RESOURCE HUB *Correspondents*

BioMalPar XIX: biology and pathology of the malaria parasite

Complete series

MESA Correspondents bring you cutting-edge coverage from the BioMalPar XIX: biology and pathology of the malaria parasite

23 - 25 May 2023

Heidelberg, Germany & Virtual Conference

The MESA Alliance would like to thank Yunuen Avalos Padilla (Institute for Bioengineering of Catalonia - IBEC, and Barcelona Institute for Global Health - ISGlobal, Spain) and Alba Pérez Cantero (Barcelona Institute for Global Health - ISGlobal, Spain) for providing senior editorial support.

The MESA Alliance would like to acknowledge the MESA Correspondents Faith Hungwe (KTH Royal Institute of Technology, Karolinska Institute and Stockholm University, Sweden), Aaron Lartey (Noguchi Memorial Institute for Medical Research, Ghana) and Rinter Karimi Kimathi (University of Glasgow, United Kingdom) for their coverage of the conference.

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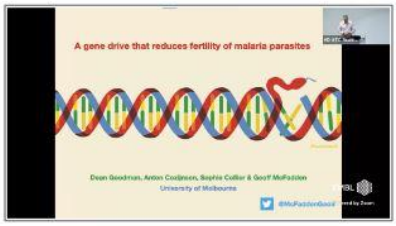
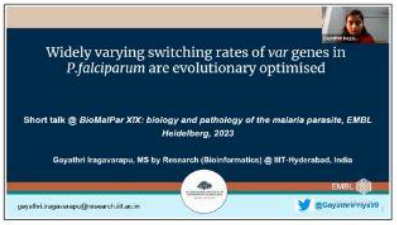
Day 1: Tuesday, 23rd May 2023

Opening Ceremony Remarks

The BioMalPar XIX conference was opened by **Borko Amulic** (University of Bristol, United Kingdom) one of the organizers who welcomed everyone to the EMBL Heidelberg and virtual. Before starting with the sessions, he mentioned that a total of 275 participants were present, 220 on-site and 55 in virtual attendance.

Scientific Session 1 – Evolution and Ecology





Geoffrey McFadden (University of Melbourne, Australia) spoke about the potential of gene drives as an alternative approach to successfully eliminate entire parasite populations. The current gene drive research targets the vector, whereas targeting *Plasmodium* parasites has not been explored. To determine the success rate of the gene drive technology, McFadden et al. developed a homing gene drive that was introduced into *Plasmodium berghei* parasites to disrupt the male development 4 (md4) gene necessary for male gamete production. This gene drive included a Cas9 cassette and a gRNA, and generated an exclusive female progeny. To understand the effects of the gene drive, two mice lines were infected, one carrying the md4- gene drive line, and another where the female development gene (fd1) was disrupted to produce male gametes only. Mosquitoes were allowed to feed on infected mice and the resulting oocyst and sporozoites were genotyped to evaluate whether the cassette had been inserted. Results showed that after a single crossing, all the progeny had the cassette inserted into the md4 locus causing a sex bias leading to population collapse which would bring the transmission chain to a halt. McFadden remarked on the efficiency with which the gene drive could eliminate parasites. In the end, he shared a precautionary warning regarding the ethical and safety implications that need to be thoroughly discussed prior to deploying gene drives and he mentioned that Target Malaria are following all the necessary steps to overcome these issues.

Victoria Ingham (Heidelberg University Hospital, Germany) highlights the importance of understanding metabolic changes in mosquitoes that contribute to insecticide resistance. She stated that 80% of all averted malaria cases until 2015 can be attributed to insecticide use. Malaria cases have plateaued after 2015 owing to the emergence of stronger resistance in mosquitoes (especially to pyrethroids), therefore, her study attempts at understanding this phenomenon. By investigating the effects of insecticide resistance and insecticide exposure on reactive oxygen and nitrogen species,

a multi-omics approach was used on a resistant mosquito population from Burkina Faso. Ingham's findings demonstrated that resistant populations were associated with an increase in respiratory rates and expression of oxidative phosphorylation genes compared to susceptible ones. Conversely, the opposite effect was observed after pyrethroid insecticide exposure with resistant mosquitoes having more respiration-related transcripts expressed. Finally, an increased respiratory rate indicated a direct link between metabolism and resistance. In summary, this study highlights intricate phenotypic alterations in mosquitoes that contribute to the development of insecticide resistance.

Catherine Oke (University of Edinburgh, United Kingdom) explained why the extrinsic incubation period (EIP) is an important parameter in vector control and parasite biology but also addressed gaps and assumptions found in the definition of EIP. One assumption is that a mosquito is infectious once sporozoites reach the salivary glands. By investigating the influence of mosquito age and incubation time on infectivity rates using *P. chabaudi*-infected mosquitoes, Oke examined how parasite burden in mosquitoes varied across days post-infectious blood meal (pIBM). The sporozoite prevalence was found to be significantly impacted being much lower on day 7, spiked on day 9 and plateaued thereafter. Meanwhile, the sporozoite burden peaked around day 12. This data reveals that day pIBM highly impacts sporozoite prevalence and burden as the likelihood of infection increases over time whilst the probability of onward transmission increases when bitten on a later day. These findings suggest that sporozoites become more infectious with time. In conclusion, Oke noted that the results make EIP measurement more complex than the definition implies.

Sarah Auburn (Menzies School of Health Research, Australia) began her talk by introducing the vivaxGEN programme which is a collaborative network with representatives from malaria-endemic countries to generate markers that can be used within endemic countries to improve malaria diagnosis. Auburn highlighted that vivaxGEN sets out to exploit the potential of genetic epidemiology to understand the transmission, resistance and opportunities for intervention at a deeper level together with rapid diagnostic tests (RDTs) and microscopic diagnostic techniques. Through capacity building and engagement, the aim is to make the program sustainable. One of the vivaxGEN projects aims to explain why *Plasmodium vivax* treatment is failing. Global genomic datasets were used to compare endemicity and relapses, in an Ethiopian cohort, to describe within-host identity-by-descent (IBD) patterns observed. Data revealed that approximately 60% of patient pairs (relapses and recurrences) are highly related. To identify polyclonal recurrences and differentiate between their haplotypes, a genome-wide IBD microhaplotyping marker panel was developed. This genotyping tool will enable scale and depth when deciphering clinical polyclonal types.

Gayathri Iragavarapu (International Institute of Information Technology Hyderabad, India) presented a study on the switching rates of *var* genes in *P. falciparum*, the human malarial parasite. The research aimed to explore the evolutionary optimization of these switching rates. Computational and mathematical tools were employed to investigate the hierarchy and intrinsic switching rates of *var* genes. Single-cell RNA sequencing data from the Malaria Cell Atlas was analyzed, revealing a preference for a specific *var* gene variant in the population. A clear hierarchy of *var* genes was observed across different stages of the blood cycle. Iragavarapu's team developed an evolutionary simulation model that demonstrated how lower switching rates made the parasite more vulnerable to immune clearance, while higher switching rates provided an advantage for immune evasion. Experimental data supported these findings, indicating that highly expressed genes interacted more

with the immune system and were easily cleared. Iragavarapu concluded by saying that *var* genes with higher switching rates offered an evolutionary advantage by maximizing the parasite's growth and persistence within the host.

Scientific Session 2 – Clinical and Field Studies

Watipenge Nyasulu (Malawi-Liverpool Wellcome Clinical Research Programme, Malawi) discussed the neutrophils' intricate involvement in cerebral malaria (CM) and their impact on disease progression. While neutrophils aid at clearing parasites, excessive neutrophil extracellular traps (NETs) formation, and pro-inflammatory environment can worsen the disease. Nyasulu aimed to identify and characterize neutrophil subsets in children with CM and assess their NET-forming ability. Blood samples were collected from CM, non-CM coma, and healthy children (control). Low-density neutrophils (LDNs) and normal-density neutrophils (NDNs) were isolated, phenotypically characterized, and their NETotic ability assessed. CM children had significantly more LDNs which were susceptible to activation, prone to form NETs, and exhibited immature expression that could contribute to the pathogenesis of CM. These findings suggest the neutrophils' dual role in malaria and have implications for targeted therapies against severe cerebral malaria.

Nora Nghochuzie Nganyewo (Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine, The Gambia) began by talking about the recent increase in low complexity polygenomic infections and sialic acid-independent invasion pathways in *Plasmodium falciparum* from Western Gambia. *P. falciparum* uses different receptor-ligand interactions to invade human red blood cells. Some clones rely on sialic acid (SA) residues on glycophorin receptors, while others use alternative receptors. Nganyewo hypothesized that intensified malaria control and reduced prevalence in the Gambia could be the reason that *P. falciparum* parasites have more efficient invasion pathways and ligand expression. Blood samples from 65 mild malaria patients across three years were analyzed. Neuraminidase, trypsin, and chymotrypsin enzymes determined erythrocyte invasion phenotypes. Genetic diversity was assessed through MSP2 genotyping, and the transcript levels of 6 *P. falciparum* genes were examined. SA-independent invasion phenotypes increased from 2015 to 2021, with 2021 isolates exhibiting high inhibition by chymotrypsin. Similarly, transcript levels varied across the years, suggesting an increase in sialic-acid independent invasion. These findings imply rising mixed infections and utilization of alternative invasion pathways by *P. falciparum* in The Gambia.

Aubrey Cunnington (Imperial College London, United Kingdom) focused on enhancing mechanistic inferences from blood transcriptome analysis and validating findings through comparative transcriptomics. Cunnington highlighted the potential of transcriptomics in overcoming limitations in malaria diagnostics, particularly in identifying targets and diagnosing cerebral malaria. The study conducted in The Gambia explored interactions between parasite genes and human gene expressions associated with severe malaria, specifically investigating the role of matrix metallopeptidase 8 (MMP8) in cerebral malaria pathology. Comparative transcriptomic analysis showed agreement in differentially expressed genes (DEGs) between infected humans and mice, although less consistency was observed in severe malaria cases. Emphasis was placed on selecting models with concordant genes or pathways of interest. The presentation also discussed the use of transcriptomes for diagnosis, citing Mike Levin's work in utilizing gene patterns as diagnostic tools to overcome the binary nature of current malaria tests. Molecular taxonomy based on 161 genes demonstrated the ability to differentiate between disease classes and individual diseases. However, distinguishing incidental parasitemia from true malaria remained challenging, as asymptomatic individuals displayed no noticeable differences. The presentation concluded by highlighting the potential of lab-on-chip technology, which enables sample analysis and direct delivery of results to mobile devices.

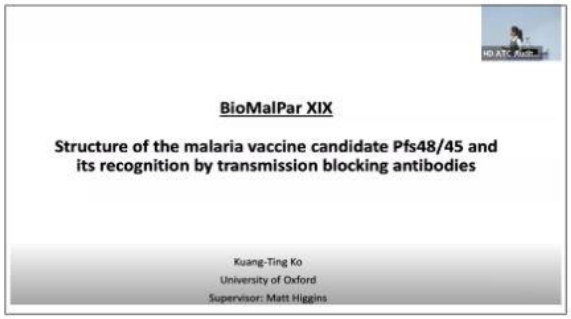
Melissa Kapulu (the University of Oxford and KEMRI Wellcome Trust, United Kingdom) highlighted the importance of Controlled human malaria infection (CHMI) as a model for malaria research and vaccine development. Kapulu's study examined healthy adults from different transmission areas: Ahero (a high malaria transmission area), Kilifi South (a moderate transmission area), and Kilifi North (an area with low to no exposure) to understand pre-existing immunity and identify key parasites targets. Following sporozoite injection, the data revealed variations in parasite growth control, leading to susceptible, slow-growing, clearer-phenotype and highly immune groups. Comparing CHMI's predictive strength of schizont antibody with field studies, having adjusted for exposure and infection heterogeneity, demonstrates CHMI's superior ability to study past exposure immunity. In addition, preliminary vaccine efficacy data on R21 and ChAd63 ME-TRAP, administered intradermally and intravenously, show higher efficacy for R21, particularly intradermally. This research sheds light on the benefits of CHMI for advancing malaria research and optimizing vaccine development.

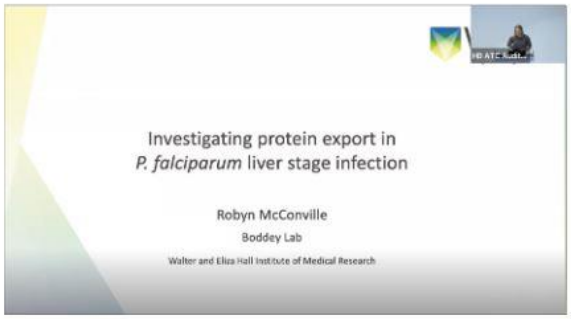
Priscilla Ngotho's (University of Glasgow, United Kingdom) talk explored how gametocytes remodel host red blood cells (RBCs) during maturation, providing valuable insights into the underlying mechanisms over time. Using transgenic lines, Ngotho's research revealed that the expression of parasite antigens coincides with flipping in the infected RBC (iRBC) membrane, exposing surface antigens and phosphatidylserine (PS), reaching peak levels during Stage II gametocytes but reverses as maturation progresses. Furthermore, a study targeting both the host and parasite scramblase to block PS exposure, suggests that scramblase blocks late PS surface loss. Notably, the study highlights that interfering with host cell modifications affects immune recognition and clearance of gametocyte-infected RBCs by immune cells. These findings contribute to developing innovative transmission-blocking approaches by enhancing the understanding of these mechanisms.

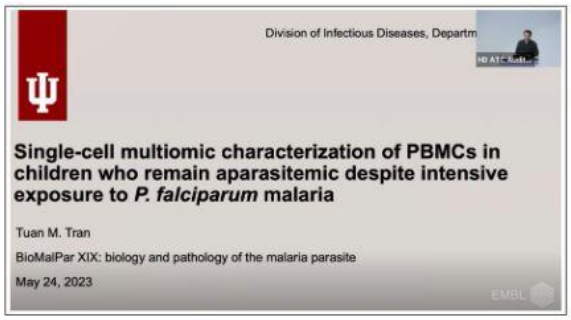
This report is brought to you by the MESA Correspondents Faith Hungwe, Aaron Lartey and Rinter Karimi Kimathi. Senior editorial support has been facilitated by Yunuen Avalos Padilla and Alba Pérez Cantero.

Day 2: 24th May 2023

Scientific session 3 – Immunology and Vaccines







Kamija Phiri (University of Malawi, Malawi) presentation focused on severe malaria anaemia (SMA). Affected children face a higher risk of in-hospital mortality, readmittance to the hospital and post-discharge mortality. To address this gap, a post-discharge malaria chemoprevention (PDMC) case-control trial was conducted to assess the effectiveness and safety of chemoprevention with artemether–lumefantrine (AL), for children with SMA. Results suggested that this strategy decreased further hospitalizations and post-discharge mortality. In addition, a confirmatory study was conducted in Kenya and Uganda, expanding the target group to include severe malaria (SM) and switching the drug to dihydroartemisinin-piperaquine (DHA-PiP) while shortening the drug administration time with the same positive results. Furthermore, data from a meta-analysis revealed a reduction in overall mortality and hospitalization rates. In addition, prolonged use of piperaquine was deemed safe, and models indicated that this treatment approach was feasible and cost-effective. Finally, the data obtained from these studies were instrumental in informing the updated World Health Organization's (WHO) recommendations for malaria chemoprevention. In conclusion, Phiri highlighted the challenges of implementing these strategies as the diversity of health systems across countries warrants the need for country-specific delivery mechanisms and raised questions about how longer-term protection could be provided for children with severe malaria.

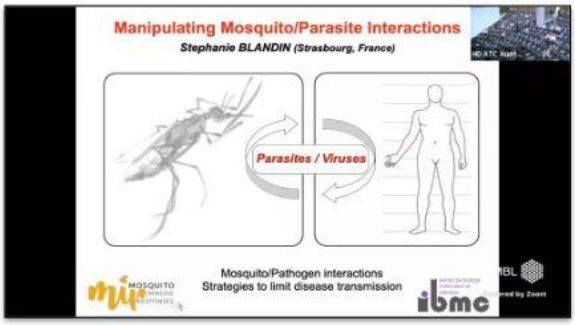
Kuang-Ting Ko (University of Oxford, United Kingdom) began the talk by addressing the importance of understanding the structure and function of Pfs48/45, a potential target for transmission-blocking vaccines. Using an AlphaFold2 model, Ko's research group discovered that Pfs48/45 forms a dynamic triangular disc-like structure, with all three domains exposed on the gamete surface. In addition, the study also identified specific antibody binding sites on Pfs48/45 and demonstrated that most antibodies with transmission-blocking activity could bind across whole molecules. Following this, using the surface plasmon resonance (SPR) binding assay, two of the most potent transmission-blocking

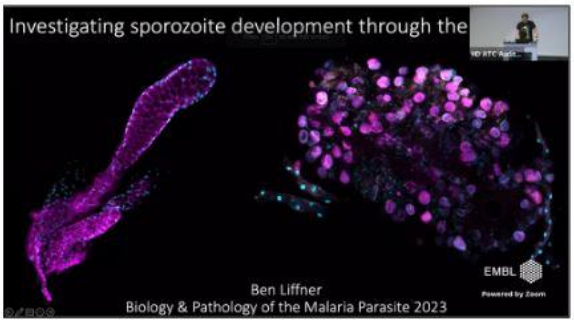
antibodies binding to the C-terminal domain of Pfs48/45(85RF45.1 and 32F3) were compared t. The study found that the two antibodies bind at slightly different angles, and the binding interaction involves different surface contacts, thus resulting in different binding kinetics. This research provides valuable insights for the design of future Pfs48/45-based vaccine immunogens.

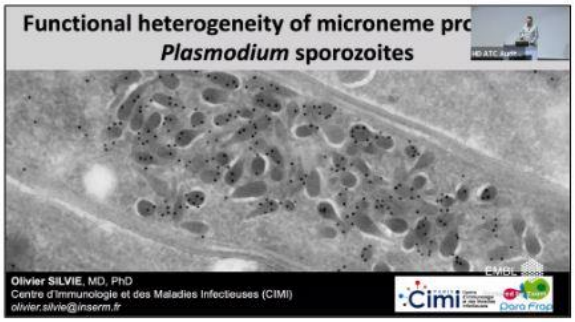
Robyn McConville (Walter and Eliza Hall Institute, Australia) spoke about how liver stages of *P. falciparum* exploit the *Plasmodium* Translocon of EXported Proteins (PTEX), a transmembrane complex consisting of 3 subunits. McConville investigated the roles of the EXP2 and PTEX150 subunits, in host-parasite manipulation by characterising PTEX150 and exploring its functionality in *P. falciparum* liver stage development. For this, an FLp/FRT system was developed and humanized mouse models were used. The results obtained demonstrated a reduced sporozoite burden in PTEX150-deficient parasites as well as developmental defects when EXP2 was excised, highlighting the essentiality of PTEX in parasite development. Subcellular localisation studies were also conducted to identify exported proteins expressed during liver infections, and the liver stage antigen-3 (LSA3) was found to be essential for both localisation and *P. falciparum* liver stage development. To conclude, McConville expressed the complexity of the export pathway and highlighted how much is still to be studied.

Tuan M Trang (Indiana University School of Medicine, United States) discussed the findings of a study aiming to understand the molecular and cellular differences in malaria-exposed aparasitemic children. The study involved 695 children and adults in Mali, where 25% of the youngest children (6 months - 2 years) remained uninfected despite living in a malaria-endemic area. Trang pointed out that similar findings were observed in previous studies conducted in Tanzania and in Kenya during a malaria vaccine trial. Employing a protein microarray assay, it was found that boosting IgG to malaria antigens evidenced malaria exposure in these children. Further comprehensive system analysis was also conducted on age- and sex-matched pairs of aparasitemic and parasitemic children using protein microarray and scRNA sequencing. Interestingly, aparasitemic children exhibited increased Pf-specific IgG reactivity, particularly against RON2 and Pfs16 antigens. Although the cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) analysis revealed similar frequencies of immune cells, distinct transcriptional states between aparasitemic and parasitemic children were observed. The tumour necrosis factor (TNF) signalling via NF-kB pathways and upregulation of pathogen sensors were observed in CD14+ monocytes of aparasitemic children compared to parasitemic children. These findings provide valuable insights into the molecular and cellular mechanisms underlying the aparasitemic phenotype, contributing to our understanding of efficient control of parasitemia.

Scientific session 4 – Transmission and Vector–Parasite Interactions







Stephanie Blandin (National Institute for Health and Medical Research (INSERM) and Institute of Molecular and Cell Biology, France) delved into molecular mechanisms which orchestrate parasite interactions and how this knowledge can be exploited to design novel control strategies. Blandin introduced the Saglin protein, a sporozoite receptor only expressed in female mosquitoes. Blandin's primary aim was to characterize the expression patterns of the Saglin protein using *An. coluzzi*. With the generation of transgenics, they were able to show that Saglin was transported to the midgut after a blood meal and was necessary for ookinete development. The absence of Saglin prevented transmission in low infection contexts with no fitness cost to mosquitoes and so a population modification strategy was developed using a dual gene drive that targeted the locus of the Saglin protein while co-driving the spread of a lipophorin allele expressing the antibody 2A10 (Lp-2A10) that binds sporozoites. With the Lp-2A10+Saglin (Sc-2A10) gene drive, transmission was drastically reduced (90%). She concluded by reiterating the need to further understand the function of Saglin in ookinete development and to assess whether parasites will develop resistance to gene drive pressures.

Ben Liffner (Indiana University School of Medicine, United States) illustrated the cellularization of sporozoites and invasion to the salivary glands processes using ultrastructural expansion microscopy (U-ExM). By visualising the overall tissue architecture, Liffner gained 3D insights into the ultrastructure of sporozoites within whole mosquito midgut and salivary glands and found that rhoptries varied in size, shape or number depending on if they were in mature oocyst-sporozoites or salivary gland sporozoites. Specifically, the ultrastructure images showed that armadillo repeats only proteins fused to rhoptries suggesting that they are a probable vehicle for rhoptry biogenesis. Liffner also noted that rhoptry biogenesis ends when segmentation completes. As egression took place, Liffner *et al.* were able to quantify rhoptries and found that their numbers decreased rapidly when sporozoites invaded epithelial cells and only 2 rhoptries entered the secretory cavity. These rhoptries would later become a vacuole. Overall, this study enhanced the understanding of sporozoite development and invasion.

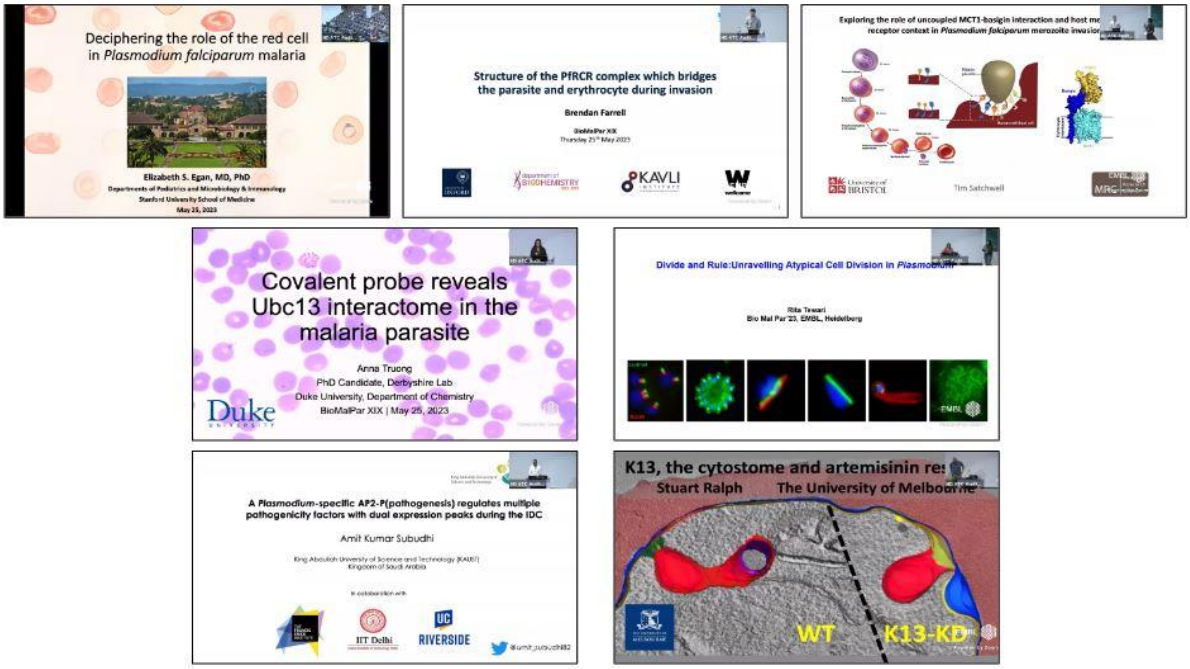
Olivier Silvie (National Institute for Health and Medical Research (INSERM) and Sorbonne University, France) focused on proteins that aided sporozoite invasion into salivary glands and host cells. It aimed at elucidating the ligand (B9)-receptor (P36 and P52 heterodimer) interactions and the sporozoite moving junction proteins (AMA1 and RONs), key factors in sporozoite egression. Silvie showed that B9 and P36 colocalise to a subset of microneme proteins, but there was no interaction detected. It was also discovered that B9 and P36 are required for sporozoite rhoptry discharge. To nuance the role of junction proteins, the role of the AMA1-RON complex was investigated and AMA1 was found to be essential for efficient sporozoite invasion of mosquito salivary glands and human hepatocytes. Lastly, Silvie talked about how claudin-like apicomplexan microneme protein (CLAMP), found in both merozoites and sporozoites, was essential to sporozoite infectivity in *P. berghei* and involved in sporozoite gliding motility. Results also showed that CLAMP had a regulatory role for tryptophan-regulated attenuation protein (TRAP), a protein involved in parasitic evasion mechanisms, absent in AMA1. This suggested that CLAMP has varying functions in sporozoite formation and invasion. Silvie concluded by stating that more work needs to be done to understand microneme secretion regulation.

Knowing that *P. falciparum* Ca2+-dependent kinase 4 (Pfcdk4) and *P. falciparum* Serine/arginine-rich protein kinases 1 (PfSRPK1) play a role in male gametogenesis, microtubule and cell motility, **Sudhir Kumar's** (Seattle Children's Research Institute, United States) aimed to investigate the role of a microtubule-binding protein in *P. falciparum* male gamete fertility. Through comparative phospho-proteome and RNA-seq analyses, Kumar studied the nuclear microtubule-associated protein EB1 (Pfeb1) bound to the growing strand of microtubules and upon deleting Pfeb1. Two major results were found. Male gametes became sterile due to a lack of nuclear incorporation in the microgametes but Pfeb1 was not required for asexual development, gametocytogenesis, macro or micro gametogenesis and exflagellation. These findings emphasise the crucial roles of cdpk4, srpk1, and EB1 in axoneme formation during gametogenesis. Kumar concluded the talk by discussing the potential that Pfeb1 has as a transmission-blocking drug target.

This report is brought to you by the MESA Correspondents Faith Hungwe, Aaron Lartey and Rinter Karimi Kimathi. Senior editorial support has been facilitated by Yunuen Avalos Padilla and Alba Pérez Cantero.

Day 3: 25th May 2023

Scientific Session 5 – Systems Biology of Malaria: Molecules, Cells, Physiology and Pathobiology



Elisabeth Egan (Stanford University, United States) spoke about the significance of the red blood cell (RBC) receptor, CD44 during *P. falciparum* invasion and the importance of implementing an improved screening approach to identify key host membrane receptors for malaria. Using nucleated human hematopoietic stem cells (HSPCs), Egan's group developed forward genetic screens of differentiated HSPCs tagged with green fluorescence protein (GFP) plasmids to identify host factors related to *P. falciparum* invasion. CD44 and CD55 were identified as essential for host cell invasion. CRISPR/Cas9 technology was used to further investigate the role of CD44s in RBCs. CD44-null cultured RBCs (cRBCs) obtained from primary HSPCs were compared to isogenic wild-type cRBCs. The results revealed a greater reduction in *P. falciparum* invasion in the CD44-null cRBCs compared to the wild type. Further exploration uncovered that ligands EBA175 and EBA140 from the parasite interacted with the CD44 receptor. Additionally, monoclonal antibodies raised against CD44 including BRIC 222, BRIC 170, BRIC 235, KZ1, and IM7 were successfully used to promote *P. falciparum* invasion. This effect was due to CD44 cross-linking, which in turn increased basigin detection on the RBC surface. Overall, Egan *et al.* discovered that both BRIC 222 and EBA175 induced changes in the RBC membrane, which increased basigin accessibility and promoted invasion. These findings enhance the understanding of the mechanisms behind *P. falciparum* infections and suggest potential targets for future therapeutic interventions.

Brendan Farrell (University of Oxford, United Kingdom) highlighted the importance of the PfPCRCR complex (formed by PfPTRAMP, PfCSS, PfRIPR, PfCyRPA and PfRH5) in the interaction between merozoites and basigin on erythrocytes. Cryo-EM analysis of this complex revealed how PfCyRPA, PfRIPR, and PfRH5 proteins interact at a molecular level. For instance, PfRH5 and PfRCR were found to be binding with equal affinity to basigin and the former showed no significant conformational changes during invasion. Surprisingly, PfRH5 possessed a crucial disulfide bond that enabled PfRH5 to act as a pore. The study also determined that the elongated tail of PfRIPR interacted with the PfCSS-PfPTRAMP

complex in merozoites. Finally, antibodies targeting the basigin-PfRH5 binding disrupted the PfPCRCR complex's bridging function, hindering invasion. Farrell concluded by stating that because PfPCRCR bridges the parasite and erythrocytes, these findings could contribute to rational vaccine design for malaria prevention.

Tim Satchwell (University of Bristol, United Kingdom) discussed the role of PfRH5-basigin in mediating invasion and elucidated the properties and traits of basigin that make invasion possible. Using *in vitro* erythropoiesis and a CRISPR/Cas9 approach, Satchwell investigated the role of uncoupled MCT1-basigin interaction in RBCs during parasite invasion. Through genetic manipulation, reticulocytes with uncoupled basigin and reduced MCT1 expression were created. The knockout of basigin led to an 80% reduction in MCT1 expression. However, reintroducing basigin mutants lacking the transmembrane helix responsible for MCT1 interaction, allowed the surface presentation of the extracellular domain for binding with PfRH5. This facilitated merozoite invasion independent of MCT1, suggesting that the interaction between basigin and MCT1 may not be essential for the invasion process. Overall, Satchwell's presentation provided valuable insights into the functional significance of MCT1-basigin in the host cell membrane context, ultimately contributing to the understanding of the invasion mechanism.

Anna Truong (Duke University, United States) began the talk by emphasizing the significance of ubiquitination in *P. falciparum*, specifically focusing on the gene PfUbc13. Ubiquitin, an 8-kDa protein, acts as a signaling molecule that attaches to substrate proteins through enzymatic reactions involving E1, E2, and E3 enzymes. The study aimed to uncover PfUbc13 interacting partners, decipher their regulatory mechanisms and identify target substrate proteins associated with PfUbc13. Initial studies showed that deletion of PfUbc13 amplified the sensitivity of the parasite to the antimalarial drug dihydroartemisinin, alluding to its involvement in drug activity. To assess PfUbc13 activity, a chemical probe was employed, and downstream target proteins were identified using mass spectrometry-based proteomic techniques. With this analysis 64 potential substrate proteins of PfUbc13 were successfully identified. Subsequently, gene ontology enrichment revealed that these proteins were clustered into four major areas: metabolic processes, regulation of biological processes, intracellular transport, and protein organization. This research offers valuable insights into the interactions of PfUbc13 and the mechanism of antimalarial drugs thus contributing to the ongoing efforts in malaria drug discovery.

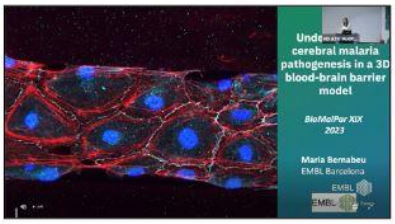
Rita Tewari's (University of Nottingham, United Kingdom) presentation began with a brief introduction to the diverse cell division mechanism in *Plasmodium*. She addressed the significance of atypical parasitic cell division and its implications for parasite survival. *Plasmodium* exhibits asynchronous division, including two modes of mitosis (closed and end mitosis) and a brief meiosis phase. Aurora related kinases and microtubule associated proteins were identified as major regulatory molecules governing cell division in *P. berghei.* Tewari's research focused on defining the functions of ARK2 in the centrosomal complex microtubule-organizing center (MTOC) during *P. berghei's* schizogeny. ARK2, associated with scaffold proteins, was found to colocalize with the spindle, displaying dynamics similar to Ndc80 suggesting that it has a polar distribution in the nuclear compartment. During male gametogenesis, ARK2 influenced early oocyte formation, leading to exflagellation. Additionally, Tewari and colleagues found that ARK2 was associated with EB1 at the centrosome, which had similar spindle dynamics as Ncd80, exhibiting a comparable profile. The Ark2-

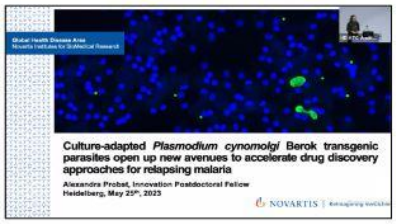
EB1 association was found to have a role in endomitosis during oocyst development. Tewari ended her presentation by mentioning the need for further research exploring the function of ARK2-EB1 interactions to understand spindle dynamics and spatial organisation during cell division in *Plasmodium*.

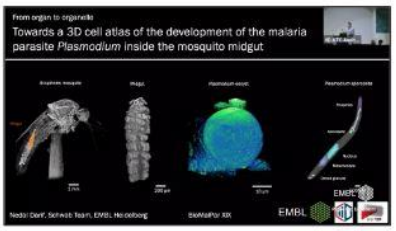
Amit Kumar Subudhi's (King Abdullah University of Science and Technology, Saudi Arabia) presentation focused on the crucial role of PfAP2-P, an Apicomplexan AP2 transcription factor, in malaria pathogenicity. The study highlighted PfAP2-P's involvement in *var* gene regulation, merozoite development, and parasite egress during the intraerythrocytic developmental cycle. ChIP-seq experiments revealed PfAP2-P's binding to gene promoters of other AP2 proteins, controlling antigenic variation and host cell remodeling. Deletion of PfAP2-P resulted in derepressed *var* gene expression and overexpression of early gametocyte markers, indicating its role in sexual stage conversion. Chromosome conformation capture experiments showed reduced chromatin interactions upon PfAP2-P deletion. Overall, the findings emphasized PfAP2-P as a vital upstream regulator governing key pathogenic processes in distinct parasite developmental stages.

Stuart Ralph's (University of Melbourne, Australia) presentation was focused on the significance of the Kelch 13 (K13) protein. K13, which has been associated with artemisinin resistance, seems to be important for the formation of cytostomes, which are invagination structures involved in haemoglobin uptake by *P. falciparum*. Specifically, Ralph revealed that K13 stabilizes the cytostome by forming doughnut-shaped rings around the cytostomal neck, consistent with electron microscopy findings. The absence of K13 resulted in abnormal cytostomal structures as shown by electron tomography and serial-block-face SEM. Additionally, fractionation experiments indicated reduced haemoglobin uptake, haem release, and artemisinin activation in K13 mutants, leading to lower haem and hemozoin production. In addition, a connection between K13 and ubiquitin signalling was suggested but requires further investigation. Overall, these findings shed light on the crucial role of K13 in cytostome formation and its link to artemisinin resistance, aiding the development of strategies to combat drug-resistant malaria.

Scientific Session 6 – New Topics and Tools for Malaria Research and Future Innovation







Maria Bernabeu's (European Molecular Biology Laboratory, Barcelona, Spain) talk focused on the use of a three-dimensional blood-brain barrier (3D-BBB) in vitro model to study malaria pathogenesis. The model consists of a microfluidic network within a collagen hydrogel, simulating various flow conditions in the brain vasculature. Two models were developed consisting of either primary human brain microvascular endothelial cells or induced pluripotent stem cell-derived endothelial cells (iPSC-EC) in combination with astrocytes and pericytes. The iPSC-EC model demonstrates improved barrier properties, such as physiological permeability rate and enhanced expression of tight junction markers. With these models, Bernabeu's team aims to identify host and parasite factors driving cerebral malaria and further explore the underlying molecular mechanisms. Consequently, 3D-BBB models were exposed to different *P. falciparum* stages or parasite product media, which disrupted the endothelial cells of the 3D-BBB. Through various molecular techniques, changes in barrier integrity and the impact on BBB cell composition were examined. Additionally, scRNAseq revealed that BBB cells have a unique transcriptional profile after exposure to parasite toxins which altered signaling pathways in endothelial cells, astrocytes, and pericytes. Overall, the 3D-BBB model uncovers novel insights into the molecular mechanisms of cerebral malaria and could potentially contribute to the development of therapies to reduce patient mortality.

Alexandra Probst (Novartis Institute for Tropical Diseases, United States) discussed genetic modification of *P. cynomolgi* as a model to gain insights into targeting *P. vivax* parasites at the liver hypnozoite stage. First, using CRISPR/Cas9, Probst's team replaced the endogenous circumsporozoite protein (CSP) gene, which is essential for parasite development in the mosquito and for liver infection, with PvCSP from *P. vivax*. The transgenic parasites completed their life cycle and generated infectious sporozoites, which could cause relapsing infections in monkeys. Then, to pursue drug discovery purposes, transgenic parasites expressing HaloTag at the host-parasite interface during the liver stage were generated to disrupt the host ubiquitin-proteasome system. The HaloTag was bound to bifunctional molecules potentially targeting hypnozoites for degradation. In addition, Probst highlighted the potential of employing transgenic lines for various applications in malaria research, such as live fluorescence microscopy, proteomics, and protein degradation studies. Furthermore, Probst mentioned the prospect of utilizing positron emission tomography (PET) for in vivo imaging of infected monkeys, enabling the monitoring of liver burden during malaria infection. This research offers potential strategies to target hypnozoites and combat relapsing *P. vivax* malaria infections.

Yunuen Avalos Padilla (Institute for Bioengineering of Catalonia, Spain) started by presenting the endosomal sorting complex required for transport (ESCRT), and in particular, the ESCRT-III sub-complex which is involved in extracellular vesicles (EVs) formation. Even though RBCs lost the majority of their organelles, they still produce EVs with an alternative pathway involving PfBro1, PfVps32 and PfVps60 proteins. By using antibodies raised against PfVps32, a reduction of parasite viability was observed. To overcome the cost limitations of antibodies, the study used aptamers as an alternative approach. Aptamers specific to PfVps32 and the AAA-ATPase PfVps4 were isolated and were shown to bind to *Plasmodium-infected* RBCs. Furthermore, fluorescence microscopy confirmed the binding of the aptamers to parasites and vesicles present on the surface of the host. The aptamer targeting PfVps32 exhibited the lowest IC50 value in inhibiting parasite growth. Despite its low level, it demonstrated its potential as an antiparasitic agent. Lastly, Avalos-Padilla discussed future plans, which included analyzing the entire pool of aptamers and determining their IC50 values through next-generation sequencing (NGS). Additionally, efforts will be made to enhance aptamer stability and reduce degradation by formulating them into polymersomes, potentially as carriers for other antimalarial drugs. The study highlighted the potential of aptamers as an efficient and cost-effective tool for targeting *P. falciparum*.

Nedal Darif (European Molecular Biology Laboratory, Heidelberg, Germany) spoke about oocyst formation and the use of advanced imaging techniques such as volume electron microscopy (vEM) as a proof of concept to differentiate the stages of oocyst biogenesis. Darif proposed using a combination of vEM, Synchrotron X-Ray Tomography (SXCT), confocal targeting, and electron microscopy to capture high-resolution images of oocysts and sporozoites. In the presentation, Darif detailed images identifying the location of oocytes at various developmental stages. By utilizing a blend of targeted imagery and pre-trained neural networks, the team achieved the remarkable feat of predicting and recreating the parasite's developmental process. This innovative approach also led to the identification of novel structures, including an electron-dense tubular organelle within the sporoblast, which presents an avenue for further investigation. Additionally, Darif discussed the ongoing efforts to construct a comprehensive cell atlas of oocysts and sporozoites, employing advanced imaging techniques to unveil the intricate stages of *Plasmodium* development.

Jacquin Niles (Massachusetts Institute of Technology, United States) began the talk by discussing genetic-based approaches for developing new anti-malarial treatments. With the current challenge of drug resistance in malaria and relatively few compounds with novel mechanisms of action, Niles's team employed advanced technologies to manipulate gene expression in the malaria parasite and study potential target proteins, such as tRNA synthases. As a matter of fact, significant fitness defects in *P. falciparum* were observed when the expression of tRNA synthases was reduced. Moreover, specific chemical compounds that selectively inhibited certain members of the tRNA synthase family were identified, aiming to minimize off-target effects. In parallel, Niles's team have developed strategies to discover new molecules that act on specific targets by screening large compound libraries, which allows the identification of novel compounds interacting with validated targets and facilitates the development of antimalarial drugs. Overall, this study highlights the importance of using an integrated approach that combines genetic techniques with target-guided chemical screening to identify and validate potential drug targets for antimalarial therapies.

Closing remarks

The conference came to an end with the conference organizers emphasizing the exceptional level of research that had been presented throughout the meeting. They also spoke on the importance of being part of a research community that shares a common goal in a collaborative nature. Furthermore, they encouraged everyone to participate in the upcoming 20th anniversary BioMalPar Conference, scheduled for 2024. The conference organizers also extended their gratitude to the EMBL and technical team for running a smooth conference.

This report is brought to you by the MESA Correspondents Faith Hungwe, Aaron Lartey and Rinter Karimi Kimathi. Senior editorial support has been facilitated by Yunuen Avalos Padilla and Alba Pérez Cantero.

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