RESOURCE HUB *Correspondents*

Molecular Approaches to Malaria (MAM) Conference 2024

Complete series

MESA Correspondents bring you cutting-edge coverage from the Molecular Approaches to Malaria (MAM) Conference 2024

18 - 22 February 2024

Lorne, Melbourne, Australia

MESA would like to thank Carla Proietti (Institute for Molecular Bioscience, The University of Queensland, Australia), Emma McHugh (Department of Biochemistry and Pharmacology, The University of Melbourne, Australia) and Danushka Marapana (The Walter and Eliza Hall Institute of Medical Research (WEHI), Australia) for providing senior editorial support.

The MESA Alliance would also like to acknowledge the MESA Correspondents Angela Rumaseb (Menzies School of Health Research, Australia), Katelyn Stanhope (Burnet Institute, Australia), Mohini Anjna Shibu (The University of Melbourne, Australia) and Nutpakal Ketprasit (The University of Melbourne, Australia) for their coverage of the conference.

Table of contents

Opening Day: 18th February 2024

Opening Ceremony

The opening ceremony, moderated by **Michelle Boyle** (Burnet Institute, Australia) and **Chris Tonkin** (Walter and Eliza Hall Institute of Medical Research – WEHI, Australia), commenced with an acknowledgement to the country by Ebony Hickey. The Eastern Maar Aboriginal Corporation (EMAC) welcomed conference delegates with a series of traditional dances. Boyle introduced Jean-Luc Bodmer from the Bill & Melinda Gates Foundation, who delivered the plenary talk on behalf of Janice Culpepper who was unable to attend the conference in person. The opening ceremony continued with a touching and inspirational tribute to Dominic Kwiatkowski by **Julian Rayner** (University of Cambridge, UK) and a compilation of memories from colleagues who highlighted Kwiatkowski's passion, excellence and mentorship in malaria genomic research.

Plenary talk – New Tools and Strategies for Malaria Elimination

Jean-Luc Bodmer (Bill & Melinda Gates Foundation – BMGF, USA) reported on the Gates Foundation's three pillars of strategies aimed at defining the pathway to malaria eradication by 2040. The three pillars involve driving down the malaria burden (using surveillance, chemoprevention, and case management), shortening the endgame (via tools that target remaining cases e.g. long duration prophylaxis) and getting ahead of antimalarial resistance. Using the lessons learnt from the polio endgame, Bodmer highlighted the need to get ahead of resistance and prioritise investment in endgame research and development (R&D) tools. BMGF is currently investing in four portfolios of transformational tools, which are (1) self-sustaining gene drive, (2) malaria elimination vaccine, (3) single-encounter radical cure drug with long-acting endectocide, and (4) improved package tools for annual control. Bodmer emphasises the need for new tools that are cost-effective and deployable for use in impacted countries. When investing and developing new tools, Bodmer encourages the delegates to reflect on the stagnancy of funding in malaria research. He emphasises the need to reallocate funds from other areas to support the development of new tools and incentives. Additionally, he reminds delegates to adopt a regional perspective, take local action, and foster global partnerships. The plenary closed with the statement 'Malaria delenda est, malaria must be destroyed'.

This report is brought to you by the MESA Correspondents Angela Rumaseb, Katelyn Stanhope, Mohini Anjna Shibu and Nutpakal Ketprasit. Senior editorial support has been facilitated by Carla Proietti, Emma McHugh and Danushka Marapana.

Day 1: 19th February 2024

Session 1 – Life Cycle Biology – pre-Erythrocytic

The conference kicked off with the keynote presentation by **Photini Sinnis** (John Hopkins University, USA). She spoke on the current paradigm that infected mosquitos are infectious by delving into the quantitative understanding of malaria transmission. Sinnis and her team used a mouse model to determine the likelihood of *Plasmodium yoelli* infection after exposure to infected mosquitoes. She presented the association between mosquito parasite burden, inoculum size and infection likelihood, and suggested that parasite numbers mattered for onward transmission. This association of mosquito sporozoite salivary gland load and infection rate is non-linear, with mosquitoes carrying over 10000 salivary gland sporozoites being more likely to transmit disease. She concluded by suggesting the possibility that as little as 20% of infected mosquitoes might account for a substantial 80% of the infections, suggesting the presence of super-spreader mosquitoes within the population.

Friedrich Frischknecht (Heidelberg University, Germany) delivered a comprehensive presentation on gametogenesis and parasite development in mosquitoes. He underscored the importance of some of the cytoskeleton proteins in gametes and elucidated how studying mosquito stages can offer potential intervention strategies. He highlighted the "upside-down" assembly of axonemes and the microtubule "push-pull mechanism", crucial for gamete formation. He also emphasised the essential role of the microtubule interacting protein EB1 in nuclear segregation of gametes. Furthermore, Frischknecht and his team reported the essential role of a divergent Plasmodium actin-related protein 2/3 (Arp2/3) complex subunit, the ARPC1, in DNA segregation during male gametogenesis. They found that deletion of the actin binding protein profilin prevented mice infection. Frischknecht and his team were also able to generate 3D maps of oocyst maturation and sporozoite formation, providing valuable insights into the developmental processes of the parasite.

Hardik Patel (Seattle Children's Hospital, USA) presented research on the liver stage of *Plasmodium falciparum (P. falciparum)*. He discussed how blood stage host responses during concurrent infections can potentially suppress liver stage parasites, impacting the efficacy of live attenuated whole sporozoite vaccine. He reports that contrary to previous studies, the blood stage induced iron-regulating hormone hepcidin was not found to be responsible for liver stage development suppression. Instead, Patel observed that the host's cytokine response, especially interferon gamma (IFNγ), played a pivotal role in suppressing the growth of the liver stage parasite, with this effect varying among different *Plasmodium* species. Furthermore, Patel reported that the effect of IFNγ resulted in an increase in GABARAPs (mediators of lysosomal fusion) as well as reactive oxygen species, but not nitric oxide production.

Liliana Mancio-Silva (Institute Paster, France) opened her presentation by addressing the current knowledge gaps and challenges associated with the dormant liver parasites, hypnozoites. Her talk focused on the application of single cell transcriptomics in *Plasmodium vivax* (*P. vivax*) hypnozoites. Mancio-Silva's team used a bioengineered human microliver platform to culture patient-derived *P. vivax* parasites for transcriptional profiling. Dual scRNA-seq was used to analyse parasite and host transcripts in individual hepatocytes. Through their research, they identified potential biomarkers of hypnozoites based on ten genes. She also showed that hypnozoites keep the proteolytic activity to stay viable. Moreover, Mancio-Silva presented the findings of their transcription analysis, revealing that hypnozoite-infected hepatocytes are transcriptionally distinct, with two genesHLA-C and B2M, being notably expressed. The work gives insight into the mechanisms regulating *P. vivax* dormancy and may lay a foundation for the development of new vaccines and drugs.

Lauren Carruthers (University of Glasgow, Glasgow) explores and discusses the different gene expression across different life cycles, focusing on the ZINGER protein family. Carruthers shows that these proteins, characterised by 3x CCCH zinc finger domains, exhibit varying expression patterns throughout the parasite's life cycle. Through knockout studies, Carruthers showed that ZINGER proteins play important roles in different developmental stages, including gametocyte, ookinete, and oocyst formation. This underscores the pivotal roles played by ZINGER proteins in regulating *Plasmodium* gene expression, thereby offering potential targets for controlling parasite development and malaria disease. Carruthers' research advances understanding of gene regulation mechanisms with implications for future interventions aimed at tackling malaria.

Abhinay Ramaprasad (Francis Crick Institute, UK) focused on the malaria parasite's asexual blood stages particularly emphasizing the essential genes during egress stage. While the DiCre system offers conditional gene manipulation, its scalability poses challenges. To overcome this hurdle, Ramaprasad introduced the SHIFTiKO method, which is a scalable knockout system for simultaneously querying the essential and biological function of multiple genes in the malaria parasite. Ramaprasad also discussed the use of pCas9-duo, which utilises two guides, thereby increasing the efficiency of the system. He highlighted the advantage of this method which facilitates high-throughput screening and promises accelerated discovery of critical genes across various stages of malaria development. This scalable strategy holds potential for transforming the pace of gene function discovery, advancing efforts towards combating malaria effectively.

Session 2 – Molecular Epidemiology & Population genetics

Isabella Oyier (Kenya Medical Research Institute – Wellcome Trust, Kenya) gave an insight into the parasite population and drug resistance in West Africa by integrating malaria molecular epidemiology into routine surveillance (IMMERSE). Oyier underscored the importance of surveillance of allele distribution and diversity, as this significantly impacts vaccine design and administration strategies. Oyier also discussed the parasite genetic diversity between asymptomatic and febrile infections, highlighting a higher degree of clonality in the asymptomatic infections. Oyier displayed the timeline of national antimalarial policy changes in Kenya and highlighted the drug resistance markers associated with the administered drugs. Longitudinal analysis in the Kilifi County Hospital revealed a decline in chloroquine resistance alleles post-chloroquine cessation, demonstrating the efficacy of temporal Malaria Molecular Surveillance (MMS). Oyier emphasised the advantage of amplicon sequencing from dried blood spot samples, now deployed nationwide in Kenya in collaboration with the National Malaria Control Programme (NMCP), enabling real-time monitoring of drug resistance.

Alexander W. Macharia (Kenya Medical Research Institute – Wellcome Trust, Kenya) presented the outcome of a recall-by-genotype study where β-thalassaemia carriers were matched with a control to determine the impact of β-thalassaemia on the invasion of red blood cells (RBCs) by *Plasmodium falciparum* (*P. falciparum*). When compared to controls, flow-cytometry assays revealed a reduction in invasion efficiency in β-thalassaemia heterozygotes. Macharia also explored whether this effect persisted in individuals co-inheriting α-and β-thalassemia traits and found that the effect of reduced infection was lost upon acquiring the α-thalassaemia trait. Results from RBCs surface proteins analysis showed differences between β-thalassaemia and normal RBCs, including increased basigin expression and reduced CD71 and CD49d. Macharia highlighted the importance of understanding these mechanisms to guide strategies for malaria elimination.

Amy Ibrahim (London School of Hygiene & Tropical Medicine – LSHTM, UK) delved into an overlooked malaria parasite, *Plasmodium malariae* (*P. malariae*) and the associated challenges, including low parasitemia infection rate and the absence of *in vitro* culture methods. Ibrahim introduced a selective

Whole Genome Amplification (sWGA) method that was developed to address these obstacles. Additionally, she also highlighted the development of the first database for *P. malariae* genomics. Population genetics analysis revealed distinct genetic differences between Asian and African *P. malariae* isolates. Given the absence of in vitro culture methods for *P. malariae*, Ibrahim and her team developed a CRISPR-Cas9 model using *P. knowlesi* to assess pyrimethamine susceptibility, unveiling both sensitive and resistant *P. malariae* genotypes. The genomic analysis sheds light on *P. malariae* biology and drug resistance mechanisms.

Sarah Auburn (Menzies School of Health Research, Australia) highlighted challenges associated with eliminating *Plasmodium vivax (P. vivax),* including the parasite's ability to form hypnozoites, the lack of effective treatment markers and the absence of reliable molecular markers for drug resistance. Auburn presented novel tools that measure the relatedness of parasites through identity-by-descent (IBD), aimed at deciphering the origin of recurrent infections as a proof-of-concept. She demonstrated the use of genomic data to deconvolve polyclonal infections using DEploid software, revealing improved data resolution and uncovering different relatedness characteristics. Another tool presented was a microhaplotype genotyping assay on the MiSeq platform that is suitable for use in malaria endemic-countries. Using this novel tool and time-to-event modelling, Auburn and her team aimed to classify recurrent infections in clinical trials. She concluded by highlighting the diverse range of questions that molecular surveillance tools can address to support the NMCP.

Yannick Höppner (Bernhard-Nocht Institute for Tropical Medicine, Germany) discussed how protection against malaria is dependent on acquired immunity, although sterile immunity is rarely achieved. The pathogenesis of malaria, caused by *P. falciparum*, is closely linked to the expression of var genes, which encode a protein called PfEMP1 on the parasite's surface. This protein plays a crucial role in how the parasite interacts with human cells. Höppner demonstrated that var gene profiles differ between malaria naive individuals and those who have been previously exposed. Sample analysis from a longitudinal study identified that parasitemia cyclical peaks seen in *P. falciparum* infections were associated with sequential switching of expressed var genes. He also presented the correlation observed between antibodies against PfEMP1 and var gene expression, concluding that infections exhibit two distinct patterns: B-type infections persist until the end stage, while C-type infections are maintained but at the expense of parasite sequestration.

Nguyen Thanh Thuy Nhien (Oxford University Clinical Research Unit, Vietnam) opened by highlighting the burden of malaria cases in Vietnam as the country approaches elimination. Nhien showcased the evolution of molecular surveillance in Vietnam highlighting how genetic surveillance informs the decision-making of NMCP. Nhien informed the audience about how drug resistant phenotypes detected through genotyping during sentinel site monitoring are reported to the NMCP. Nhien also presented the increasing prevalence of artemisinin and piperaquine resistance throughout the years. She highlighted that the change in drug resistance trend coincided with drug treatment change and hence the importance of continuing molecular surveillance to track the drug's effectiveness, the spread of drug resistance and the identification of parasite strains causing outbreaks.

Session 3 – Life Cycle Biology – Blood stage – 1

Yi–Wei Chang (University of Pennsylvania, USA) and his team used in situ cryo-electron tomography to study the rhoptry secretion system in related blood parasites such as *Cryptosporidium parvum* and *Toxoplasma gondii.* This system, which utilises a molecular ultrastructure called the rhoptry secretory apparatus (RSA), is essential for parasite invasion. Chang utilised cryo-electron tomography to examine the RSA in *P. falciparum* merozoites. Through subtomogram averaging of over a hundred images, his team revealed that the rhoptries have three distinct morphologies, determined by their interaction with the apical vesicle (AV). Additionally, he

introduced cryo-confocal fLM and cryo-FIB-SEM which have been used to create stacks of images to study the surface of infected-red blood cells. He also suggested that rhoptries and AVs may develop from different biogenesis processes. He hypothesised that rhoptries evolved from a common ancestral structure similar to the trichocysts used by ciliates.

Kirk Deitsch (Cornell University, USA) elucidated how var genes express and switch in *P. falciparum*. His team used RNA-seq to study epigenetic switching. In their dataset, parasite populations switch between "high singles" (single *var* gene expression resulting in high binding parasites) and "low manys" (multiple *var* gene expression resulting in low binding parasites (a.k.a *var*-null parasites)). Results showed that *var*-null parasites were not recognised by antibodies and could cause asymptomatic infections. He also explained the availability of intracellular S-adenosylmethionine (SAM), a methyl group donor which could influence *var* genes to switch. In conclusion, he suggested a shifting bias in *var* genes wherein parasite populations express only a select 5-6 *var* genes every switch.

Emma Jones (University of Cambridge, UK) used optical tweezers to better understand the mechanism of *P. falciparum* merozoite attachment to the red blood cell surface. Using tweezers to detach merozoites from red cell membranes allowed the identification of the roles of different merozoite ligands in parasite-red cell attachment. Using a knockout model, the loss of MSP1 and GAP45 proteins did not reduce detachment forces, however, knockouts of EBA and RH proteins did reduce detachment force. The team explored how shaking speeds affected parasitic growth rates in culture, and determined a complex relationship between shaking speed and growth. To more accurately emulate invasion within blood vessels the team created a microfluidic device through which infected red blood cells travel and are visualised via fluorescence staining. By moving beyond the traditional static methods exploring invasion, this new tool expands our understanding of the invasion process *in vivo*.

Danny Wilson (The University of Adelaide, Australia) and team investigated the function of merozoite surface proteins (MSPs) across *Plasmodium* species due to their potential as vaccine candidates. Previous studies of MSP2 and MSP4 determined that these proteins are essential whereas MSP5 had been determined to be non-essential. To explore and challenge this, Wilson used CRISPR-Cas9 knockouts and found that loss of *Pf*MSP2 simultaneously allowed parasite invasion as well as potentiated antibodies against Apical Membrane Antigen 1. Knock-down experiment results showed that MSP4 in *P. falciparum* was essential, but conversely, was not found to be important in merozoite invasion in *P. knowlesi*. Opposite results were found for MSP5, which was found to be essential for *P. knowlesi* but not for *P. falciparum.*

Ross Waller (University of Cambridge, UK) spoke of an evolutionary cell biology approach to parasitology. His group analysed the evolution of genes in common between species of the Myzozoa phylum such as *Plasmodium* and *Toxoplasma*, and addressed the question of "what has changed and what has stayed the same". They used a spatial proteomic method called hyperplexed localization of organelle proteins by isotope tagging (hyperLOPIT) to study the cellular locations of new and old proteins. They identified Maurer's clefts, the RBC membrane, and micronemes to be compartments that are evolving the fastest. Waller also suggested that the "spatial proteome" changes throughout the lifecycle of the parasite.

Vasant Muralidharan (University of Georgia, USA) presented studies on rhoptry neck protein 11 (RON11), which was shown to localise to the rhoptries. A knockdown of RON11, using the DOZI system, impacted parasite growth and merozoite invasion. However, the knockdown did not impact rhoptry secretions as the release of RAP1 into the infected cells was observable. Ultra-expansion microscopy revealed that RON11 knockdown merozoites only contained a single rhoptry. Muralidharan concluded

that RON1 may not be essential for production of the first developing rhoptry but was required for biogenesis of its partner. To conclude he suggested that RON11 may also have an independent function in merozoite invasion that is less understood.

Tobias Spielmann (Bernhard Nocht Institute for Tropical Medicine, Germany) discussed *Pf*EMP1 which is encoded by the multigene *var* family, which is canonically understood to have mutually exclusive expression. Spielmann introduced a novel Selection Linked Integration (SLI) system that allowed activation of a single *Pf*EMP1 of interest and prevented switching. This was confirmed by the inclusion of an epitope tag (HA-tag) to detect the *Pf*EMP1. Such modified parasite strains 3D7 and IT4 were used to study the binding to receptors such as CSA, CD36, ICAM-1, and EPCR. Biotinylation identification (BioID) with *Pf*EMP1 as bait was used to identify new proteins that were important for cytoadhesion in infected parasites. The candidates identified are now being characterised using a modified SLI system.

This report is brought to you by the MESA Correspondents Angela Rumaseb, Katelyn Stanhope, Mohini Anjna Shibu and Nutpakal Ketprasit. Senior editorial support has been facilitated by Carla Proietti, Emma McHugh and Danushka Marapana.

Day 2: 20th February 2024

Session 4 – Drug Development and Resistance

Susan Wyllie (University of Dundee, UK) presented advanced methods in antimalarial discovery. Her laboratory employed chemical proteomics approaches to deconvolute irresistible drug targets. Chemical proteomics provide evidence of compound-target interaction. To date, the mode of actions of 45 compound series were identified, with 4 clinical candidates (success rate 85%). For chemical pulldown, phenotypic active compounds underwent Structure-Activity Relationship (SAR) and were attached with linkers that were coupled with beads used to pulldown targets. Wyllie's team developed expanded forms of this assay to functionalize linkers. Molecules of interest are linked with photo-activatable groups and chemical handles for bioorthogonal reactions such as click chemistry to enable pulldown. She introduced a new generation of functionalized linkers, microMap, to further enrich targets.

Deus Ishengoma (National Institute for Medical Research, Tanzania) presented recent data on the evaluation of artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *Plasmodium falciparum (P. falciparum)* malaria in North-western Tanzania. The first K13 mutation in Tanzania was found in 2021. He hypothesised that the mutations of parasites carrying K13 R561H found in the region have arisen on their own as the haplotypes were different from those observed in neighbouring Rwanda. He showed the number of districts with detected resistance increasing from three districts in 2021 to five districts in 2023. While the current treatment of artemisinin combination therapy is still effective, he highlighted the need for ongoing molecular surveillance for drug resistance in other parts of the country.

Belen Tornesi (Medicine for Malaria Venture, Geneva) discussed the use of triple combination therapies to minimise the risk of resistance development and increase drug efficiency. Tornesi talked about a checkerboard method for the identification of pharmacodynamic antimalarial drug-drug interactions (DDIs). The method can be used to identify antagonistic, additive, or synergistic effects between multiple drugs. A triple combination parasite reduction ratio (PRR) can be used to qualitatively assess DDIs. She concluded by stating that these tools are important to determine new drug combinations to combat artemisinin resistance.

Kelly Chibale (University of Cape Town, South Africa) and team investigated dual inhibitors that target *Plasmodium* kinases (PKs) and hemozoin formation in *P. falciparum*. There are many advantages to studying PKs since they can be targeted easily, are essential to parasite survival, and are expressed at multiple stages. However, there is a risk of parasite resistance, especially due to mutations in the ATP binding site. It was found that there is a structural similarity between inhibitors of hemozoin formation due to haemoglobin digestion and certain kinases. His lab validated this finding by showing that PI4K inhibitors disrupted and reduced the levels of haemoglobin-derived peptides. This provides a new avenue in antimalarials that couple protein and non-protein targets.

Ashley Vaughan (Seattle Children's Research Institute, USA) presented genetic crosses in *P. falciparum* that identified crucial insights into drug resistance mechanisms. His group used bulk segregant analysis (BSA) to identify loci and mutations associated with drug resistance. Genetic crosses showed a blood stage fitness cost associated with mutant *pfcrt*, and BSA results revealed mutant *pfcrt* and *pfaat1* after the presence of chloroquine drug pressure. Vaughan revealed and discussed the evidence of evolutionary dynamics and fitness trade-offs, particularly in the case of *pfaat1* mutations.

Session 5 – Immunity

Gemma Moncunill (Barcelona Institute for Global Health – ISGlobal, Spain) spoke on the newly approved RTS,S/AS01 vaccine recommended by WHO for use in African children. She highlighted the need to improve and develop new vaccines for malaria with high efficacy and durable protection. Moncunill's team focused on the cellular response to the RTS,S/AS01E vaccine, particularly the role of IgG antibodies. Moncunill utilised samples from children enrolled in an RTS,S/AS01E phase 3 trial from Tanzania and Mozambique. Through the use of cytokine, chemokine and growth factor profiling, they explored how T cell functions in children are altered in response to vaccination. She reported that the protection induced by RTS,S/AE01E vaccination was associated with T-helper 1 cell released cytokines specific to circumsporozoite-protein. Additionally, effector and central memory CD4+ T cells were established after the 3-dose vaccine regime. Finally, Moncunill reported that transcriptomic studies from the vaccine participants reflected that interferon signatures are associated with protection.

Noah Butler (The University of Iowa, USA) talked about the lack of long-term immunity after malaria infections. He showed data suggesting that this may be due to the 60-70% fraction of all activated B cells being short-lived plasmablasts (PB). Deletion of PB enhanced germinal centre (GC) responses and protection. He used a metabolomic approach to understand this in mice infected by *Plasmodium yoelii*. Butler hypothesised that host metabolism limits long-lasting memory responses and identified that high glutamine availability results in an increase in GC responses and elevated resistance to blood stage *Plasmodium* infections. This data was supported by the identification of a high magnitude of circulating PB in controlled human malaria infection studies using *P. falciparum*. They conclude that PB functions as a "nutrient sink" that limits nutrient availability for GC, B cells, and Cd4 T cells and that this may be overcome with exogenous glutamine. He suggested that such malaria induced metabolomic shifts may be due to epigenetic programming.

Brendan McMorran (Australian National University, Australia) talked about host defence antimicrobial peptides (AMPs) with membrane active properties. AMPs kill the parasite by inducing lysis of the digestive vacuole (DV). His team designed PF4 Derived Internalization Peptides (PDIP), which are synthetic peptides with the structural properties of AMPs. Using an array of techniques they were able to prove that PDIP accumulated within the cytosol of intra-erythrocytic parasites very rapidly and did not affect uninfected red blood cells. Additionally, they were able to show that PDIP traverses the multiple membranes surrounding an intra-erythrocytic parasite, namely the host cell plasma membrane, the parasite parasitophorous and plasma membranes to selectively lyse the DV. He suggested that this selectivity may be due to the distinct phospholipid compositions of these membranes. McMorran concluded that PDIP and its drug conjugates were specific for parasitised cells and could be used for cargo delivery such as drugs, antibodies and other molecular probes.

Lynette Beattie (The University of Melbourne, Australia) discussed the immune response generated by intravenous injection with radiation-attenuated *Plasmodium* sporozoites (RAS), which provides protection against malaria infection via the priming and expansion of antigen-specific CD8+ and CD4+ T cells in the spleen. Beattie determined that γδ T cells played a crucial role within the first 24 hours post-vaccination by producing IL-4, essential for effective CD8 T cell expansion. This was confirmed through various mouse-model experiments including antibody-mediated blockade, bone marrow chimaeras, adoptive transfer, and CRISPR-based gene knockdown. The studies revealed that γδ T cells facilitated CD8 T cell memory generation, which could play a role in improving vaccine responses.

Gonzalo Acevedo (University of California, USA) talked about the role of CD4+ T cells in immune protection in liver stage parasites. His work identified *Plasmodium falciparum* liver stage antigens and epitopes that trigger T cell immunity. They identified 11 antigen candidates that were recognised by CD4+ T cells from Ugandan children exposed to malaria. From this dataset, they identified an epitope

that was detectable in a high percentage among donors from the Program for Resistance, Immunology, Surveillance, and Modeling of Malaria in Uganda (PRISM) study. Acevedo also noted that immune responses against liver stage antigenic peptides were more frequently detected at longer times after a malaria episode.

Hedda Wardemann (German Cancer Research Institute, Germany) discussed the rationale behind improving the current circumsporozoite protein (CSP) based malaria vaccines through cellular and molecular analysis of human anti-PfCSP immune cell responses. *In vitro* stimulation and expansion-based enrichment of CSP reactive T cells were investigated to identify target epitopes of human anti-PfCSP antibodies. PfCSP is considered an ideal target as CD4 T cells recognise a small polymorphic epitope of PfCSP and lack cross-reactivity. Using this information, the team designed a second generation pre-erythrocytic malaria vaccine that consisted of ferritin-based nanoparticle scaffolds displaying PfCSP repeat epitopes.

Wiebke Nahrendorf (University of Edinburgh, UK) isolated a novel *P. vivax* clone (PvW1) which was used in an infection model to establish controlled human malaria infection. Nahrendorf utilised this clone to introduce infection into malaria naive individuals and followed their immune responses. It was found that *P. vivax*-infected participants became febrile at lower parasite densities when compared to *P. falciparum*-infected participants. For individuals who had not experienced malaria infection before, a large T cell response which resulted in tissue damage was observed. The response was seen to be more dramatic in *P. falciparum* infections compared to *P. vivax.* Upon re-infection the severity of the physical responses to infection were diminished, however, parasite densities remained similar. These findings demonstrate that after initial *P. vivax* infection the immune system is capable of reducing responses which may harm the body.

Session 6 – Life Cycle Biology – Sexual Development

Rita Tewari (University of Nottingham, UK) presented studies on regulatory proteins such as mitotic kinases/phosphatases, cyclins and molecular filaments that play a role in gametogenesis in *Plasmodium berghei*. First, she talked about a type of mitotic kinase called Nima-like kinases (Nek). Nek1 was found to localise to the outer centriolar microtubule organising centre (MTOC) during male gametogenesis. Knockdown studies of Nek1 showed that it was essential for male gametogenesis and parasite transmission. The team showed that Nek1 was important for MTOC splitting and chromosome segregation and that it partially co-localized with centrin but not Ndc80 (a component of the kinetochore). In the later part of her talk, Tewari briefly showed data on the study of another mitotic kinase, Aurora kinase 2 (ARK2), which was similar to Ndc80. Despite the lack of ARK2 the parasites still produced male gametes, however, the presence of ARK2 blocked transmission to oocyst stages.

Oliver Billker (Umeå University, Sweden) talked about using stage-specific gene expression data to understand sexual reproduction in *Plasmodium berghei*. Billker and colleagues used barcoded PlasmoGEM vectors to create mutant parasite lines that only produce single sex gametocytes. They identified over 12,000 sex-specific phenotypes from 3000 mutants. From this data, they were able to describe and assign functions to hundreds of previously unstudied genes that regulate fertility. They further studied 125 male genes associated with egress and motility. Interestingly, a SUN protein was identified and found to link the nucleus to axonemes. He introduced a strategy to study the diploid stage of the parasites by using Cas9 homing technology. Pilot screens identified new homozygous oocyst phenotypes. As an example, he briefly mentioned a *P. berghei* gene which was identified to be a CRT homolog in oocysts.

Danushka Marapana (Walter and Eliza Hall Institute, Australia) talked of a study conducted in *Toxoplasma* where a CRISPR screen identified a multi-component E3 ubiquitin ligase, which was named glucose induced degradation deficient (GID) complex. This complex was critical for parasite transition from the tachyzoite to bradyzoite stages. Homologs of the GID complex components were identified in *P. falciparum*. PfGID was localised to the nucleus and cytoplasm. From gene knockout studies it was found that PfGID was not essential for asexual development or sexual commitment, yet arrested development of the parasites and prevented parasite transmission. The team performed various transcriptomic and whole-cell proteomic analyses to understand the function of PfGID. They were also able to identify a few potential substrates ubiquitinated by PfGID.

Michaela Petter (University Hospital Erlangen, Germany) gave a talk on epigenetic regulation mechanisms by bromodomain proteins in *Plasmodium falciparum*. The talk was focused on bromodomain protein 1 (BDP1) which is a protein highly expressed throughout gametocytogenesis. Conditional knockdown of BDP1 resulted in a reduction in gametocyte rate, with the resulting parasites displaying abnormal morphology. They observed that a lack of BDP1 led to progressive gene deregulation. Chip-seq and RNA seq analyses revealed that there was an increase in antigenic variation in the BDP1 knockdown cells. The team also identified a *Pf*BDP1-specific inhibitor which affects both asexual and sexual parasite stages. The conclusion was that BDP1 co-regulates genes in the early gametocyte stage.

Björn Kafsack (Cornell University, USA) gave a talk on the investigation of Homeodomain-like Protein 1 (HDP1). Kafsack's lab found that HDP1 acted as a nuclear DNA-binding protein which regulates gene expression during the early development of *P. falciparum* gametocytes. Upon knocking out the HDP1 gene, it was found that this loss of HDP1 resulted in the failure to upregulate genes required in early gametocytes, indicating its role as a positive transcription regulator. To further elucidate the protein function, Kafsack performed targeted truncations and deletions within HDP1. This identified that one area of the gene is responsible for the development of all gametocytes, whereas the opposite end is distinctly responsible for male gametocyte development.

Gigliola Zanghi (Center for Global Infectious Disease Research, USA) and team combined GFP-tagged parasites and transcriptomics to study genes associated with liver stage development. This study utilised a human liver-chimeric mouse model and a PfNF54 parasite line to analyse *P. falciparum* infected hepatocytes. Using RNA-seq analysis they revealed that translation regulation is required for the transformation from sporozoites to liver stage, alongside sustaining factors such as fatty acid biosynthesis and iron metabolism. The *P. falciparum* transcriptome was compared to *Plasmodium vivax* liver stage gene expression profiles, which showed conservation of liver-stage specific genes. Interestingly, Zanghi identified that *P. falciparum* parasites are not sexually committed at the liver stage in contrast to *P.vivax*. Furthermore, components of the export translocon PTEX are expressed during the liver stage of *P. falciparum*.

Scott Lindner (Pennsylvania State University, USA) worked on the mosquito stage of parasite development. His work was focused on identifying proteins required for host-to-vector transmission. RNA-seq data showed that female gametocytes repress certain mRNA transcripts in anticipation for protein production during mosquito stages. This process involved DDX6 (DOZI), LSM14 (CITH), and ALBA proteins which form a repressive complex which stabilises certain mRNA transcripts. Lindner utilised comparative transcriptomics and proteomics across host-to-vector transmission in female *P. yoelii* parasites to identify a large amount of transcripts which were essential for zygote function and released for translation post-fertilization. It was also found that certain transcripts remained repressed beyond the ookinete stage.

This report is brought to you by the MESA Correspondents Angela Rumaseb, Katelyn Stanhope, Mohini Anjna Shibu and Nutpakal Ketprasit. Senior editorial support has been facilitated by Carla Proietti, Emma McHugh and Danushka Marapana.

Day 3: 21st February 2024

Session 7 – Lifecycle Biology – Transmission

Flaminia Catteruccia (Harvard University, USA) stated that the best technique to combat malaria is eradicating mosquitoes. However, the use of insecticides has led to the emergence of resistance in mosquitoes. She suggested an alternative approach: using antimalarial drugs on mosquito nets. Catteruccia demonstrated this approach by showing that *Plasmodium* parasites can be killed in mosquitoes using atovaquone-treated nets. She suggested that using combinations of drugs to treat humans and kill mosquitoes may reduce the risk of parasite and mosquito derived resistance. Catteruccia and team tested over 100 promising new transmission-blocking compounds. The targets of these compounds included cytochrome b, elongation factor 2 and acetyl CoA synthetase. After identifying the most effective of the tested compounds, the team worked on developing more compounds with similar properties. She concluded her presentation by speaking about using single-cell RNAseq (scRNAseq) to identify genes highly expressed in oocyst which may be targeted to stop the oocyst development and thereby block transmission.

Benjamin Liffner (Indiana University School of Medicine, USA*)* talked about the use of ultrastructure expansion microscopy (U-ExM) to examine oocysts and sporozoites. This technique could enlarge samples between 4 to 4.5 fold which is especially useful for studying organogenesis and proteins of interest in *Plasmodium* parasites. This technique was used to define a timeline for sporozoite rhoptry biogenesis. He observed the number of rhoptries during two events – sporozoite release and salivary gland invasion. Four rhoptries were found in parasites located in the midgut, but only two rhoptries were observed in parasites in the salivary glands. The four midgut rhoptries were in two pairs – congruent and dimorphic. He reported that the congruent rhoptry pair was used to invade the salivary gland, leaving behind the dimorphic pair. He implicated RON11 in correct rhoptry formation wherein RON11 knockdown parasites showed only half the number of rhoptries.

Lauriane Sollelis (University of Zurich, Switzerland) talked about the switch of *Plasmodium falciparum* from asexual blood stage parasites to transmission-competent gametocytes. Environmental factors regulate this switch and also epigenetic factors such as the activation of AP2-G, via the nuclear protein GDV1. Sollelis and colleagues employed transcriptomic data from Tracking Resistance to Artemisinin Collaboration (TRAC-I) studies in Southeast-Asia to investigate the impact of natural selection on stage switching. Data showed an association between Ben, a long non coding RNA, and sexual commitment. It was proposed that Ben was an internal sensor of host lysophosphatidylcholine levels which was considered a sensor for metabolic adaptation and cell-fate determination. Interestingly, they found that a high AP2-g expression also coincides with K13 mutations and that dihydroartemisinin treatment reduces stage conversion.

Fiona Angrisano (Burnet Institute, Australia) highlighted the importance of blocking transmission for malaria eradication. Her lab used stage specific proteomics to identify new transmission-blocking targets. Nine protein disulphide isomerases, essential for transmission, were identified to be expressed on the surface of gametocytes. Of the nine, PDI-Trans is transcribed throughout the lifecycle. When it was knocked out, the ookinete conversion rate dropped, and complementation with PDI-Trans restored function. Angrisano showed bacitracin, a known PDI inhibitor, inhibits exflagellation and fertilisation. She thus suggested the use of PDI inhibitors as possible antimalarials, to develop novel transmission-blocking drugs.

Matthias Rottmann (Swiss Tropical and Public Health Institute, Switzerland) emphasised that the currently used Artemisinin-based combination treatments (ACT) fail to eliminate gametocytes, and that novel drugs with potent transmission-blocking activity were needed. His work was focused on the discovery and profiling of stage-specific gametocytocidal compounds *in vitro,* and the *in vivo* pre-clinical validation of transmission blocking drug activity using humanised mouse models. His team used the genetically-engineered inducible Gametocyte Producer (iGP) line to develop a simple *in vitro* assay for drug/compound screening. The immuno-deficient NOD/SCID/IL2rγ null (NSG) mouse model was used to assess Stage V gametocytocidal effect of compounds. *In vivo* transmission efficacies were determined by membrane feeding assay.

Sophie Collier (The University of Melbourne, Australia) talked about organellar inheritance in *Plasmodium berghei*. *Plasmodium* mitochondria and apicoplast are both organelles that are maternally inherited. In her work, she studied mechanisms underpinning such uniparental inheritance. Using several microscopy techniques, including lattice light sheet microscopy, Collier observed the mitochondrion and the apicoplast during gametogenesis and noted that these organelles were excluded from exflagellating male gametes. This data was supported by digital-droplet qPCR, and results showed a decrease in the relative copy number of the apicoplast and mitochondrial genomes in male gametocytes, confirming the exclusion of these organelles from the microgamete. Forced crosses were set up between single sex lines with a selectable polymorphism in the mitochondria of the male parent. After screening 1.4 million sporozoites from such crosses, a single male leakage event was identified, demonstrating that, while being an extremely rare event, it is possible for drug resistance encoded by the mitochondrial genome to be inherited from the male parent.

Session 8 – Vaccine Design

Matthew Higgins (University of Oxford, UK) presented the structure of the PfPCRCR complex, utilised in erythrocyte invasion, which appeared to be a promising blood stage vaccine target. This complex included PfRH5, PfCyRPA, PfRIPR, PfPTRAMP and PfCSS. To further understand the molecular mechanism of the PfPCRCR complex, Higgins utilised cryogenic electron microscopy to elucidate its structure in high resolution. The protein PfRIPR was found to bind to a complex containing PfCSS and PfTRAMP. As PfRIPR was bound to PfCyRPA and PfRH5, which attached to basigin on the erythrocyte membrane, it was shown that PfRIPR was responsible for bridging the space between the erythrocyte and merozoite membranes. Higgins then mapped identified neutralising antibodies onto the model of the PfRCR complex, which demonstrated that these antibodies target PfRH5 and PfCyRPA to prevent the binding of PfRCR to the erythrocyte surface. Interestingly, it was found that highly effective PfRIPR antibodies act towards the parasite membrane. Higgins concluded that furthering the understanding of invasion proteins could inform rational vaccine design.

Azza Idris (National Institutes of Health – NIH, USA) highlighted that despite progress in control measures like insecticide-treated nets (ITNs) and rapid diagnostic tests (RDTs), efforts have stalled due to parasite derived resistance. Developing effective vaccines has been challenging due to the parasite's complex life cycle and limited durability of existing vaccines. Idris emphasised the value of monoclonal antibodies (mAbs) that target circumsporozoite protein to induce passive immunity. Idris highlighted the advantages of antibodies as therapeutics, including their ability to safely and efficiently instigate immediate and high level protection independently of the immune system while being unaffected by prior exposure and administered through a simple dose regimen. Using samples from individuals who had received the attenuated *P. falciparum* whole sporozoite vaccine, Idris identified CIS43, a CSP-specific antibody. This antibody conferred protection in a humanised mouse model by recognising the junctional epitope on CSP. She introduced L9LS, a genetically engineered

version of CIS43, which has a longer half-life and has now reached clinical trials. Idris concluded by reiterating the potential of mAbs as a safe and effective intervention against malaria.

Liriye Kurtovic (Burnet Institute, Australia) evaluated phase IIb clinical trials in which children received the CSP-containing RTS,S vaccination. Kurtovic explored the antibody responses of the children longitudinally and found a strong induction of IgG with a more moderate induction of IgM and IgA. To further explore these antibody responses, Kurtovic utilised surrogate cell-free assays to look at the antibodies' functional activities. It was determined that antibodies fix the complement initiation protein C1q. Further, the antibodies from vaccinated children were found to recognise the NANP and C-terminal of the CSP protein, albeit with variable affinity. Finally, Kurtovic identified antibodies with FcγR binding activity targeting specific CSP domains.

Danielle Stanisic (Institute for Glycomics, Australia) performed proof-of-principle studies to explore the use of whole parasite vaccines. Stanisic first inoculated rodents with blood-stage malaria parasites chemically attenuated with Tafuramycin-A (TF-A) and showed the induction of protective immunity. This concept was advanced through vaccinating malaria naive humans with TF-A attenuated ring-stage *P. falciparum* parasites. Limited parasite-specific IgG was induced through the vaccination and surprisingly, two volunteers achieved complete protection. Stanisic highlighted the challenges with this novel approach which include the requirement of the vaccine to be delivered to the patient freshly and the risk of inducing allo-antibodies.

Krishanpal Karmodiya (Indian Institute of Science Education and Research – IISER, India) highlighted the main obstacles to controlling and eliminating malaria, which included a lack of effective vaccines. Traditional vaccine approaches focusing on single antigens have shown limited success due to the parasite's genetic complexity and immune evasion tactics. Karmodiya introduced a novel strategy which involves combining multiple immunodominant peptide sequences from various blood stage antigens to overcome these challenges. Three chimeric antigens, created from selected B-cell epitopes, demonstrate effectiveness in eliciting neutralising antibodies against the parasite. Using microfluidics, Karmodiya identified that these antibodies reduce cytoadhesion, a crucial step for parasite virulence, and inhibit parasite growth.

Justin Boddey (Walter and Eliza Hall Institute – WEHI, Australia) presented that seasonal malaria chemoprevention (SMC) aims to protect vulnerable populations during malaria season, but current drugs mainly target blood stage parasites, which are increasingly resistant. He talked about a new approach focused on late liver stage parasites, showing promise in providing both chemoprevention and long-lasting immunity. Boddey revealed that targeting aspartyl proteases, plasmepsin IX and X, with the novel compound WM382 kills liver parasites just before blood infection, enabling sterilising immunity against reinfection in mice. Boddey introduced how integrating late liver stage-active drugs, like the clinical candidate MK7602, developed through WEHI-Merck collaboration, into SMC protocols could revolutionise malaria control. These drugs offer dual benefits by preventing blood stage malaria yet allowing immunity to develop against locally circulating strains, addressing drug resistance, and enhancing prevention efforts in endemic regions.

Session 9 – Pathogenesis

Ana Rodriguez Fernandez (New York University, USA) studied anti-phosphatidylserine (anti-PS) antibodies in malaria patients. An *in vitro* human malaria model showed that the anti-PS was a panel of broadly reactive natural antibodies. The team also showed that infected-red blood cells (iRBCs) induced endothelial barrier disruption, but it was not the cause of cell death in cerebral malaria and RNAseq data confirmed this observation. iRBCs were found to downregulate the protein prenylation pathway, and this pathway was observed to modulate the integrity of the blood-brain barrier. Results

revealed that haemozoin, but not the biomolecules attached to it, induced the disruption of the endothelial barrier. The team also investigated the proteins secreted from endothelial cells upon incubation with *P. falciparum*. It was found that inhibin-beta E was elevated 2 days before cerebral malaria symptoms appeared. This finding was useful for diagnosing cerebral malaria and preventing malaria-associated deaths.

Hannah Fleckenstein (EMBL Barcelona, Spain) and team developed a workflow to study tissue organisation and interaction between parasites and blood brain barrier endothelial cells. This pipeline employed correlative x-ray and electron microscopy to build a 3D map. This model was further improved by including micro CT-based X-ray imaging and synchrotron-based X-ray imaging, in addition to volume electron microscopy. High throughput tomography was also used to visualise the blood brain barrier upon infection. The key observation from the study was that remodelling of endothelial cells occurs and involves wrapping around attached iRBCs.

Tracey Lamb (University of Utah, USA) delivered the results of a study on CD8+ T cells derived from a mouse *Plasmodium berghei* infection model associated with pathogenesis of cerebral malaria. Lamb described the use of a novel 2D micropipette adhesion frequency assay to determine the presence of responding T cells. This determined the adhesion frequency and affinity showing broad ranges of CD8+ T cells affinities. Utilising a T-cell depleted mouse model and the transfer of only low affinity T cells, Lamb determined that CD8 T cells which travel to the brain and cause pathogenesis were low affinity. Additionally, it was found that higher affinity cells were not pathogenic. This finding contrasts research on other cerebral infections.

Steven Kho (Menzies School of Health Research, Australia) introduced the contribution of the spleen as a compartment that harbours parasites during the life cycle of *P. falciparum* and *P. vivax*. Through splenectomy cohorts in Timika (Indonesia), Kho found that a vast majority of patients had asymptomatic infections, with a substantial portion of parasites within their spleens. Spleen and peripheral blood samples confirmed the spleen as a hidden compartment of parasites for *P. vivax*, since *P. vivax* biomass was significantly higher in the spleen than in peripheral blood. Kho et al. revealed that differences in spleen architecture and parasite distribution between *P. vivax* and *P. falciparum* infections were observed. They found that in patients who had had a splenectomy, the number of circulating parasites was higher but the total biomass lower when compared to patients with an intact spleen. Kho stated that the future aim of the spleen research program is to better understand the biology of the spleen as a reservoir for malaria parasites. Some of the areas Kho discussed were the development of *P. vivax* serological exposure markers to identify individuals with hidden infections, analysis of the genetic diversity between parasites in the spleen and in the peripheral blood, immune cell phenotyping in the spleen and spatial phenotyping of spleen cells.

Nathan Schmidt (Indiana University School of Medicine, USA) talked about host, parasite, and environmental factors influencing malaria severity. Schmidt highlighted that the composition of the gut microbiota modulates the severity of malaria, with *Bacteroides fragilis* implicated in susceptibility to hyper-parasitaemia. This was studied in a mouse model experiment, where mice treated with antibiotics had a lower parasitaemia compared to control mice. However, samples from a study in Ugandan children showed an increased abundance of multiple *Bacteroides spp* in severe malaria cases. A study on germ-free mice colonised with stool samples from children susceptible to severe malaria revealed higher parasitaemia when compared to those from resistant children, suggesting a causal role of gut *Bacteroides.* He also suggested conducting metagenomic analysis of the gut microbiome to understand malaria better. Targeting gut bacteria therapeutically may prevent severe malaria in children, indicating a potential intervention strategy.

Lauren Galloway (University of Glasgow, UK) presented l *P. falciparum* schizont sequestration and introduced the bone marrow and spleen as important reservoirs of malaria parasites independent of classical vascular sequestration. Her study focused on the comparative analysis of post-mortem spleen, bone marrow, and lung tissue collected from paediatric fatal malaria cases and non-malaria controls in Malawi. The study revealed significant accumulation of non-phagocytosed parasites (mostly ring-stage parasites) observed in the spleen. However, there was no significant difference in the splenic biomass when compared to other tissue, and there was minimal evidence of an intrasplenic life cycle. She also observed that the splenic biomass had very few gametocytes and that the bone marrow had many more gametocytes. The study suggested that the spleen plays a different role in acute paediatric malaria compared to chronic adult malaria, hinting at a more complex host-pathogen interaction.

Rhea Longley (Walter and Eliza Hall Institute, Australia) highlighted that the challenge of eliminating *P. vivax* infections was largely caused by the presence of hidden liver stage parasites called hypnozoites. Therefore detecting individuals with these hypnozoites is crucial for effective elimination strategies. Longley introduced a novel approach that involves measuring antibodies induced by 60 *P. vivax* proteins as markers of hypnozoite presence. Studies across the Asia-Pacific and Americas have identified and validated a panel of serological markers for recent infections. More recently, Longley and team found these markers could be used to identify people with hypnozoites who may have relapsing infections. Longley highlighted that by testing and treating populations at risk based on serological markers, malaria elimination could be accelerated. This approach was introduced by the Vivax Serology Partnership (VISPA), which aims to integrate serology-based identification and treatment interventions into national policies. She also talked about successful interventions using serological testing and treatment (SeroTAT) in Thailand.

Closing Remarks

Matthew Dixon closed the conference by thanking all funding partners, sponsors and exhibitors. Special mention and thanks were given to the principal funders, Open Philanthropy and Johns Hopkins University. Dixon thanked the Mantra Group staff and the Leishman Associates for their great hospitality throughout the conference. Dixon also thanked everyone who contributed to the success of the conference, which included invited speakers, oral and poster presenters, co-chairs, poster and oral judges as well as the organisers and contributors of the two workshops. Dixon closed by announcing the 15 travel award recipients, three oral presentation award recipients and two poster award recipients.

This report is brought to you by the MESA Correspondents Angela Rumaseb, Katelyn Stanhope, Mohini Anjna Shibu and Nutpakal Ketprasit. Senior editorial support has been facilitated by Carla Proietti, Emma McHugh and Danushka Marapana.

Discover more content in the Resource Hub