduced circulating parasite-specific IgG, and increased susceptibility to homologous re-challenge. Our findings suggest that anti-malarial drugs impose quantitative but not qualitative limits on B-cell differentiation in the spleen. This raises a hypothesis that anti-malarial drug treatment, combined with approaches that quantitatively boost endogenous B cell responses, would result in improved long-term immunity to malaria.

LT17 Multi-Antigen mRNA Vaccine Development for Blood-Stage Malaria

Timothy Ho[1], Adam Thomas[1,2], Tom Rünz[1,4], Linda Reiling[1,2,3], Lee Yeoh[1,3], Stewart Fabb[5], Chee Leng Lee[5], Colin Pouton[5], James Beeson[1,2,3]

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Next-generation malaria vaccines with more durable and potent protection than RTS,S and R21 are urgently needed. Blood-stage vaccines targeting merozoite antigens could prevent severe illness and control infections. We identified a region in the *P. falciparum* blood-stage merozoite surface protein MSP2 to which functional antibodies from infected Kenyan adults were highly reactive. This region was effective at activating multiple downstream immune functions *in vitro*. Guided by these findings, we engineered MSP2 mRNA vaccines and incorporated enhancing elements that dramatically improved the immune response in mice. We also achieved earlier peak immune responses against MSP2 with fewer doses using the new self-amplifying RNA vaccine platform. Additionally, our circular MSP2 RNA vaccine showed promising immunogenicity consistent with the conventional mRNA vaccine, offering a more thermostable option that improves vaccine accessibility. Excitingly, our multivalent MSP2 mRNA vaccines combining both alleles (3D7 and FC27) or the sporozoite CSP immunodominant region elicited strong and balanced functional immune responses to all antigens. These results demonstrate that the strategic targeting of protective epitopes, optimising antigen design, and using mRNA vaccine platforms can collectively generate potent functional immune responses. This approach can be extended to other malaria antigens to accelerate the development of novel multi-antigen, multi-stage mRNA vaccines.

LT18 Can JAK/STAT inhibition improve humoral immunity in malaria?

Julianne Hamelink[1,2,3], Damian Oyong[1], Zuleima Pava[1], Dean Andrew[3], Jessica Engel[3], Megan Soon[2], Luzia Bukali[3], Reena Mukhiya[3], Nicholas Dooley[1], Mayimuna Nalubega[1], Jessica Loughland[1], Fabian De Labastida Rivera[3], Teija Frame[3], Rebecca Webster[3], Bridget Barber[3], Christian Engwerda[3], Michelle Boyle[1]

[1]Burnet Institute, Australia; [2]The University of Queensland; [3]Queensland Institute of Medical Research.

Background: Antibody responses to malaria develop slowly, partially due to parasite induced immune-regulatory pathways. Regulatory pathways initiate via type-1 interferon signalling, which drive regulatory T cells that can inhibit antibody development. We tested if blockade of type-I interferon signalling, via the JAK/STAT inhibitor ruxolitinib, could boost humoral immunity in a controlled human malaria infection.

Methods: 20 malaria-naïve individuals were enrolled in a controlled human malaria infection, and randomised to receive either ruxolitinib or placebo therapy alongside antimalarial treatment. We examined the induction of protective immunity, including to a secondary malaria challenge. We analysed B-cell responses with scRNAseq and flowcytometry, and humoral immunity via MSP1, MSP2 and merozoite ELISA.

Results: Ruxolitinib inhibited interferon-stimulated gene expression in B-cells, and blocked the emergence of interferon stimulated B-cells. Ruxolitinib also delayed plasmablast proliferation in first infection, and allowed greater clonal expansion and somatic hypermutation in secondary infection. Whilst antibody titres were lower in ruxolitinib treated individuals in second infection, their antibodies exhibited enhanced functional capacity, showing increased FcγRIII binding.

Conclusion: Ruxolitinib successfully inhibited type-I interferon responses via JAK/STAT inhibition, resulting in higher quality humoral responses. Data suggest that host directed type-I interferon inhibition has the potential to improve infection or vaccination induced immunity in malaria.

LT19 Repeated *Plasmodium* infection in mice progressively modulates CD4+ T cell recall

Zihan Liu[1], **Brooke Wanrooy[1]**, Marcela Moreira[1], Ashlyn Kerr[1], Oliver Skinner[1], Zheng Ruan[1], Marcus Tong[1], Takahiro Asatsuma[1], Chen Zhu[1], Jason Nideffer[2], Prasanna Jagannathan[2], Ashraful Haque[1]

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In high-transmission, malaria-endemic regions, children can experience repeated malaria episodes. Clinical evidence suggests repeated *Plasmodium* infection may shift $CD4^+$ T cell responses from a pro-inflammatory state toward a disease-tolerant state associated with IL-10 production. However, the correlation between the number of infections experienced and IL-10-producing capacity remains to be fully delineated. Here, we developed a mouse model of repeated blood-stage *Plasmodium* infection, in which parasite-specific TCR-transgenic (PbTII) and polyclonal $CD4^+$ T cell recall was assessed over primary infection and four re-challenges, each interspersed by antimalarial drug treatment. First, we determined $CD4^+$ T cell recall was best assessed at 3-days post-rechallenge, when proliferation and cytokine production were evident. Importantly, we discovered a positive linear correlation between the number of infections experienced, and a capacity to exhibit a regulatory "Tr1" phenotype, defined by co-expression of IL-10 and IFN-g. This suggested that $CD4^+$ T cell recall had been re-wired by multiple infections, which was then tested by scRNA-seq, comparing $CD4^+$ T cell recall after one versus four rechallenges. Preliminary analysis to date reveals substantial transcriptional re-wiring after four rechallenges. Thus, splenic $CD4^+$ T cell recall is progressively modulated during multiple infections with blood-stage *Plasmodium*.

Session chairs: Mohini Shibu & Elena Lantero-Escolar

LT20 Characterising the role of glideosome-associated proteins throughout *P. falciparum* development

Katrina Larcher[1,2], Sash Lopaticki[1,2], Dylan Multari[2], Vineet Vaibhav[2], Jumana Yousef[2], Cindy Evelyn[2], Niall Geoghegan[2], Niva Jayakrishnan[1,2], Yuri Shibata[1,2], Hayley Buchanan[1,2], Laura Dagley[2], Kelly Rogers[2], James McCarthy[1,2], Matthew Dixon[1,2]

[1]Department of Infectious Diseases, Doherty Institute, University of Melbourne; [2]Walter and Eliza Hall Institute.

Plasmodium falciparum uses a highly conserved organelle to facilitate transition between different cellular niches throughout its lifecycle. The inner membrane complex acts as a scaffold for cytoskeletal components to support cell structure and anchor the actin myosin motor essential for invasion. Despite being well studied little is known about the organisation and assembly of the IMC and the function of many of its resident proteins. In this work we use a gene tagging and conditional knockout (cKO) approach, integrated with super-resolution microscopy and proteomic analyses, to determine the location and function of all three Glideosome Associated Protein(s) with Multiple membrane spans (GAPM) within the inner membrane complex. Quantitative proteomics indicated that loss of either GAPM1-3 leads to a decreased abundance of multiple IMC components, compromising the integrity of the structure. 3D Structured Illumination Microscopy of cKO gametocytes highlighted abnormal tubulin deposits due to incorrect coupling to the collapsed IMC whereas ultrastructure expansion microscopy of segmented schizonts indicated a total loss of microtubules along with abnormal rhoptry formation. This dysfunction prevented cKO merozoites from reinvading new red blood cells and gametocyte elongation. This work proposes the GAPM protein family acts as key anchors of cytoskeletal components to the inner membrane complex.

LT21 The Bromodomain Protein PfBDP2 Regulates Chromatin Structure and Gene Expression in *P. falciparum*

Nicholas C. Lauw[1,3], Suffian Azizan[2,3], Scott A. Chisholm[2,3,4], Lee M. Yeoh[3,5,6,7], Shamista A. Selvarajah[3,6,8], Michaela Petter[8,9], Michael F. Duffy[3,5,6]

[1]Faculty of Science, The University of Melbourne; [2]School of Biosciences, The University of Melbourne; [3]Bio21 Institute; [4]Cambridge University; [5]Department of Microbiology and Immunology, The University of Melbourne; [6]Peter Doherty Institute; [7]Burnet Institute; [8]Department of Medicine, Royal Melbourne Hospital, Western Hospital, The University of Melbourne; [9]Erlangen University.

Plasmodium falciparum is an apicomplexan parasite responsible for the majority of malaria infections and deaths. P. falciparum uses epigenetic regulation to modulate gene expression, allowing it to rapidly adapt to changes in its environment. Bromodomain-containing proteins such as PfBDP2 bind acetylated lysines in histones and are involved in epigenetic regulation through a diverse range of functions including chromatin remodelling, and recruitment of transcription factors. Here we show that PfBDP2 was important in modulating gene expression across the parasite's life cycle, particularly at genes involved in host interaction and variant surface antigens. PfBDP2 was enriched in intergenic regions and heterochromatin, and was positively associated with levels of gene expression at euchromatic gene promoters. Loss of PfBDP2 resulted in the downregulation of some euchromatic genes. Loss of PfBDP2 resulted in de-repression of var genes in ring-stage parasites, and multiple ApiAP2 transcription factor binding sites were found in regions of high PfBDP2 enrichment. In conclusion, we identified PfBDP2 as a key player in the regulation of gene expression across time, particularly those related to host interaction and in the repression of var genes.

LT22 PfPTRAMP-CSS as a multi-stage malaria vaccine target – Part 1

Pailene S. Lim[1,2], Nicolai Jung[1,2], Kitsanapong Reaksudsan[1,2], Mikha Gabriela[1,2], Danushka Marapana[1,2], Rainbow W.B. Chan[1], Alan F. Cowman[1,2], Stephen W. Scally[1,2]

[1]Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; [2]Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia.

Next-generation malaria vaccines that can inhibit multiple parasite life-cycle stages and species are highly desired to progress malaria elimination. In *P. falciparum*, the pentameric PTRAMP-CSS-Ripr-CyRPA-Rh5 (PCRCR) complex is essential for invasion into red blood cells. Rh5, CyRPA and Ripr are leading blood-stage vaccine candidates that have been well characterised in comparison to PTRAMP-CSS. Although nanobodies raised to PTRAMP-CSS are growth inhibitory, these were of moderate efficacy. Furthermore, the expression and function of the PCRCR complex in both the pre-erythrocytic and mosquito-stages of the parasite has not been explored. Here, we characterise an extensive panel of nanobodies raised to the PTRAMP-CSS complex that are more potent than any described to date. We identified three novel invasion inhibitory epitopes on CSS and two on PTRAMP that are highly conserved across *P. falciparum* strains. The crystal structures of all inhibitory nanobodies to PTRAMP-CSS and two non-inhibitory nanobodies to PTRAMP have been solved which has significant implications for structural-guided design of immunogens to the PCRCR complex. We have additionally engineered nanobody-Fc (Nb-Fc) fusion proteins and have found that PTRAMP-CSS specific Nb-Fcs can prevent transmission of the parasite in the mosquito stages. This highlights the potential of the PCRCR complex as a multi-stage vaccine target.

LT23 PfPTRAMP-CSS as a multi-stage malaria vaccine target – Part 2

Nicolai Jung[1,2], Pailene Lim[1,2], Mikha Gabriela[1], Alan C. Cowman[1], Stephen W. Scally[1]

[1]WEHI, Australia; [2]University of Melbourne.

Recent years have seen a stagnation in malaria case decline, despite widespread deployment of preventive measures and frontline therapies. This highlights the urgent need for novel biologics that can complement existing interventions and address persistent transmission. Nanobodies, single-domain antibody fragments derived from camelid heavy-chain antibodies offer high specificity, stability, and ease of engineering, making them attractive candidates for next-generation malaria therapeutics. In this study, we engineered bispecific nanobody-Fc (Nb-Fc) fusion proteins by combining previously identified and characterized nanobodies targeting two essential Plasmodium falciparum blood-stage antigens: the Plasmodium Thrombospondin-Related Apical Merozoite Protein (PfTRAMP) and the Cysteine-rich Small Secreted Protein (CSS). Individual nanobodies with the highest inhibitory activity were rationally selected and fused to generate bispecific constructs. These bispecific Nb-Fcs demonstrated markedly enhanced affinities compared to their parental monospecific bivalent nanobody-Fc. Furthermore, they exhibited increased cross-reactivity against P. vivax and P. knowlesi, highlighting their potential for a multispecies biologic. Growth inhibition assays with blood-stage parasites revealed that bispecific Nb-Fcs significantly outperformed their monospecific counterparts. Together, these findings demonstrate that rational design of bispecific nanobody-Fcs can greatly improve binding, cross-reactivity, and parasite inhibition. This work establishes a foundation for developing versatile nanobody-based biologics as novel interventions against malaria.

LT24 Improving the capacity of vaccine-induced antibodies to arrest the growth of *Plasmodium falciparum*

Alysha Literski, Hayley Bullen, Paul Gilson

Burnet Institute, Australia.

Plasmodium falciparum causes malaria, and while vaccines targeting the liver-stage have been developed, they are not fully protective, underscoring the urgent need for novel vaccines that target the parasite's blood stage. To survive in the bloodstream, *P. falciparum* invades and replicates within red blood cells (RBCs), using its essential *Pf*RH5 protein to bind to the RBC receptor basigin to help trigger invasion.

*Pf*RH5 is a leading blood-stage vaccine candidate as anti-*Pf*RH5 monoclonal antibodies (mAbs) sterically block the *Pf*RH5-basigin interaction, preventing RBC invasion. However, as mAb concentrations are reduced, their blocking efficiency declines. To boost the efficiency of RH5 mAbs, we successfully combined them with invasion-slowing compounds that give the RH5 mAbs more time to inhibit invasion. We have also successfully applied this strategy to polyclonal RH5 IgG from previously vaccinated individuals. These polyclonal RH5 IgGs, when combined with invasion-inhibiting compounds, also show a synergistic inhibitory interaction. Underscoring that this approach is effective not only in the laboratory but also has potential in real-world contexts, particularly if it can be used to boost natural immunity in endemic populations. By providing valuable insights into the neutralising mechanisms of anti-RH5 mAbs, we will help inform the development of more effective blood-stage malaria vaccines.

Session Chairs: Chiara Drago & Patience Nayebare

ST13 The GID E3 ligase complex controls cell fate programs for sexual development of *Plasmodium falciparum*

Danushka Marapana[1,2], Sash Lopaticki[1,2], Balu Balan[1,2], Kitsanapong Reaksudsan[1,2], Simon Cobbold[1,2], Niall Geoghegan[1,2], Sachin Khurana[1,2], Stephen Scally[1,2], Peter Hickey[1,2], Niva Jayakrishnan[1,2], Vineet Vaibhav[1,2], Sukhdeep Spall[1,2], Jumana Yousef[1,2], Laura Dagley[1,2], Dean Goodman[2], Anton Cozijnsen[2], Geoff McFadden[2], Kelly Rogers[1,2], Aaron Jex[1,2], David Komander[1,2], James McCarthy[1,2], Christopher Tonkin[1,2], Matthew Dixon[1,2], Alan Cowman[1,2]

[1]WEHI, Australia; [2]University of Melbourne, Melbourne, Australia.

Plasmodium falciparum, humankind's deadliest parasite, orchestrates precisely timed developmental transitions, yet the molecular mechanisms orchestrating these shifts have remained elusive. We identify the PfGID E3 ubiquitin ligase complex as a master regulator of sexual development in *P. falciparum*, revealing a sophisticated control system integrating protein degradation and RNA regulation. The PfGID complex is required for gametocyte maturation and parasite transmission to mosquitoes, underscoring its pivotal role. PfGID governs gametocyte development by fine-tuning levels of two substrates: the ZPF36 family RNA binding protein GD1, and PfDPL, a cryptochrome-like protein. GD1 is a key regulator of P-body dynamics by maintaining the delicate balance of translational repression essential for gametocyte maturation. Our findings reveal PfDPL regulates expression of male-specific proteins essential for gametogenesis. These findings illuminate the intricate molecular choreography underlying *Plasmodium* sexual development and provide insights into how single-celled eukaryotes execute cell-fate programs to navigate complex life cycles and adapt to diverse host environments.

ST14 Developing a Human Lymphoid Organ Model to Decode Immune Response to Malaria

Rama Dhenni[1], Julianne Hamelink[1,3,4], Jessica Canning[1,2], Amaya Ortega Pajares[1], Mayimuna Nalubega[1,3,4], Adam Thomas[1,2], Olivia Ventura[1], Catherine Pitt[2], Dean Andrew[3], Luzia Bukali[3], Nankya Felistas[5], Kenneth Musinguzi[5], Annet Nalwoga[5], Evelyne Nansubuga[5], Sedrack Matsiko[5], Moses Kamya[5], Abel Kakuru[5], Gareth Lloyd[6], Brian Wilson-Boyd[6], Nicola Slee[6], Jodie Ballantine[6], Katherine Girling[7], Sarju Vasani[7], Prasanna Jagannathan[8], Danika Hill[2], Fiona Angrisano[1], James Beeson[1,2], Michelle Boyle[1,2,3]

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Vaccines have eliminated and reduced the burden of many diseases, but highly protective vaccines remain unavailable for major infectious diseases like malaria, which is a top 5 killer of children globally. Antibodies play a crucial role in malaria immunity, and their development occurs predominantly in the germinal centres (GCs) within the secondary lymphoid organs. However, in vivo studies of the human immune system are often constrained by the limited accessibility of lymphoid tissues. Thus, there is an urgent need to develop a high-throughput, robust, and accessible system that models the dynamics of antigen-specific humoral and GC responses. Here, we utilised tonsil-derived lymphoid culture systems recently developed to study humoral and cellular responses to different types of vaccines and malaria parasites. Using this system, we show that our tonsil-derived lymphoid culture model recapitulates key aspects of human lymphoid tissue and allows investigation of antigen-specific B cells, GC formation, and antibody production in response to many types of vaccines, including inactivated, live-attenuated, and mRNA vaccines as well as malaria parasites. With tonsil cells from malaria-naïve, malaria-exposed, and asymptomatically infected children, this model provides a scalable platform to study human vaccine responses and potentially accelerate the development of effective vaccines against malaria.

ST15 Validating antibody prevalence to PkSERA3 antigen 2, a *Plasmodium knowlesi*-specific biomarker of exposure.

Zi Kang Ooi[1], Anju Abraham[1], Caitlin Bourke[2], Giri Rajahram[3], Timothy William[3], Pailene Lim[1], Jetsumon Sattabongkot[4], Kevin Tetteh[5], Chris Drakeley[6], Nicholas Anstey[2], Matthew Grigg[2], Rhea Longley[1]

[1]Walter and Eliza Hall Institute of Medical Research, Australia; [2]Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Australia; [3]Infectious Diseases Society of Kota Kinabalu, Sabah, Malaysia; [4]Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [5]FIND Global Alliance for Diagnostics, Geneva, Switzerland; [6]Department of Infection Biology, London School of Hygiene & Tropical Medicine, London, UK.

Malaria elimination in Southeast-Asia is threatened by *Plasmodium knowlesi*, with clinical cases underestimated despite potential severity. Limited molecular surveillance outside of Malaysia necessitates alternative tools to monitor transmission. Serological surveillance shows promise for monitoring falciparum and vivax malaria transmission. Integrating *P. knowlesi*-specific antigens into *Plasmodium* multiplex panels requires validating specificity against closely-related species. A recent study identified PkSERA3 antigen 2 as the most specific *P. knowlesi* biomarker antigen, where higher responses were observed in Malaysian *P. knowlesi* PCR diagnosed samples at all time-points (D0, 7, and 28) compared to Ethiopian *P. vivax*-positive and malaria naïve samples ($p<0.0001$). To assess specificity in our own experiment, antibodies to PkSERA3 antigen 2 were tested by ELISA in patients with confirmed infections (33 *P. knowlesi* and 31 *P. vivax*) at Day 0, 7, and 28 post-treatment, plus 90 healthy control samples. We found no significant difference in antibody levels to PkSERA3 antigen 2 between the Malaysian *P. knowlesi* samples and Ethiopian *P. vivax*-positive samples, indicating high cross-reactivity ($p>0.05$, ordinary one-way ANOVA). Further experiments are ongoing to determine reasons behind these discrepancies and the potential value of PkSERA3 antigen 2 for *P. knowlesi* sero-surveillance.

ST16

Poster abstracts

P1 Cut to the point: m6A facilitates 3' end mRNA processing in *Plasmodium falciparum*

Joshua Levendis, Amy Distiller, Lakvin Fernando, Emma McHugh, Stuart Ralph

Department of Biochemistry and Pharmacology, University of Melbourne, Australia.

Harnessing RNA modifications in therapeutics has enabled breakthroughs in vaccines. Despite their importance in RNA stability and translation, the mechanisms of RNA modifications are poorly understood. N^6-methyladenosine (m^6A) is the most abundant RNA modification in eukaryotes and changes translational efficiency or reduces mRNA stability. We studied this modification in *Plasmodium falciparum* by disrupting the methyltransferase that makes m^6A with a knock-sideways mislocalisation system. Recent developments in sequencing by Oxford Nanopore Technologies (ONT) have enabled the direct detection of m^6A in RNA. We therefore disrupted the methyltransferase and used Nanopore direct RNA-sequencing to study differential methylation at multiple points during the *P. falciparum* lifecycle. We were able to detect differentially methylated transcripts after mislocalising the methyltransferase, confirming the utility of both the knock-sideways system and Nanopore RNA-sequencing in studying m^6A in *P. falciparum*. We detected differentially expressed genes after disrupting the methyltransferase, and found removing m^6A affected the 3' end position of transcripts. Our work shows Nanopore RNA-seq can be used to detect m^6A abundance and location in *P. falciparum*, proving it a valuable technique for studying the impact of RNA modifications on parasite biology.

P2 Light-activatable Tafenoquine-Methylene Blue Conjugate for Blocking Transmission of Malaria

Sean A. Lynch[1,2], Angelica F. Tan[1], Juanita M. Heunis[1], Jenny M. Peters[1], Dean Andrew[1], Jeremy S.E. Gower[1], Fiona H. Amante[1], Bridget E. Barber[1,2]

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Malaria elimination requires interventions that target both blood-stage parasites and transmission. Primaquine is the only antimalarial currently indicated for interrupting transmission, but its use is limited by haemolysis in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals. Tafenoquine (TQ), a long-acting primaquine analogue, shares similar haemolytic potential but demonstrates transmission blocking activity at doses below the recommended therapeutic regimen. Methylene blue (MB) has multistage antimalarial activity and is a WHO-listed Essential Medicine. Using a novel light-activatable drug delivery platform, we evaluated a TQ-MB conjugate designed to release active compounds upon far-red light exposure (λ=660 nm). Transmission blocking activity was assessed using *P. falciparum* NF54 gametocyte cultures and standard membrane feeding assays with *An. stephensi* mosquitoes. TQ-MB achieved complete transmission blockade at sub-micromolar concentrations when stage V gametocytes were exposed to drug and far-red light for 24 hours prior to mosquito feeding. No transmission blocking effect occurred without light activation, confirming light-dependent drug release. These findings suggest that TQ-MB has potential as a potent, long-acting transmission blocking agent. Further investigations will determine optimal drug concentrations and light parameters, and whether effective TQ-MB concentrations correspond to safe TQ levels in G6PD-deficient individuals.

P3 Optimization and comparative evaluation of MAGPIX Luminex and INTELLIFLEX platforms for measuring antibody responses against *Plasmodium vivax* malaria

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Plasmodium vivax is the most widespread species and dominant malaria parasite outside sub-Saharan Africa, remains a major global health burden due to its high morbidity and mortality. Naturally acquired clinical immunity, driven by strong antibody responses to malaria, develops slowly through repeated exposure in endemic areas. Previous studies supported that studying natural acquired immunity to malaria provide a promising insight for vaccine development. Reliable and reproducible multiplex platforms are therefore essential to quantify antibody responses against *P. vivax* antigens. In this study, we utilized Luminex MAGPIX and xMAP INTELLIFLEX dual reporter systems to measure antibody responses to a 70 panel of *P. vivax* antigens. The INTELLIFLEX platform allows simultaneous detection of IgG and IgM antibodies against multiple Plasmodium vivax antigen within a single assay. Optimization steps include protein-bead coupling concentration, secondary antibody dilution and plasma sample dilution for detecting IgG and IgM. Consistency of antibody assays across both platforms was assessed using log-linear standard curves generated on MAGPIX data with malaria-positive Ethiopian samples using them as the references. This work will ensure optimized workflow and supports standardization of MAGPIX and INTELLIFLEX platforms for future work on validating novel antigenic targets of protective immunity to malaria across diverse transmission settings.

P4 Exploring the regulation of sex ratios in *Plasmodium berghei.*

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Malaria, caused by single-cell parasites of the genus *Plasmodium*, currently has a widespread impact on global public health security. As a sexually reproducing species, understanding the sexual biology of *Plasmodium* is fundamental to developing approaches to control malaria transmission. It is widely accepted that *Plasmodium* exhibits a female-biased sex ratio (male to female is <1) during gametocyte development and actively adjusts this ratio to promote outcrossing.

However, due to the lack of straightforward research models, how *Plasmodium* controls and changes its sex ratio is still unclear. By genetically manipulating sexual differentiation in *Plasmodium berghei*, we could artificially bias sex ratio. Using this system, we found that, contrary to existing evidence, when the growth of the parasite stabilizes, the sex ratio of the gametocytes (male to female) is close to 1:1. Furthermore, isogenic parasite lines do not modify sex ratio in response to an overabundance of one sex.

P5 Development of an experimental human blood-stage model for studying *Plasmodium knowlesi*

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The zoonotic parasite, *Plasmodium knowlesi,* is an increasing cause of human malaria in Southeast Asia. This study aimed to develop a *P. knowlesi* blood-stage volunteer infection study (VIS) to enable further study of parasite biology, host responses to infection, and activity of antimalarial treatments.

We manufactured and characterised a *P. knowlesi* master cell bank using the YH1 *P. knowlesi* strain adapted to grow in human serum (YH1-HS). The cell bank was evaluated in a VIS involving four malaria-naïve healthy adults intravenously inoculated with *P. knowlesi*-infected erythrocytes using a dose escalation strategy. Parasitemia was monitored by qPCR and participants received curative treatment with artemether-lumefantrine. Endpoints included safety and infectivity.

Two participants became infected. Participant 2 (administered ~85,000 parasites) had detectable parasites (3 parasites/mL) immediately prior to protocol-defined treatment on day 21. Participant 3 (administered ~1,000,000 parasites) developed detectable parasites on day 17, peaking at 202 parasites/mL just after protocol-defined treatment on day 24. The parasite multiplication rate over 32 hours (PMR_{32}) was 2.33 (95% CI 1.66 – 3.25). Adverse events were mostly mild and unrelated to the challenge agent.

Although successful, infectivity was suboptimal. Further studies are required to optimise parasite and host factors to improve infectivity.

P6 Uncovering the mechanism of action of second generation bis-triazines, a potent new class of antimalarials

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Widespread resistance to all current antimalarials threatens the control and eradication of malaria. Second-generation bis-triazines display low nanomolar potency, fast-killing activity and no known cross-resistance. However, the novel mechanism of action (MOA) remains unknown. In vitro combination drug-pulse assays using various inhibitors were performed to identify potential modulators of bis-triazine activity. We also included two antimalarial candidates with unknown MOAs currently under development with MMV. Only E64d, a cysteine protease inhibitor, caused modest antagonism of activity against trophozoites for the bis-triazines and one of the MMV candidates (2-fold increase in IC50), whereas both E64d and chloroquine decreased activity of the other candidate (6-fold increases in IC50), indicating the involvement of the haemoglobin digestion pathway in its MOA. To identify molecular targets of the bis-triazine analogues we used solvent proteome profiling. Two proteins were identified across two independent SPP experiments; RING finger E3 ubiquitin-protein ligase (PF3D7_1004300) and SART1 (PF3D7_0323700). Alongside these two hits, most other significantly stabilised proteins are localised to the nucleus with involvements in mRNA splicing, RNA processing and proteolysis. Further investigations will use supplementary proteomics and transcriptomics studies to confirm these targets and elucidate the antimalarial mechanism of this promising new class.

P7 Developing a novel health system readiness assessment tool for malaria elimination in the Greater Mekong Subregion

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Background

Countries in the Greater Mekong Subregion (GMS) are committed to eliminating malaria by 2030. Achieving this goal requires health systems to be adequately prepared to integrate new policies, processes, and interventions. However, a comprehensive tool that evaluates readiness for malaria elimination across all WHO health system building blocks is currently lacking.

Methods

To address this, qualitative interviews with 39 malaria stakeholders across the GMS identified key health system requirements for malaria elimination. Based on these findings, a draft readiness assessment tool was developed and refined through two Delphi consultation rounds with 24 experts in June and September 2025.

Results

Stakeholders highlighted the need for targeted interventions for high-risk populations, mandatory G6PD testing, and private sector inclusion in surveillance. Delivering these requires a skilled workforce, sustainable human resource planning, efficient supply chains, reliable funding, political commitment, and technical leadership.

Discussion

WHO recommends that malaria elimination strategies consider both transmission intensity and health system capacity and readiness. A structured framework is essential for evaluating readiness and guiding the shift from control to elimination.

Conclusion

The resulting tool provides policymakers and programme managers with a systematic approach to assess readiness, identify gaps, and meet WHO standards for validating zero local transmission.

P8 Understanding the translational repression landscape in gametocytogenesis

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The malaria-causing parasite *Plasmodium falciparum* has a complex lifecycle transitioning through diverse cellular niches within the human host and the Anopheles mosquito vector. Transmission of the parasite from the human to the mosquito requires the dramatic differentiation (cell fate change) from an asexually dividing short-lived cell (48 hrs) to a long-lived (12 days) sexual stage gametocyte capable of forming gametes in the mosquito midgut. While gametocyte development is a significant lifecycle bottleneck that could be exploited in transmission blocking interventions, a fundamental understanding of the cell fate decisions driving the development of gametocytes is unknown. *Plasmodium* parasites lack sex chromosomes and have a reduced repertoire of transcription factors, making them heavily reliant on post-transcriptional mechanisms for gene regulation. One such mechanism is translation repression, a process where RNA-binding proteins (RBPs) regulate stage-specific gene expression, through the selective binding of transcripts stopping them from being translated. The stage specific release of these held transcripts allows for rapid protein translation and stage switching. In this work we will use a combined genetic approach targeting both canonical and novel RBPs, with transcriptomics and proteomics analysis to define the landscape of translational repression driving gametocytogenesis.

P9 When SWEETs turn sour: a hypothesis for the origin(s) of parasitism in Apicomplexa

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The Myozoa supergroup includes both apicomplexan parasites like *Plasmodium* and photosynthetic dinoflagellates, some of which form symbioses with animal hosts. In mutualistic relationships, such as those between dinoflagellate algae and corals, metabolic exchange is key: the algae export photosynthetically derived glucose to their host using SWEET (Sugars Will Eventually be Exported Transporter) proteins, and in return, receive nitrogen and carbon. SWEETs are passive, bidirectional sugar transporters with seven transmembrane domains that are found acros all kingdoms of life. We hypothesise that apicomplexan SWEETs could mediate sugar uptake from the host. We identified a SWEET gene (*PbSWEET*) in the rodent malaria parasite *Plasmodium berghei* and generated a knockout line. Surprisingly, *PbSWEET* was dispensable across the entire life cycle, including blood, mosquito, and liver stages, making it one of a handful of malaria parasite genes with no observable knockout phenotype. This suggests that *PbSWEET* may be functionally redundant, consistent with prior reports that a 12-transmembrane hexose transporter mediates glucose uptake during blood stages. We are currently characterising *PbSWEET*'s localisation and substrate specificity to better understand its potential contribution to the evolution of parasitism in Apicomplexa.

P10 Quantification of cure for pharmacodynamic models of antimalarial drugs: deterministic versus stochastic approaches

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Within host models of malaria infection and treatment are used to inform dose optimisation during antimalarial drug development, to maximise the probability that a drug can effectively 'cure' infection. These *in silico* models capture the change in parasite count over time due to exposure to antimalarial drug(s). Current 'deterministic' models simulate parasite numbers that decline proportionally each hour during treatment, approaching (but never reaching) zero. Therefore, a threshold on the parasite count must be selected, below which patients are deemed 'cured'. We implemented a 'stochastic' model, where parasite-time profiles were simulated using a binomial distribution at each hour, according to a parasite survival probability in the presence of treatment. This stochastic model enables a time of exact cure to be calculated when zero parasites remain.

We simulated parasite-time profiles for antimalarial combination therapy, artemether-lumefantrine, for 1000 paediatric patients with *Plasmodium falciparum* malaria across 16 drug resistance scenarios. We found 28-day cure rates predicted by the deterministic and stochastic models to be equivalent. This confirms the standard deterministic method is an adequate proxy for the underlying stochastic process of parasite death during antimalarial treatment.

P11 Identifying novel biomarkers of Asian-Pacific *Anopheles* vector bite exposure

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Progression towards malaria elimination requires sensitive and scalable surveillance tools to monitor human interactions with *Anopheles* mosquito vectors. Current methods of entomological surveillance are logistically challenging and only provide population-level estimates of mosquito human biting rates, which cannot capture heterogeneous transmission. Alternatively, the measurement of antibodies against *Anopheles* salivary proteins injected during a bite could provide a proxy measure of individual-level bite exposure. While IgG responses to the current leading candidate antigen from the African vector *An. gambiae* have been shown to be a reliable marker of biting in Africa; no reliable biomarkers have been validated for the Asia-Pacific region and its vector species.

To address this, we screened antibody responses against salivary antigens belonging to Asian-Pacific *Anopheles* species to identify novel biomarker candidates. We identified several region-specific biomarker candidates that showed higher responses in individuals from the Asia-Pacific compared to African and naïve Australian donors ($p<0.05$). Furthermore, individuals with malaria infection had 23%-30% higher odds of IgG seropositivity to certain salivary antigens evaluated. These findings suggest that the identified biomarker candidates may be indicators of exposure to Asian-Pacific *Anopheles* species bites and could be further validated to improve vector serosurveillance and aid malaria elimination efforts in the Asia-Pacific region.

P12 Modelling SP resistance through dhfr and dhps haplotypes

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The emergence and spread of antimalarial drug resistance has greatly hindered efforts towards global malaria elimination. Drug resistance can be tracked through mutations at particular genetic markers within malaria parasites. In particular, it is the co-occurrence of marker mutations (haplotypes) that best informs on the effectiveness of a specific drug. Resistance to the drug sulfadoxine-pyrimethamine (SP) has been linked to mutations within the *dhfr* and *dhps* genes. While resistance to SP as a clearing agent is widespread, it can still lead to improved birth outcomes when used as an intermittent preventive treatment in pregnancy (IPTp). We propose a model to estimate the prevalence of *dhfr* and *dhps* haplotypes across sub-Saharan Africa, with a particular focus on the quintuple and sextuple *dhps* and *dhfr* mutations that have been linked to resistance to SP as a clearing agent and IPTp respectively.

P13 Understanding the role of the RNA binding protein FD4 in malaria transmission

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The malaria-causing parasite *Plasmodium falciparum* relies on the formation of specialised sexual cells, known as gametocytes, for successful transmission. Male and female gametocytes develop in the human bloodstream and are ingested during a mosquito blood meal. Following ingestion, they rapidly differentiate into male microgametes and female macrogametes, which undergo fertilisation, initiating further development within the mosquito. This human-to-mosquito transmission represents a critical bottleneck in the parasite's lifecycle and is therefore an attractive target for therapeutic intervention. Previous studies have shown that translational repression operates during gametogenesis. This process involves the sequestration of mRNA transcripts by RNA-binding proteins (RBPs), preventing their translation into proteins until the precise time they are required. In this work, we will investigate the function of a putative RBP, Female Development 4 (FD4). Preliminary data indicate that deletion of FD4 leads to transmission failure and significant dysregulation of pathways essential for gamete differentiation and fertilisation. We aim to characterise the RNA-binding capacity of FD4 protein and define the biological programmes it regulates during this key developmental stage.

P14 Predicting the impact of antimalarial resistance in West African parasites

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Background

Africa bares the highest malaria burden, with 569,000 deaths in 2023. Artemisinin combination therapies (ACT) remain the frontline treatment but are becoming less efficacious due to delayed parasite clearance linked to mutations in the *Plasmodium falciparum* Kelch 13 (K13) gene. While well documented in East Africa, similar resistance is predicted to emerge in West Africa.

Aims and Methods

This study investigates artemisinin phenotypes in contemporary West African parasites. A total of 380 isolates from Ghana and The Gambia will be genotyped, with 15 multi-drug resistant isolates selected for whole-genome sequencing. Known resistance markers, including *pfcrt*, *pfmdr1*, and *plasmepsin 2/3*, will be assessed and correlated with phenotype across adapted parasite lines. Three genetically diverse culture-adapted Ghanian strains will be transfected with key K13 mutations (R561H, 622I, A675V, C469Y) to analyse their resistance phenotypes. Multi-omics approaches will identify biochemical hallmarks linked to artemisinin resistance.

Results

Preliminary genotyping has revealed pyrimethamine resistance markers typically associated with mild resistance. However, phenotypic assays suggest strong resistance, indicating additional mechanisms may be involved. Initial work will correlate genotypic markers with phenotypic profiles across the same parasite populations.

Conclusion

These findings will inform ACT resistance surveillance and treatment strategies in West Africa.

P15 Blood-stage *Plasmodium* infection transcriptionally modifies stromal cell populations in the spleen

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The spleen protects against malaria by removing *Plasmodium*-infected red blood cells and initiating immune responses. To coordinate and recruit immune cells, the spleen is organised by a dynamic scaffold of stromal cells. Studies in steady-state and viral infections reveal splenic stromal cells are diverse and adaptable. Responses of these cells during *Plasmodium* infection remain uncharacterised. In this study, we employ *Plasmodium chabaudi chabaudi AS* parasites to determine whether and how splenic stroma respond during blood-stage infection of mice. Using flow cytometry, we found that stromal cells associated with T and B-cell zones in the white pulp significantly increased in number by day 28 post-infection, when splenomegaly was evident. These increases were retained when splenomegaly was ameliorated by anti-malarial drug intervention. Next, we noted follicular dendritic cells, associated with B-cell zones, had down-regulated CD157 (BST-1), suggestive of qualitative changes occurring in stromal cells. To test for this, we employed droplet-based scRNA-seq of enriched stromal cell populations. scRNA-seq analysis resolved diverse splenic stromal populations, as expected, with differential gene expression analysis suggesting some cell-types, but not all, had been altered transcriptionally. Thus, our data reveal for the first time that blood-stage *Plasmodium* infection induces cell-type specific quantitative and qualitative changes to splenic stroma.

P16 Chemo-attenuated liver-stage merozoites (CALM): a novel vaccine for malaria.

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Malaria is a disease of global importance, causing >600,000 deaths annually. Despite WHO approval of two vaccines targeting the circumsporozoite protein on sporozoites, robust and broadly protective immunity remains elusive. Our lab has developed a novel chemo-vaccination strategy that involves live sporozoite infection under the cover of a novel drug class that targets the late liver stage, transforming malaria parasite infection in the liver into a disease-free immunisation event. The drug class targets the essential aspartyl proteases plasmepsin IX & X, producing chemo-attenuated liver merozoites (CALM), who's death stimulates robust and durable sterile immunity in mice. The potent efficacy from whole-sporozoite vaccination approaches obtained in clinical trials with naive volunteers can be reduced in malaria pre-exposed populations. This reduction in immune efficacy appears to involve immune modulation during blood-stage malaria infections that occur before or concurrent with vaccination. It is unclear whether prior malaria infection influences the protective efficacy of CALM chemo-vaccination. Here, we will present our latest findings addressing this important question. Using the *P. berghei* BALB/c infection model, we will assess the effect of a concurrent or prior malaria blood-stage infection on the efficacy of CALM chemo-vaccination.

P17 Investigating primary and recall humoral immune responses in malaria and the role of type I interferon signaling

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Protective immunity to malaria relies primarily on high level of circulating antibodies. However, individuals in high-endemic areas often fail to mount a protective antibody level efficiently following vaccination, with antibody levels declining after each subsequent booster. The mechanism to establish and maintain the humoral and memory responses against the parasite is unclear. This study aims to investigate the qualitative and quantitative differences between primary and recall anti-malarial humoral immune responses, and to determine how malaria-induced immunoregulatory network via type I interferon, influences these responses.

We will use plasma and PBMCs samples collected from healthy volunteers participating in controlled human malaria infection (CHMI) study treated with Ruxolitinib, a licensed orally bioavailable small molecule inhibitor of type I IFNs signalling pathway, and anti-malaria drugs artemether/lumefantrine. We will investigate the specific epitope and breadth of the primary and recall antibody responses against blood-stage plasmodium antigen, MSP2, using peptide array while total IgG and IgM antibodies will be quantified using the Luminex assay. The phenotype of plasmodium-specific peripheral B cells will be detected and analysed with high dimensional spectral flow cytometry and scRNAseq with the aid of B cell tetramers. Collectively, this study will give important insights into the development of next-generation malaria vaccines.

P18 Investigating the function and phylogenetic conservation of *Plasmodium falciparum* vaccine candidate MSP2

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Plasmodium falciparum merozoite surface protein 2 (PfMSP2) has been a target of blood stage malaria vaccines, which have reached phase I/2b clinical trials. Despite decades of interest in MSP2, characterisation of its function remains limited. Though originally thought unique to the *Laverania*, comparison of published genomes revealed MSP2 orthologues in avian malarias, with conservation of N and C terminal domain amino acid composition. A common feature found in the *Laverania* MSP2 was a disordered repeat region of varying composition near the N terminus, suggesting this unstructured region provides an evolutionary advantage. Despite previous studies reporting MSP2 is essential for blood stage growth, we successfully knocked out MSP2. MSP2 knock out did not influence blood stage growth but did increase potency of antibodies targeting other vaccine candidates, such as AMA1. To investigate this phenotype, erythrocyte invasion by wild-type and MSP2-knockout parasites will be observed in the presence of anti-AMA1 serum using live cell microscopy. Thus, while not essential for *in vitro* growth, the evolutionarily conserved features of PfMSP2 and its effect on immune evasion suggest an important role, which will be further characterised in ongoing work.

P19 Antibodies to mosquito salivary proteins following controlled mosquito biting exposure

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Measurement of human antibodies specific for mosquito salivary antigens has been proposed as an outcome measure to assess human exposure to vector bites. However, only a handful of antigens have been identified and the specificity and longitudinal dynamics of antibody responses are not well known. We conducted a world-first clinical trial of controlled exposure to mosquito bites to identify and validate biomarkers of exposure to malaria and dengue mosquito vectors in Southeast Asia. This trial was an exploratory randomized controlled trial of controlled exposure to mosquito bites with 10 arms corresponding to different species (Aedes aegypti, Ae. albopictus, Anopheles dirus, An. maculatus and An. minimus) and biting levels (35 or 305 bites total over 6 weeks) and its effect on species-specific antibody levels longitudinally (17 weekly measurements). Antibodies against species-specific candidate antigens were measured by high-throughput ELISA in sera collected from participants (n=210) before, during and after mosquito challenges. We found that antibody levels against Anopheles and Aedes salivary peptides decayed slowly overall but with small boosts during and after mosquito biting challenges. This research generates important knowledge on species-specific antigens for vector sero-surveillance in Southeast Asia.

P20 The Effects of N6-methyladenosine on the Proteome of *Plasmodium falciparum*

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Plasmodium spp. utilises many mechanisms to regulate gene expression, including the modification of mRNA bases. One such modified base, N^6-methyladenosine (m6A) is the most common modification found on mRNA and is known to influence mRNA functions such as export from the nucleus, cytosolic abundance and translational efficiency in yeast and mammalian cells.

Adenosine is converted to m6A by the nuclear protein complex known as the m6A writer. Orthologs of the human m6A writer, such as *Pf*MT-A70 have been detected in *P. falciparum*. We use the rapamycin inducible knock sideways system to mislocalise *Pf*MT-A70 away from the nucleus to the parasite plasma membrane to study the effects of m6A. The aim of this study was to determine the effects of m6A on the proteome of *P. falciparum* using label-free quantitative proteomics.

Human erythrocytic stages that were 28±2 HPI and 36±2 HPI were used for the analysis. Knock sideways was induced for 4 and 8 hours for each time-point along with uninduced controls. Peptides for 1,548 and 2,117 were detected for the 28 HPI and 36 HPI time-points respectively, however a small proportion of these (6.9% and 11.8% respectively) had statistically significant changes to their abundance following 8 hours of knock sideways.

P21 Update on the prevalence of malaria infection at first antenatal visit in Madang Province, Papua New Guinea

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Infection with *Plasmodium falciparum (Pf)* and *Plasmodium vivax (Pv)* in pregnancy is associated with adverse outcomes. This study assessed the prevalence and predictors of malaria infection at first antenatal visit (ANC) in Madang Province, PNG.

Between August 2022 – August 2024,1,171 pregnant women (12 – 26 week's gestation) attending three rural and two urban health facilities were screened as part of a clinical trial (NCT05426434). Venous blood and blood slides were analysed by light microscopy (LM) and quantitative PCR (qPCR). Genus qPCR to detect infection; species-specific qPCR identified and quantified *Pf* and *Pv*. A subset of low-density samples underwent ultra-sensitive species PCR.

Overall malaria prevalence was 15.7% by LM and 38.2% by qPCR. Among 429 participants with species-specific qPCR results, *Pf* was the predominant parasite detected: *Pf* mono infection (59.7%), followed by *Pv* mono-infection (28.4%) and mixed infection (12.3%). Prevalence was higher in women attending rural vs. urban health facilities (qPCR: 41.2% vs. 29.9%) and among primigravid vs. multigravid women (qPCR: 38.8% vs. 37.7%; microscopy: 19.2% vs. 13.7%).

Over one-third of women carried malaria parasites at ANC, indicating a resurgence in burden (2009-12:10). Interventions targeting early pregnancy, prior to antenatal care, are urgently needed to reduce maternal and neonatal risks.

P22 Metabolic Tracing in *P. falciparum* Using a Stable Isotope Labelling Strategy

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Plasmodium falciparum, the causative agent of malaria, undergoes complex metabolic transformations across its life cycle stages, presenting opportunities for targeted therapeutic interventions. This study employs stable isotope labeling (SIL) with 13C6-glucose to investigate metabolic fluxes in infected red blood cells (iRBCs) under different developmental conditions. High-resolution mass spectrometry and bioinformatic analysis enable the identification of both native and labeled metabolites, offering insights into metabolic pathway activities and potential novel metabolite discovery. The approach captures dynamic metabolic changes across various stages, contributing to a comprehensive understanding of *Plasmodium* metabolism and supporting efforts to develop novel antimalarial strategies.

P23 Applying tensor-structured decomposition to identify patterns of protective immunity against malaria

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Both the quantity and quality of antibodies are important for immunity to malaria. 'Systems serology' involves measuring antibodies to multiple antigens, as well as multiple antibody Fc features, like isotype, subclass and leukocyte receptor binding. Such studies generate large, high-dimensional data that is challenging to interpret.

We have applied a technique called 'Tensor-structured decomposition' to simplify systems serology data into 'components' that summarize unique points of variation. Importantly, the contribution of the original variables to each component can be visualized across multiple dimensions (for example, across subjects, times, antigens and Fc features), offering a key advantage over other dimension reduction techniques. We have applied tensor-structured decomposition to two systems serology datasets, to uncover patterns of protective immunity against severe malaria in Papua New Guinean children and Kenyan children.

In Papua New Guinean children, we determined that overall cytophilic antibodies to all variant surface antigens measured were associated with protection from severe malaria. In Kenyan children, we identified a subset of variant surface antigen targets of antibodies that are associated with protection from cerebral malaria across three time points. Tensor-structured decomposition provides novel insights into naturally acquired protective immunity to severe malaria and can be applied to other multi-dimensional datasets.

P24 Development of next-generation mRNA vaccines against malaria

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Current malaria vaccines (RTS,S/AS01 and R21/Matrix-M) provide modest and short-lived efficacy against clinical malaria, emphasising the need for innovative vaccine approaches to achieve greater efficacy. The mRNA platform has advantages for more efficient and robust construct design and multi-antigen formulation, needed to target multiple *Plasmodium* species, compared to conventional platforms. As proof-of-concept, we aimed to evaluate the RTS,S/R21 vaccine antigen based on the *P. falciparum* circumsporozoite protein (*Pf*CSP) using the mRNA platform and a novel trivalent mRNA vaccine formulation combining the RTS,S/R21 and two *P. vivax* antigens. We vaccinated mice with our RTS,S/R21 mRNA alone or in combination with the *P. vivax* mRNA (trivalent) in a three-dose regimen. IgG levels were monitored over 9 months to examine the immunogenicity and durability of the immune response.

We found that the RTS,S/R21 mRNA induced high IgG levels against *Pf*CSP, while the trivalent mRNA vaccine successfully induced IgG responses to all three antigens. In future studies, we will evaluate vaccine-induced IgG subclasses and antibody functional activities with next-generation mRNA platforms, including self-amplifying RNA and circular RNA. The findings will inform the next-generation malaria vaccine development and provide insights into developing vaccines against *P. falciparum* and *P. vivax*.

P25 Investigating the effect of plasma primaquine and carboxyprimaquine concentrations on the risk of *Plasmodium vivax* recurrence

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Background:

Primaquine is the most widely used treatment to prevent relapsing *Plasmodium vivax* malaria. It is administered at the same weight-adjusted dose in all ages. Recent studies suggest that children have a higher risk of recurrence following primaquine compared with adults. We aimed to define the relationship between plasma primaquine concentrations and the risk of *P. vivax* recurrence across different age groups.

Methods:

A subset of data from the IMPROV trial were used. IMPROV was a randomised trial to compare high-dose primaquine regimens. The association between day 7 plasma primaquine and carboxyprimaquine concentrations and the risk of *P. vivax* recurrence by day 120 were investigated using Cox proportional hazards regression. Mixed-effects linear regression was used to investigate the association between the daily dose of primaquine and day 7 plasma primaquine and carboxyprimaquine concentrations.

Results: Data were available for 317 patients administered 7-day primaquine (PQ7 - 1.0mg/kg/day) and 377 patients administered 14-day (PQ14 - 0.5mg/kg/day) primaquine. The median plasma primaquine concentrations was 17.5ng/mL (IQR:7.2-39.1) after PQ7 and 4.2ng/mL (2.1-9.6) after PQ14. The respective carboxyprimaquine concentrations were 1670.0ng/mL (876.0-2370.0) and 610.0ng/mL (347.0-938.0). The relationship between plasma concentrations, the daily primaquine dose and the risk of recurrence will be presented.

P26 Variability in malaria exposure estimates: an exploratory methodology for integrating GPS data

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Forests are considered a high risk environment for malaria transmission which makes the forest-going population a key demographic for targeted interventions. GPS trajectories which provide fine scale spatio-temporal information on individual movement can be used to identify individuals in this demographic and validate hypotheses about environmental exposure. However, producing reliable estimates of exposure from raw data requires a sequence of processing choices particular to the available data and context. Each alternative option in the analysis brings with it different assumptions about the validity and meaning of the data. To provide concrete demonstrations of when these choices matter, we analyse GPS trajectories collected in a rural area of Cambodia with a focus on estimating exposure to mosquito biting and therefore the risk of malaria infection. Integrating GPS data with local geospatial information raises additional questions about the best approach to linking multiple data sources together. We identify key decision points in the data processing pipeline, using a structured framework to explore how different choices modify the data and ultimately affect the estimation of exposure.

P27 Monoclonal antibodies against *Plasmodium vivax* work synergistically to recruit immune factors

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Monoclonal antibodies (mAbs) are a promising new avenue for malaria elimination. Recent data indicate that recruitment of the complement system by antibodies is strongly correlated with clinical protection against malaria.

Here, we demonstrate that mAbs can be modified to become more potent recruiters of complement, surpassing that of unmodified mAbs and highlighting a powerful strategy to boost protective immunity and confer greater protection.

C1q is a hexameric complement protein that activates when bound to multiple sites on separate Fc antibody tails. Utilising this knowledge, we hypothesised that complement recruitment depends on the arrangement of multiple antibodies. We established two novel approaches for improving mAb design to maximally bind C1q. Firstly, we trialled and identified specific combinations of mAbs that are capable of working synergistically to fix more complement. Secondly, we created hexamers of mAbs by inducing a Stellabody® single point mutation in their Fc region, enhancing their ability to bind to the hexameric C1q.

This current research builds upon previous work in which we identified mAbs to a specific malaria antigen (*Pv*AMA1) that bind to C1q and emphasises the critical need for more targeted approaches in future.

P28 Investigating malaria specific B cells in secondary lymphoid tissues in children.

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Background: Clinical immunity to *Plasmodium falciparum* malaria is mediated by protective antibodies acquired over years of repeated infections in children. Antibody production depends on B cell activation within germinal centres (GCs) in secondary lymphoid tissues. No studies have yet characterised malaria-specific B cells within GCs.

Methods: Tonsil cells were collected from Ugandan children aged 2-11 (n=103), 33% of whom had an asymptomatic malaria infection. Malaria-specific B cells were quantified using tetramers for MSP1, MSP2, and AMA1 and compared to RBD (SARS-CoV-2) and haemagglutinin (HA) (influenza) -specific cells by spectral flow cytometry.

Results: Malaria-specific B cells were detected in tonsils and were significantly higher in the Ugandan children compared to Australian malaria-naïve controls. Malaria-specific B cells had a GC phenotype and were class-switched whereas RBD and HA -specific cells were enriched for memory B cells. Unbiased clustering analysis to quantify B cell phenotypes and associations with infection status and humoral responses is ongoing.

Conclusion: Tonsil cells are a readily available secondary lymphoid tissue suitable for studying malaria-specific B cells in the GC. Future studies will analyse spatial locations of parasites and B cells in the tonsil. Data may inform our understanding of B cell development during malaria infection in children.

P29 Profile and functions of antibody responses in children given the RTS,S vaccine with repeated annual booster doses

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There remains an urgent need for effective malaria interventions to reduce disease burden in children. Combining RTS,S vaccination with seasonal anti-malarial chemoprevention was shown to greatly enhance protection among children compared to either intervention alone. However, over four years, efficacy of this combination steadily declined despite annual booster. RTS,S primarily induces antibody responses that contribute to clinical immunity. Only one antibody type, IgG, has been studied in this trial and was shown to peak following primary vaccination, but progressively declined after each annual booster. The drivers of poor IgG responses to boosters, and whether these also affect other antibody response types, remain unknown. Understanding the induction, decay, and boosting of immunity is essential to inform the next generation of malaria vaccines with greater efficacy and longevity.

We evaluated young children in West Africa who received RTS,S and subsequent boosters with or without seasonal chemoprevention as part of a phase-III clinical trial. We quantified the induction and boosting of antibodies over four years (n=1,929), evaluating antibody isotypes, multiple functional activities, and epitope specificity. We observed novel differences in the boosting and kinetics of different antibody isotypes and functions to different epitopes, shedding light on why vaccine efficacy wanes over time.

P30 Explorative analysis investigating VAR2CSA antibodies previously identified as protective against placental malaria in Papua New Guinea and their associations with foetal and maternal outcomes in pregnant women in Malawi

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The *Plasmodium falciparum* protein VAR2CSA which binds placental receptor chondroitin sulfate-A (CSA) is a vaccine and therapeutic candidate for reducing placental malaria. This study aimed to investigate if six previously identified antibody features associated with protection against placental malaria in PNG women were also associated with reduced risk of small gestational age and maternal anaemia in women exposed to *Plasmodium falciparum* in Malawi.

Six antibody features were antibody-dependent phagocytosis by THP1 and neutrophils, IgG3 to CSA-binding infected erythrocytes (IE), IgA2 and IgG3 to VAR2CSA subdomain DBL2, and antibody-dependent CSA IE binding inhibition, in 466 Malawian women at mid-pregnancy and delivery.

In primigravidae at enrolment, antibodies promoting neutrophil or THP1 phagocytosis, and IgG3 to CSA-binding IE were associated with reduced risk of maternal anaemia at delivery and similar associations were observed for antibody-dependent THP1 phagocytosis and IgG3 levels to IE at delivery. However, no clear associations were found between the six antibody features and small gestational age.

Antibody features identified in PNG also confer protection against maternal outcomes in Malawi. Immunity to VAR2CSA that protects against maternal anaemia may develop as early as mid-first pregnancy. Phagocytosis of CSA-binding IE is an important protective mechanism to consider for VAR2CSA-based vaccines and therapeutics.

P31 Molecular surveillance of pfhrp2 and pfhrp3 gene deletions in *Plasmodium falciparum* field isolates from the Central Highlands of Vietnam

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In Vietnam, the most commonly used malaria rapid diagnostic tests (RDTs) rely on detection of *Plasmodium falciparum* histidine-rich proteins 2 and 3 (HRP2/3) antigens for diagnosis of falciparum malaria. However, the emergence and spread of *P. falciparum* parasites with *pfhrp2* and *pfhrp3* gene deletions in countries worldwide pose a significant challenge for malaria diagnosis, leading to the occurrence of false negative results. The aims of this study were to determine the prevalence of *pfhrp2/3* gene deletions in *P. falciparum* isolates in malaria endemic regions of Vietnam and characterize the *pfhrp2/3* genetic profile of the parasite population.

Of 233 *P. falciparum* isolates collected between 2018-2021 and analysed in this study, the majority of samples were collected in Dak Nong province (47.6%, 111/233), followed by Dak Lak (36.9%, 86/233), Kon Tum (9.9%, 23/233) and Gia Lai (5.6%, 13/233) provinces. No *pfhrp2* deletions were detected, while the estimated prevalence of *pfhrp3* deletions was 6.4% (15/233, 95% confidence interval 3.6 -10.4%). The analysis of the HRP2/HRP 3 amino acid sequences will be presented. The absence of *pfhrp2* gene deletions suggests that there is no immediate need to change HRP2-based RDTs for malaria diagnosis in the Central Highlands of Vietnam but ongoing monitoring is essential.

P32 Diagnostic accuracy of a malaria rapid diagnostic test (RDT) used in Lao People's Democratic Republic (Lao PDR): a secondary data analysis

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To support the malaria elimination agenda in Lao PDR, a one-year (1 March 2022 and 28 February 2023) trial evaluating effectiveness of personal protection package was conducted in 423 villages across five malaria-endemic provinces in southern Lao PDR. Both residents and mobile migrant people in the study areas were tested for malaria using RDT (Abbott Malaria Ag *Plasmodium (P) falciparum/ P. vivax*) by village malaria workers. Archival RDTs were used to determine presence of *P. falciparum* and *P. vivax* by polymerase chain reaction (PCR). Taking PCR as the gold standard, diagnostic accuracy of the RDT was estimated with exact binomial 95% confidence intervals (CI). The sensitivity and specificity of RDT were 36.8% (95% CI: 30%-44%) and 99.8% (95% CI: 99.7%-99.9%), respectively, among all the individuals tested by both RDT and PCR. Positive and negative predictive values of the RDT were 78% (95% CI: 68.1%-86%) and 98.9% (95% CI: 98.6%-99.1%), respectively. Performance of a RDT on diagnosis of RDT-detectable malaria is affected by various conditions of manufacture, storage and use that can impair the tests' accuracy and reliability. Further investigations on RDT deployment and use including post-deployment quality testing (lot testing) should be undertaken for appropriate public health actions.

P33 Treatment failure and post-artesunate delayed haemolysis in a returned traveler from Uganda with severe *Plasmodium falciparum* malaria with partial artemisinin resistance and reduced susceptibility to lumefantrine

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A 49-year-old man recently returned from Uganda was admitted to hospital in Brisbane with *Plasmodium falciparum* malaria, with hyperparasitemia of 1,507,220 parasites/μL. The patient had a negative blood film after completing treatment with intravenous artesunate followed by artemether-lumefantrine; however, he was readmitted 3 weeks later with recurrent parasitemia. Further testing for drug resistant phenotypes and genotypes demonstrated reduced susceptibility to lumefantrine, as well as an A675V mutation in the PfK13 gene, and increased ring-stage survival, consistent with partial artemisinin resistance. The case highlights the high risk of *P. falciparum* treatment failure in patients with hyperparasitemia and partial drug resistance.

P34 Epidemiological Study on the Efficacy of Insecticide-Treated Bednets in Malaria Transmission Control

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Although insecticide-treated nets have proven efficacy against nocturnal vectors of malaria in Africa and Asia, evidence from Central America remains scarce, and the prevalence of day-biting vectors demands rigorous, context-specific bed-net studies in this region. My PhD aims to comprehensively evaluate the efficacy of insecticide-treated bednets (ITNs) in controlling malaria transmission. Evidence was synthesized through (i) a narrative review of Integrated-Vector-Management (IVM) and emerging mosquito-vector-control tools; (ii) a systematic review and meta-analysis of randomized-controlled-trials(RCTs)/cluster-RCTs(cRCT)(iii) analysis of a cRCT study on ITNs in Ixcan, Guatemala (25 communities; seven-month follow-up).

The meta-analysis showed a 29% reduction in malaria incidence in Africa (RR=0.71; 95% CI: 0.54–0.93; p=0.013; I^2=64%) and a 68% reduction in Asia (RR=0.32; 95% CI: 0.17–0.62; p=0.0007; I^2=89%); meta-regression indicated a significant overall intervention effect (β=–0.84; 95% CI: –1.68 to –0.01; p=0.05). The narrative review found integrated strategies outperformed single malaria-vector interventions (six studies, $p<0.05$) with declines in morbidity, mortality, and entomological indicators; emerging tools (e.g. Genetically-modified-mosquitoes, green-synthesized metallic nanoparticles) show promise as complementary options. In the cRCT study, Malaria cases were 165 in the control group, compared with 64 in the intervention group, corresponding to an approximate 60% lower incidence with the intervention (RR ≈ 0.40).

P35 Investigating the mechanisms of action of repurposed compounds as antimalarials

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Malaria remains a leading cause of morbidity and mortality, with 608,000 deaths reported in 2022, predominantly among children under five in sub-Saharan Africa. The disease, predominantly caused by *Plasmodium falciparum*, faces escalating challenges due to the emergence of strains resistant to all frontline antimalarials, including artemisinins. This highlights an urgent need for new therapeutic strategies. While *de novo* drug discovery is slow and costly, repurposing existing compounds offers a rapid and cost-effective alternative.

We screened the Structural Genomics Consortium's Donated Chemical Probes Library, which consists of compounds originally developed for human diseases, to identify candidates with antimalarial potential. From 200 compounds, 11 demonstrated sub-micromolar potency against the *P. falciparum Pf*3D7 reference strain and retained activity across five resistant lines. Their equipotent profiles suggest novel mechanisms of action distinct from existing drug classes. *In vitro* pulse activity assays further characterized compound speed-of-action and asexual stage-specific antimalarial activity.

Future work will employ unbiased multi-omics approaches, including as untargeted metabolomics, solvent proteome profiling and limited-proteolysis mass spectrometry, to elucidate molecular targets and validate these candidates as starting points for drug development. These findings highlight the potential of compound repurposing to accelerate antimalarial discovery and identify new druggable pathways to combat resistance.

P36 Preparing for Complex Resistance: modelling the spread of treatment resistant malaria with multiple-mutation haplotypes

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Resistance to artemisinin is a major challenge to malaria elimination. Molecular surveillance of genetic mutations marking artemisinin resistance is a crucial tool in tracking the spread of resistance over space and time. Recently, potentially more resistant genotypes with mutations in multiple genes have been observed. Monitoring the prevalence of these co-occuring mutations is important to disease elimination efforts, but comprehensive data documenting then remains scarce. To address this, we systematically review existing literature for evidence of multi-marker artemisinin resistance, and developed bespoke modelling approaches tailored for best extracting spatio-temporal patterns from emerging multi-marker data. Our review finds that current evidence of multi-marker artemisinin resistance is limited. While multi-marker mutants do not appear to pose imminent threats, the insufficiency of existing databases in documenting multi-marker mutations undermines our ability to monitor them in the future. In preparation of such future threats, we develop geospatial models that simultaneously predict the patterns of different mutations, including when they concurrently appear. We demonstrate an application of the model approach using the more prevalent Sulfadoxine-Pyrimethamine resistance data, showcasing its utility in malaria molecular surveillance. Our findings underscore the importance of the development of database and modelling tools in supporting malaria elimination.

P37 Investigating the activation of new permeability pathways in *Plasmodium falciparum* infected erythrocytes

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With resistance across all available antimalarials, gaining deeper insights into *Plasmodium* biology could help identify novel drug targets. About 12-15 hours post invasion, *Plasmodium falciparum* modifies the host cell membrane to form 'new permeability pathways' (NPPs) to facilitate enhanced nutrient uptake and accommodate the parasite's increased metabolic demands for subsequent growth and proliferation. NPPs behave like channels and play a key role in several of the parasite's metabolic pathways that rely entirely on host-cell scavenging. Whilst a lot is understood about the transport properties of NPPs, the identity of the proteins that form NPPs remain poorly understood. The RhopH complex, a trimeric protein consisting of CLAG3, RhopH2, and RhopH3, has been implicated to play a role in the formation of NPPs though the mechanism as to how the complex does this remains unclear. This research focuses on the proposed mechanism in which the RhopH complex interacts with integral erythrocyte membrane transporters to enhance nutrient uptake, following a recent crosslinking study which consistently identified an interaction between RhopH2 and Glut1. By applying metabolomics and structural proteomics, we aim to explore the functional consequences of the interaction between RhopH2 and Glut1 and elucidate the mechanism of NPP activation in *P. falciparum* infected erythrocytes.

P38 Neutrophil extracellular trap (NET) formation is inhibited by both *Plasmodium falciparum*–infected and uninfected erythrocytes

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Neutrophils are innate immune cells, but their role in clearing *Plasmodium falciparum* in humans is unclear and requires investigation. Neutrophil extracellular traps (NETs) are DNA and protein webs that trap and stop the spread of infection. We developed a simple assay to assess the effect of infected erythrocytes (IE) and uninfected erythrocytes (UE) on NET formation. In the assay, SYTOX green-stained primary neutrophils stimulated with phorbol 12-myristate 13-acetate (PMA) and calcium ionophore (CaI) were cocultured with different doses of IE and UE for 5 hours at neutrophil: erythrocyte ratios of 1:5, 1:10, 1:20 and 1:40. NET formation was measured by fluorometric DNA quantification and confocal imaging. Extracellular myeloperoxidase (MPO), a NET enzyme, was measured by ELISA in the supernatant. IE and UE markedly reduced NETs and MPO release in response to PMA or CaI stimulation in a dose-dependent manner, with pronounced inhibition at a 1:40 neutrophil: erythrocyte ratio. However, UE showed stronger inhibition than IE, suggesting a non-parasite-specific NET suppression. Microscopy shows reduced NET formation with IE and UE. This study highlights a potential novel immune evasion strategy of *P. falciparum* and suggests that erythrocytes, regardless of infection status, can modulate neutrophil responses. The inhibitory mechanism is under investigation.

P39 CERLI1 and CERLI2 roles during invasion process into mosquito salivary and human liver cells

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Invasion is a pivotal step in the Plasmodium falciparum life cycle, occurring during sporozoite entry into mosquito salivary glands, human hepatocytes, and red blood cells. These events depend on rhoptries, specialized apical secretory organelles. While much attention has focused on rhoptry proteins, the molecular mechanisms regulating rhoptry function remain poorly understood. Recently, cytosolically rhoptry-interacting proteins (C-RIPs) have been proposed as regulators of rhoptry biogenesis, secretion, and structural organization. Among these, Cytosolically Exposed Rhoptry Leaflet Interacting proteins 1 and 2 (CERLI1/2) have been shown to be essential for merozoite invasion, yet their roles in sporozoites remain uncharacterized.

This PhD project investigates the functions of CERLI1 and CERLI2 in P. falciparum sporozoites. Initial immunofluorescence assays confirmed their rhoptry localization. Ongoing work includes phenotypic characterization of knockout parasites across mosquito and liver stages, development of an ex vivo salivary gland invasion assay, and ultrastructural analysis of rhoptries in CERLI1/2 mutants. Future studies will employ proximity labeling (mini-TurboID) to identify interacting partners.

Overall, this project will provide novel insights into rhoptry regulation in sporozoites and may identify potential targets for interventions effective across multiple stages of the malaria parasite life cycle.

P40 Profiling Antibody-mediated Neutrophil Multi-Functions Against *P. falciparum*-Infected Erythrocytes

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Background: Neutrophils, the most abundant leukocytes, may play a crucial role in defence against malaria. They exhibit diverse effector functions in response to malaria parasites. However, their specific roles during malaria infection and how they cooperate with antibodies to protect against malaria during pregnancy remain poorly understood. **Methods:** We used *Plasmodium falciparum*-infected erythrocytes (IEs; CS2 line), isolated primary neutrophils from healthy donor and pregnant women's plasma to perform multiparametric assays to investigate antibody-dependent neutrophil functions. Functions included phagocytosis, production of reactive oxygen species (ROS), degranulation, FcγRI activation, ectodomain shedding, and the formation of neutrophil extracellular traps (NETs). We assessed their association with protection against placental malaria (PM) in Papua New Guinean women. **Results:** Compared to women with PM, women without PM showed significantly higher neutrophil phagocytosis (**P=0.046**) and ROS production (**P=0.004**), accompanied by increased surface expression of CD11b (Secretory vesicles/CR3; **P=0.0007**), CD35 (Secretory vesicles/CR1; **P=0.008**), and CD66b (Secondary granule secretion; **P=0.006**). Notably, antibody-mediated NET formation was also elevated in women without PM (**P=0.009**), suggesting that NETs may contribute to protective immunity when driven by opsonising antibodies. Overall, plasma samples varied in their ability to induce markers of neutrophil activation. **Conclusion:** Antibody-dependent neutrophil functions are diverse between individuals and associated with protection against PM.

P41 Mapping protein complexes to reveal new functions in the malaria parasite

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The complexity of *Plasmodium falciparum*'s lifecycle and the limited understanding of its biology have hindered the identification of promising drug targets. This project aims to address these challenges by mapping protein–protein interactions across the asexual blood stage of the parasite using proteomics. By implementing protein correlation profiling, we will identify interacting proteins through mass spectrometry analysis of co-eluted complexes from chromatographic fractionates. Various combinations of chromatographic separation techniques will be implemented to ensure high coverage of genuine protein interactions. This approach enables the detection of dynamic changes in protein complexes, such as those regulating gene expression and the cell cycle. These are key processes driving the parasite's rapid replication; however, underlying mechanisms remain unclear. Our study will fill this gap by capturing stage-specific interaction networks, revealing how protein complexes evolve from ring to trophozoite to schizont stages. These insights will help annotate previously uncharacterized, non-conserved proteins by associating them with well-characterised proteins. The resulting network of protein–protein interactions will complement other proteomic approaches, guide functional validation of previously unannotated proteins and provide critical insights for the development of novel symptom-targeted therapeutics.

P42 Investigating Antibodies that Protect from Cerebral Malaria in Kenyan Children

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Cerebral malaria (CM), a severe manifestation of *Plasmodium falciparum* infection, results from PfEMP1-mediated sequestration of infected erythrocytes in the brain microvasculature. PfEMP1 also promotes rosetting, and antibodies against specific domains are linked to protection and reduced disease severity. In this study, plasma from Kenyan children with cerebral or uncomplicated malaria collected at hospital enrolment, six weeks post-infection, and six months post-infection was used to characterise targets and features of antibody responses. Antibody classes, subclasses, and engagement of Fcγ receptors and complement C1q were assessed using 28 PfEMP1 antigens previously associated with severe malaria, along with five merozoite and one sporozoite antigens. Using a machine learning approach that coupled LASSO regression with a partial least squares regression (PLSR) model, we identified 12 antibody measurements at enrolment that classified children with cerebral and uncomplicated malaria with a median accuracy of 99% (IQR: 89–100%). Longitudinal analysis using a linear mixed-effects model of these 12 selected antibody measurements revealed that IgG2_AMA1, FcγRIIIb_DBLα1.5, and FcγRIIIb_CIDRα1.5 were consistently higher in children with uncomplicated malaria compared to those with cerebral malaria across all three time points. These findings offer insights into the development of malaria therapeutic interventions aimed at reducing CM morbidity and mortality in children.

P43 Characterising monoclonal antibodies to placental malaria antigen, VAR2CSA

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Placental malaria, due to the sequestration of *Plasmodium falciparum*-infected erythrocytes (IE), causes adverse pregnancy outcomes. The sequestration is mediated by VAR2CSA, a protein that binds to placental chondroitin sulfate A (CSA). VAR2CSA antibodies protect against adverse pregnancy outcomes; however, no pregnancy-specific vaccine or therapeutic exists to date. We identified and expressed VAR2CSA-specific IgG1 monoclonal antibodies (mAbs) using B cells of exposed Papua New Guinean women. VAR2CSA mAbs were characterised by their ability to recognise eight heterologous CSA-binding *P. falciparum* strains to neutralise CSA binding and/or induce phagocytosis of IEs by THP-1 monocytes. We identified 16 mAbs, and all targeted just two of the six domains of VAR2CSA. Cross-reactivity varied between mAbs, with 2D9 binding to all eight strains. Although individual mAbs did not promote phagocytosis, combinations targeting distinct VAR2CSA epitopes did. None of the mAbs inhibited IEs from binding to CSA. Structural studies using Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) with 2D9 revealed a broadly conserved epitope on globular VAR2CSA structure. Our findings revealed a conserved VAR2CSA epitope as a target for strain-transcending immunity and suggest that effective mAb therapies should combine mAbs targeting distinct epitopes. Altogether, we demonstrated how mAbs can dissect naturally acquired antibody responses to inform vaccine design.

P44 Antibody-dependent activation of NK cells as a potential correlate of protection from placental malaria: Insights from Malawi and PNG cohorts with varying infection status at enrolment

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Background: In malaria-exposed pregnant women, antibodies to VAR2CSA induce NK cell activation; whether the role of antibody-mediated activation contributes to protection from placental malaria remains unclear.

Method: This study explored activation of NK92-CD16A and primary NK cells by IEs opsonised with plasma samples collected mid-pregnancy from 1) Malawian pregnant women who were infected at enrolment with (n=72) and without (n=84) evidence of past placental malaria at delivery or 2)73 PNG pregnant women (most whom were uninfected at enrolment), 46 with placental malaria at delivery and 27 with peripheral infection but not placental malaria.

Results: NK cell responses from IE opsonised with plasma from Malawian women were not associated with gravidity and were higher with plasma from women with placental malaria than without, with differences between the two groups observed for CD107a in NK92-CD16A ($p = 0.011$) and IFNγ in primary NK cells ($p=0.012$). Using PNG women's plasma, responses of NK92-CD16A cells (CD107a) and NK cells (CD107a, TNF and IFNγ) were lower for plasma from women with than without placental malaria ($P<0.039$).

Conclusion: Antibody dependent activation of NK cell responses show potential as an immune correlate of protection from placental malaria, but associations vary with the timing of infection.

P45 Investigating Antibody Responses to Endothelial Protein C Receptor Binding *Plasmodium falciparum* Erythrocyte Membrane Protein 1 Associated with Severe Malaria in Children

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Introduction: Variant surface antigens, mainly PfEMP1, on infected erythrocytes (IEs) play role in malaria pathogenesis and immunity (2-4). While antibodies to EPCR-binding PfEMP1 are linked to reduced severity, their role in protection, particularly when measured using IEs and functional assays, is not well understood. **Methods:** Plasma from PNG children with severe malaria (SM) and uncomplicated malaria (UM) was used to measure IgG binding and antibody-dependent neutrophil and monocyte phagocytosis (ADNP, ADCP) of IE expressing EPCR-binding IT4-*Var19* using flow cytometry, and antibody fucosylation was assayed. **Results:** At presentation, IgG response to IT4-*Var19* IE and ADCP by THP-1 cells were higher in children with UM than SM. ADCP but not IgG binding or ADNP increased from presentation to convalescence in SM children. Similarly, afucosylated IgG, was higher in UM than SM at presentation and increased from presentation to convalescence in children with SM. **Conclusion:** Higher IgG levels to *Var1*9-expressing IEs in children with UM may indicate prior exposure and protective immunity, while increased IgG during convalescence in SM suggests a role for IEs expressing *Var19*-like PfEMP1 in SM. Notably, the rise in ADCP at convalescence in SM despite stable IgG levels highlights the importance of measuring antibody function not just antibody levels.

P46 Manufacture of a new *Plasmodium falciparum* parasite bank for use in malaria volunteer infection studies

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Malaria volunteer infection studies (VIS) have played a major role in expediting development of antimalarial drugs, providing key insights into parasite biology, and host responses to disease. At QIMR Berghofer, *P. falciparum* malaria VIS have primarily been conducted using the 3D7-V2 master cell bank (MCB) produced in 1995. However, additional MCBs are now required for ongoing malaria VIS, even more so in malaria-endemic countries. Recently, we manufactured a new high-parasitemia *P. falciparum* 3D7 MCB in compliance with Good Manufacturing Practice requirements, for use in malaria-exposed individuals. One vial of 3D7-V2 was thawed and cultures expanded in flasks prior to seeding a 10L Cellbag™ bioreactor container at 1% parasitaemia (>95% ring stage) for further expansion using the Wave™ 25 bioreactor system. The final culture was cryopreserved with a parasitaemia of 10% (96% ring stages) in Glycerolyte 57 at 1:2.2 ratio to produce 240 x 1mL cryovials of a *P. falciparum* 3D7 MCB (Lot. No. MBE-028) and stored in vapour phase liquid nitrogen at Q-Gen Cell Therapeutics. Further characterisation and testing of this MCB is underway to ensure suitability for use in future malaria VIS. Updated findings will be presented.

P47 Investigation of the relationship between naturally acquired antimalarial antibodies and the duration and clearance of ultra-low density *Plasmodium vivax* infections

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Spontaneous clearance of subclinical peripheral *Plasmodium* spp. parasitemia, including *P.vivax*, occurs frequently in low transmission settings. Subclinical *P.vivax* infections contribute significantly to ongoing malaria transmission, therefore understanding the role of antibody mediated immunity in the spontaneous clearance of parasitemia is essential to the broader understanding of the epidemiology and transmission potential of *P.vivax*. We sought to identify IgG responses associated with clearance of peripheral *P.vivax* parasitemia. IgG against 30 *P.vivax* antigens was determined in participants of a nested cohort study of subclinical *Plasmodium* spp. infection in Laos (n=202) and Cambodia (n=150) sampled monthly for 12 months (n=3,041). *Plasmodium* spp. infections detected by ultrasensitive qPCR. Accelerated failure time models were used to determine the association between IgG and time to spontaneous clearance of peripheral *P.vivax* parasitemia. A total 293 subclinical *P.vivax* infections were detected. Spontaneous clearance was observed in 79% and 67% of participants in Laos and Cambodia, respectively. Anti-*P.vivax* IgG responses were higher in *P.vivax* infected compared to uninfected participants during the study period. Antigen-specific IgG responses were not associated with time to clearance of peripheral *P.vivax* parasitemia. Future investigations will include analysis of multi-antigen responses. These findings will further our understanding of immunity in the epidemiology of *P.vivax* infections.

P48 Identification of B cell epitopes in serological exposure markers for improved *Plasmodium vivax* surveillance

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Malaria remains a major global public health burden. *Plasmodium vivax* poses a challenge to malaria surveillance due to its unique life cycle. Recently, serology has emerged as a promising surveillance tool. It is based on antibody detection of serological exposure markers. Our previous work identified a panel of eight *P. vivax* antigen markers that demonstrated good sensitivity and specificity in detecting recent *P. vivax* exposure in endemic cohorts. However, difficulties in protein expression and purification have limited the translational potential of the serology tools. In this study, we focus on B cell epitopes, the special regions of antigen markers that bind antibodies, which can potentially overcome these challenges and may provide improved performance over the antigens.

Using computational immunology tools, we predicted both conformational and linear B cell epitopes with potentially high antigenicity. In the follow-up epitope screening experiments, we selected the best-performing epitope peptides as candidates that have superior seroprevalence in IgG responses to sera from current *P. vivax* infections in endemic regions. In comparison to the recombinant protein markers, the combined use of several epitope candidates achieved higher sensitivity and specificity. These epitope candidates were further validated in observational cohort samples.