RESOURCE HUB *Correspondents*

Keystone Symposia
"Malaria: Confronting Challenges from Drug Discovery to Treatment"

Complete series

MESA Correspondents bring you cutting-edge coverage from the Keystone Symposia "Malaria: Confronting Challenges from Drug Discovery to Treatment"

10 - 13 April 2022

In-person & Virtual Conference

The MESA Alliance would like to thank the Chairs and Speakers[1] of the Symposia who collaborated with the program.

The MESA Alliance would also like to acknowledge the MESA Correspondents Patricia Doumbe Belisse (OCEAC & University of Yaoundé I, Cameroon), Nutpakal Ketprasit (Tilley Laboratory, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne), Samuel Blankson (Université Paris Cité, France and the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana) for their crucial role in the reporting of the sessions.

[1] *Complete list of names at the end of the report.*

RESOURCE HUB Correspondents

M E S A

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Day 1: 11th April 2022

Welcome & Keynote Address

David A. Fidock from Columbia University Medical Center, USA, opened the Malaria Keystone Symposia 2022 by expressing his pleasure to take part in the conference. He warmly welcomed 145 people attending in person, 60 attendees participating virtually and around 70 speakers. Furthermore, he thanked the co-organizers of the conference including Elizabeth A. Ashley (Lao-Oxford-Mahosot Hospital - Wellcome Trust Research Unit, Lao) and Kelly Chibale (University of Cape Town, South Africa) for putting together all the hard work. Finally, he introduced the keynote speaker Arjen M. Dondorp.

Arjen M. Dondorp (University of Oxford, Thailand) summarized the evolution over the last decade of artemisinin and Artemisinin Combination Therapy (ACT) partner drug resistance in P. falciparum in the Greater Mekong Subregion of SE Asia, and the components of the highly successful malaria elimination program in the GMS, which was initiated 10 years ago to contain the resistance problem. The experience from the GMS was discussed in the context of the recent independent emergence and spread of artemisinin resistance in Rwanda and Uganda. Artemisinin partial resistance contributes to decreased ACT efficacy, but also facilitates the selection of ACT partner drug resistance, which has caused high treatment failure of a number of ACTs in the GMS. Artemisinin resistance is mainly confined to the ring stage asexual parasite, although also schizont stage parasites show decreased susceptibility. In addition, artemisinin resistant infections show higher gametocyte carriage and reduced artemisinin sensitivity of male gametocytes, contributing to increased transmissibility. In the GMS, a successful malaria elimination program was initiated 10 years ago to contain the resistance problem. Its cornerstone is a well-organized network of village malaria workers for early diagnosis and treatment of malaria, supplemented with additional interventions such as mass drug administration in target populations. Epidemiological surveillance is another important component of the program, including genetic surveillance. Single gametocytocidal low-dose of primaquine reduces transmission of drug resistant parasites, and is also safe to use in African children, independent of the G6PD status of the patient. New drugs will be needed to treat multidrug resistant falciparum malaria, but these are not expected to come to the market within 5 years. An important approach using existing drugs are Triple ACTs, combining the artemisinin component with two well-matched partner drugs. In clinical trials these have shown to be safe, well-tolerated and efficacious also against drug resistant parasites. The artemether-lumefantrine-amodiaquine combination is now being developed as a fixed dose combination, which could also be important for Africa to, in addition to improved antimalarial stewardship, delay the further spread of artemisinin resistance and protect the current ACTs.

Session 1: Innovations in Antimalarial Drug Discoveries

Elizabeth A. Winzeler (University of California, USA) began the talk by giving an introduction to the evolution of antimalarial drug discovery which includes pharmacophore-based discovery, phenotypic screening, and structure-guided drug design. The structure-guided drug discovery is more sophisticated and efficient, compared to the previous strategies. She further highlighted that combining reverse chemical genetic methods with chemical screening can yield highly validated targets such as lysyl-tRNA synthetase. She introduced the Malaria Drug Accelerator (MalDA) consortium that collaborates with international groups to build up a pipeline for structure-guided drug discovery. MalDA also uses in vitro evolution and whole-genome analysis or IVIEWGA to discover clinically relevant resistance genes. Recently, the MalDA website has been updated with a resistance database, which is useful for predicting association between phenotype and observed mutations. Dr. Winzeler also proposed the use of other species, such as yeast (Saccharomyces cerevisiae), as a model

for identifying the targets of antimalarial compounds. For future work, it is important to understand how drug resistance in the malaria parasites arise and how to prevent it.

Jacquin Niles (Massachusetts Institute of Technology, USA) shared that his laboratory is interested in establishing genetic technologies for drug discovery and target validation. Currently, there are relatively few compounds with novel mechanisms of action in the development pipeline. He described the use of conditional gene expression perturbation approaches for target validation and inhibitor discovery. In this approach, the target gene is modified using CRISPR-Cas9 genome editing to generate parasite lines that enable tunable expression of essential proteins of interest. When expression of a protein of interest decreases, this can lead to hypersensitivity to compounds targeting the protein. A rapid phenotypic screening is invented and tested with aminoacyl-tRNA synthetases (aaRSs), important enzymes in protein translation. This lead to discovery of several compounds that hit aaRSs. Dr. Niles proposes that the future work is to use his strategy to validate the biological function of genes and to discover potential antimalarials.

Susan Willye (University of Dundee, UK) started her talk by highlighting some factors hampering drug discovery programs required to combat parasitic disease such as few robustly validated drug targets within the pathogens that cause these infections thus leaving our drug discovery programs relying heavily upon phenotypic screening. She then presented target deconvolution studies as a solution to address those challenges. She presented a matrix of new methodologies in use in her lab, including high throughput genetics, cell biology, biochemical assays, and chemical proteomics that increases the chances to understand the mode of action of compounds and identify molecular targets. She emphasized using multiple methodologies in concert for identifying and validating molecular targets. Dr. Wylie's team is currently in building a toolkit to support identification of molecular targets of antimalarial drugs which includes a genome-wide cosmid-based overexpression library in P. knowlesi, high throughput in vitro translation assays for P. falciparum and adapted chemical proteomic strategies for P. falciparum. Their mode of action studies currently uses various strategies including chemical proteomics strategies, chemical pulldown, and thermal proteome profiling (TPP), thus providing critical information to drug-discovery programmes.

Jerzy Michal Dziekan (Nanyang technological University, Singapore) focused his talk on mapping the druggable landscape of malaria parasites using thermal proteome profiling. He gave an overview of the MS-CETSA-Cellular Thermal Shift assay as a tool for antimalarial drug-target deconvolution. His work evaluated a library of antimalarial compounds to identify interactions with Plasmodium proteins. Dr. Dzieken used cellular thermal shift assays to identify compound-target interactions. He presented a wide range of new interactions between a range of antimalarial compounds and Plasmodium proteins, which excitingly sets the stage for additional functional validation via orthogonal approaches..

Alison E. Roth (Walter Reed Army Institute of Research, USA) and her lab with the aim of identifying novel antimalarial compounds measurable to tafenoquine and to accelerate drug discovery, developed P. cynomolgi assay for identifying compounds targeting hypnozoite stage of the parasites. The assay also incorporates high-throughput data pipeline for data analysis. This approach has been used to screen a large number of compound libraries. Another approach is artificial intelligence (AI) is being developed to accelerate the identification of anti-hypnozoite compounds. Lastly, Alison pointed out that collaborations are key for success.

Session 2: Advancing Drug Discovery Efforts

Jeremy Burrows (Medicine of Malaria Venture - MMV, Switzerland) began his talk by giving an overview of malaria case management which is dominated by artemisinin combination therapies (ACTs). ACTs are still working in Africa but there is a risk of resistance because of the spread of de novo artemisinin resistant K13 mutations. For seasonal malaria chemoprevention, sulphadoxine-pyrimethamine-amodiaquine is approved for use in 0-5 year children. Burrows described the target product profile (TPP) for new antimalarials and presented a strategy that covers case management, prophylaxis, severe malaria management, and a radical cure for relapsing P. vivax malaria. MMV and partners have developed several assays to facilitate drug discovery. Examples of these assays include liver, blood and gametocyte stages, as well as humanised mouse model for P. falciparum infections. MMV with its partners have delivered 34 candidate drugs over the last 15 years. The four strategies used to discover these have included inspiration from natural products, delivering back-up compounds that address liabilities of compounds in the clinical portfolio, phenotypic screening and target-based approaches. Lastly, Burrows outlined some challenges for antimalarial drug discovery, one of the most important is antimalarial resistance; a newly developed resistance risk assessment is used to aid prioritization and to influence the combination strategy.

Laura Sanz (GlaxoSmithKline - GSK, Spain) presented the approaches adopted by GSK to fight malaria disease: treatment, prevention and vector control. She focused on treatment and mentioned antimalarial therapeutic requirements: safety, efficacity, oral administration, drug resilience against antimalarial resistance combination therapies, and the development of new regimens . She introduced a novel drug for treatment of malaria, GSK701 (MMV1582367), as a potential drug to replace artemisinin in combination therapies. This is a fast-acting antimalarial, with anticipated low propensity to select for parasite resistance. Toxicological studies have shown it is safe, and GSK701 acts through a novel antimalarial mechanism of action.

Kelly Chibale (University of Cape Town, South Africa) described how, using genomic and chemoproteomics, several chemotypes targeting P. falciparum kinases have been discovered. Even though there is concern about the toxicity of inhibitors targeting protein kinases, the phylogenetic differences between human and malarial parasite kinases suggest selectivity can be achieved. Chibale proposed a very efficient drug repurposing strategy that uses clinically characterized human kinase inhibitors for antimalarial discovery. The benefit of repurposing drugs is that these drugs have been previously studied in clinical trials in humans for safety and tolerance. However, the key challenge is to minimize off-target activity against human kinases.

Andrew B. Tobin (University of Glasgow, UK) dived into the details of protein kinases as antimalarial targets, focusing on his team's work on kinase inhibitors. Using biochemical and genetic validation, his team identified PfCLK3 as a promising target. A promising compound (TCMDC-135051) which inhibits PfCLK3 is currently undergoing chemical optimization. PfCLK3 is involved in RNA splicing and is therefore essential for the parasite survival in liver stage, blood stage, and gametocyte stage.

Didier Jean Leroy (Medicine of Malaria Venture - MMV, Switzerland) presented on orthology-based screening performed to obtain the MMV68853 antimalarial candidate. This is a fast killer compound which has shown efficacious exposure in humanized mice infected with P. falciparum. It is highly potent on early rings to late trophozoites, and selects for low-grade resistance via mutations in PfACG1 and PfEHD. Mutations in PfACG1 and PfEHD do not confer cross resistance with antimalarials, and PfACG1 and PfEHD are localized mainly to distinct intracellular parasite vesicles. Its target is still elusive, and the mode of action is being studied. The fast killer antimalarial candidate, MMV68853, almost as fast-acting as artemisinin, is currently in the phase I clinical trial.

This report is brought to you by the MESA Correspondents Patricia Doumbe Belisse, Nutpakal Ketprasit and Samuel Blankson. Senior editorial support has been facilitated by the Chairs and Speakers of the session.

Day 2: 12th April 2022

Session 3: Experimental Models and Other Human Plasmodia

John H. Adams (University of South Florida College of Public Health, USA) used forward-genetic screens of Plasmodium mutants created by piggyBac mutagenesis to understand mechanisms of drug response and tolerance in parasites. They have developed the QIsec methodology that identifies and quantifies the piggyBac insertions efficiently. He carried out a large-scale forward-genetic phenotype screen in P. falciparum to identify genes allowing parasites to survive febrile temperatures. Screening identifies many P. falciparum mutants with differential responses to heat shock (HS). He found that mutants are more likely to be sensitive to artemisinin derivatives as well as to heightened oxidative stress. Adams' findings highlighted an adaptation of PiggyBac approach for genome-scale mutagenesis and forward genetic screens of P. Knowlesi. He also identified common and species-specific gene essentiality between P. Knowlesi and P. falciparum. Besides, he identified genetic factors associated with altered sensitivity to chloroquine (CQ).

Flaminia Catteruccia (Harvard T.H. Chan School of Public health, USA) started her talk by introducing their project, initiated three years back, that aimed at developing new strategies based on delivering drugs to mosquitoes thus blocking the transmission. She talked about a process that blocks parasite transmission of Plasmodium falciparum by Anopheles mosquitoes without affecting the mosquito. After a tarsal exposure of mosquito to antimalarial drug atovaquone (ATQ) which is followed by infectious blood feeding, their analysis revealed an absence of oocysts in midguts presuming that Plasmodium parasites can be killed in the mosquito using ATQ. They also modeled the effect of using antimalarials on all bed nets and the results showed significant reduction in prevalence of infection particularly in areas of high insecticide resistance. They are currently testing the full potential of this strategy and also screening other active compounds. In vivo screens are revealing promising targets which are highly active (ELQ Qo, ELQ-613, ELQ-300). Catteruccia also highlighted that post -infection ATQ exposure in sugar solutions severely impairs Plasmodium growth stages.

James McCarthy (Peter Doherty Institute for infection and immunity, Australia) focused his talk on the blood stage human challenge model for the clinical development of antimalarials. At the onset of his talk, he presented a review of the antimalarials tested in this model (ferroquine, MK-5717, ZY-19489, lumefantrine among others). He expanded on the results of the clinical trial for ZY-19489 (well tolerated with a clean safety profile) that supported its progression to Phase 2 trials. He also discussed combination drug development strategy after reviewing published papers. Pharmacokinetics and pharmacodynamics (PK/PD) play a role in determining a drug's safety and efficacy and those parameters can be obtained in a short timeframe. He also presented a study design and results of a challenge study focused on evaluation of transmission blocking activity of low-dose TQ (Thymoquinone). They found that a delayed effect was seen 7 days after TQ and 50mg of TQ reduced transmission intensity by 75 % (95%CI 50-100%). Despite this positive impact some limitations are rising about its efficacy in field settings.

Christopher Anthony Bower-Lepts (University of Cambridge, UK) presented an in-vitro chemogenomic screening system that uses Bar-seq to identify essential gene targets of antimalarial drug resistance. This makes use of a *Plasmodium berghei* Artificial Chromosomes (*Pb*ACs) engineered with barcodes for identification in pools through next generation sequencing (NGS). Their investigation to validate *Pb*ACs for in vitro chemogenesis in *P. knowlesi* showed that the parasite takes up *Pb*ACs following pooled transfection, and that *P. berghei* genes are expressed in *P. knowlesi*. They also observed that an increase in the number of vectors in the transfection pool increases throughput and selection of compounds. This Bar-seq approach is also capable of identifying resistance associated genes in addition to targets and is more sensitive than conventional growth and dose-response assays.

Sachel Mok (Columbia University Medical Center, USA) talked about genetic crossing as a tool that can be used to uncover the genetic basis of drug resistance. She described *P. falciparum* genetic cross in humanized mouse: RF7 (DHA, PPQ-resistant) x NF54 (sensitive) and also showed that unique markers of artemisinin resistance are observed in comparing drugR-drugS. Pfcrt and plasmepsin 2 copy were identified as piperaquine resistance loci. Finally, tests to elucidate the molecular determinants of tolerance to ACT partner drugs showed that RF7 has differential sensitivity and that QTL maps Chr7 mutant PfCRT to Lumefantrine and Mefloquine sensitivity.

Michael T. Ferdig (University of Notre Dame, USA) elaborated on the use of quantitative trait locus (QTL) mapping for finding genes that control parasite traits such as resistance genes. Unique recombinant progeny were obtained by crossing drug resistant parent and drug sensitive parent. The resulting progeny may have desirable traits, have traits favoring one parent or the other, or may be skewed across the genome. He stated that one of the challenges is finding a locus and proposed a solution to use bulked segregant analysis (BSA)-directed QTL, as well as cloning and phenotyping individuals. He then highlighted other ongoing studies to improve and implement the application of this tool worldwide. He also mentioned Pfaat1 as an emerging mutant gene that causes drug resistance.

Donelly A. Van Schalkwyk (London School of Hygiene and Tropical Medicine, UK) and his team basically studied the use of ortholog approach to confirm resistance. They compared in vitro susceptibility of P. knowlesi and P. falciparum to a range of established and novel antimalarial agents under the same assay conditions and found that P. knowlesi have a significant difference of about 6 fold increase compared to P. falciparum. Ex-vivo susceptibility to cipargamin, an ATP4 inhibitor, regarding the two Plasmodium spp. including malariae and ovale also showed it to be highly potent against falciparum but reduced in the others. Ortholog replacement of Plasmodium berghei ATP4 with PfATP4 however restored susceptibility. PocATP4OR was the least susceptible ATP4 inhibitors compared to PfATP4OR.These findings support the necessity to add in vitro drug screens against P. knowlesi to those using P. falciparum strains to inform new drug discovery and lead optimisation.

Session 4: Antimalarial Use and Drug Resistance

Didier Menard (Institut Pasteur and University of Strasbourg, France) gave an overview of the spread of partial artemisinin resistance (ART-R) both locally and globally. He began with the origin and history of partial ART-R, which became prominent with the discovery of the molecular marker Pfkelch13. He mentioned some examples of Pfkelch13 such as 13580Y in South America and Papua New Guinea, and 561H, 675V and 469Y in Africa. He made listeners aware that a new mutant of Pfkelch13, R622I has now been observed in Southeast Asia. There was significant association of this mutation with Day 3 positivity rate, and it conferred ART-R in both Dd2 and NF54 genetic backgrounds, which have been engineered with the mutant upon ring-stage survival (RSA) expression. Pfkelch13 R622I mutant was detected in 50% in recrudescent isolates, and there was no distinct cluster between Pfkelch13 wild-type and mutant R622I. He also showed that R622I is responsible for the variable performance of hrp2 based malaria RDTs as it caused the deletions of hrp2 and hrp3 (majority of deletions)

David A. Fidock (Columbia University Medical Center, USA) stated that multiple antimalarials interact with hemoglobin import and detoxification. Multi-omics studies showed the mutant K13 to play a role in ART-R and parasite physiology as it is linked to specific biological processes such as hemoglobin endocytosis and metabolism, lipid transport, signaling etc. K13 mutations vary widely in the level of ART-R and evidence showed a failure in dihydroartemisinin-piperaquine (DP) in Cambodia and across the Greater Mekong Sub-region. Another ART-R gene that has emerged and rapidly dominated in Cambodia is haplotypes of the mutant PfCRT; and this is quite different from the PfCRT observed in Africa. PfCRT binds and transports drugs. Piperaquine resistant PfCRT mutations reverse resistance to

chloroquine (CQ). However, a new and potent antimalarial drug against P. falciparum called ZY-19489 has been discovered, which is able to select for novel PfCRT mutations and block CQ efflux.

Leann M. Tilley (University of Melbourne, Australia) talked about artemisinin resistance and the underlying mechanism. Her laboratory and colleagues demonstrated that artemisinin causes protein damage and proteasome disruption, leading to unresolved stress. Pfkelch13 mutant parasites seem to experience less stress when they are exposed to dihydroartemisinin. Also, it was suggested that partial loss of function of Pfkelch13 decreases DHA sensitivity. Mislocalization of Pfkelch13 apparently leads to cytostome dysfunction, suggesting the important role of Pfkelch13 in the cytostome. Then, she moved to a recent discovery of nucleoside sulfamates that kill P. falciparum in vitro. ML901, an exemplar of nucleoside sulfamate, found to inhibit tyrosine-tRNA synthetase (PfTyrRS), via a reaction-hijacking mechanism. Several assays were used to confirm that PfTyrRS is a target of ML901. Promisingly, ML901 kills P. falciparum in vivo but needs further optimization to improve oral bioavailability. This fascinating study might lead to other discoveries of compounds targeting aminoacyl-tRNA synthetases via the novel reaction-hijacking mechanism.

Colin J. Sutherland (London School of Hygiene and Tropical Medicine, United Kingdom) highlighted a result from field studies of patients with repeat malaria episodes over 2 years, suggesting a slow parasite clearance. Importantly, artemether-lumefantrine failure is not associated with Pfkelch13 mutations, suggesting other players are influencing ACTs treatment. He suggests that it is currently important to find alternative treatment strategies, for example, extend the curative duration of artemisinin and its derivatives to ensure complete parasite clearance. To monitor artemisinin resistance, we need molecular diagnosis for following up patients as it is more sensitive and gives more information than microscope smears.

Pierre Buffet (Inserm, France) showed that the spleen is a great clearer of infected red blood cells. A recent study showed that there was a massive accumulation of parasitized red blood cells in the liver, however, massive macro-phagocytic activity is also there to reduce the burden of hepatic infection. His team also developed a spleen-on-a-plate for screening drugs to block malaria transmission. This involved stiffening mature gametocytes with drugs and microsphiltrating them. Several safe compounds such as TD6450 and Cipargamin have been found to kill gametocytes at nano molar range or induce retention, encouraging clinical trials.

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Day 3: 13th April 2022

Session 5 - Reducing the Burden of Malaria Part I

Thierry T. Diagana (Novartis Institute for Tropical Diseases, USA) started by presenting the wide range of Novartis drug candidates for malaria elimination (lumefantrine, KAF156 (ganaplacide), KAE609, INE963 among others). Their three pillars are to meet current demands, address anticipated future needs and early access to drugs for a malaria free world. They are acting in the second major era (2020-2030) to improve existing therapies and address the threat of drug resistance. NITD aims to deliver novel combinations therapies for severe and uncomplicated malaria, chemoprevention, single-dose MDA and *P. vivax* radical cure to target numerous unmet needs for control and elimination. Diagana pointed out one of the past challenges of poor understanding of hypnozoite's biology which hindered progress of radical cure drug discovery, however with tremendous efforts over the past decade now we have robust in vitro culture systems, animal models and transcriptomic data to understand the biology. Their ongoing experiments are focused on phenotypic screening. Diagana reported promising results from a Phase IIb study for novel non-artemisinin-based combination therapy to treat uncomplicated malaria: Ganaplacide/lumefantrine combination was efficacious in children less than 12 years old. Diagana also mentioned profile QSAR (PQSAR), which is a machine learning-based method for virtual screening of drugs. Using an artificial intelligence approach, they can distinguish three distinct mechanisms of action (MOA) by phenotype.

Abdoulaye Djimde (University of science, Techniques and Technologies, Mali) presented genomics studies as a novel tool to reduce the burden of malaria in Africa. Djimde spoke about the missions of Plasmodium Diversity Network Africa (PDNA) to build capacities of African scientists and use knowledge from malaria parasite genetic diversity to inform malaria elimination policies in sub-Saharan Africa. Among other things, PDNA helps with regular monitoring of molecular markers involved in parasite drug resistance (PfK13). A report many years ago showed that Southeast Asian parasites were diverse whereas African parasites were closely related in a PCA analyses. However, diving deeper into the data shows that in fact there is high genetic diversity of *P. falciparum* African parasites. Keeping this parasite diversity in mind, accordingly there should be a diversification of treatment strategies depending on each locale. Djimde presented malaria sub-national stratification in Mali. The complexity and significant differences in the risk of malaria infection in different locations of southern Mali suggests the need of district tailored interventions. He also highlighted the use of genetic epidemiology by the national malaria control program in the region for control and elimination of malaria. In addition, Djimde mentioned that parasites with HRP2/3 deletion will lead to a false negative test using rapid diagnostic tests (RDT), which is widely used. Many places do not have a constant supply of electricity, microscopists, or microscopes, so routine diagnosis by microscopy cannot be achieved. Other cheap diagnostic tools need to be invented and distributed. Djimde ended his communication by highlighting the need to translate genomics knowledge into policy decisions.

Janice Culpepper (Bill and Melinda Gates Foundation, USA) started her talk by informing about the shift in the Foundation's malaria elimination strategy (2019 onward) now referred to as the "pathway to eradication" strategy. The Bill and Melinda Gates Foundation's strategy covers targeting high burden areas referred as "Eradication backbone", optimizing the use of existing tools and supporting the development of new tools required for elimination. Culpepper further illustrated the benefit of single exposure radical cure (SERC) which not only includes effectiveness, but also simplifies case management, and improves compliance. The next generation of drug combinations will be focused on

delivering a SERC. Currently, ivermectin is used as an endectocide to reduce and eliminate residual transmission. However, modeling suggests that a short-course ivermectin is unlikely to be an ideal elimination tool. Therefore, long-acting endectocides are needed to reduce and eliminate residual transmission, while mass drug administration will extend the duration of parasite reduction in the population. Culpepper also highlighted the importance of Chemoprevention. Culpepper mentioned that new strategies including novel and repurposed drugs, long-acting injectable drugs for seasonal prevention, and monoclonal antibodies are needed. Besides these existing tools, robust surveillance systems are needed.

Beatriz Baragana (University of Dundee, UK) and her group identified *Plasmodium falciparum* acetyl-CoA synthetase (*Pf*AcAS), as a target of MMV019721, a multistage antimalarial, by in vitro selection followed by whole genome sequencing; resistant parasites had mutations in PfAcAS that line the predicted active site of the enzyme. This was confirmed by showing that PfAcAs WT parasites genetically edited to express A597V or T648M yielded resistance to MMV019721 similar to that seen in the selected resistant lines. In addition, conditional knockdown of PfAcAS sensitized parasites to MMV019721, demonstrating that this protein is necessary for resistance to the compound. Using recombinant WT or mutant PfAcAS, they found linear non-competitive inhibition for the compound vs ATP, and linear competitive inhibition against CoA. Thus, MMV019721 inhibits CoA from binding to the enzyme, resulting in the inability of PfAcAS to catalyze formation of acetyl coA. They were able to reproduce co-crystallisation with the MMV-019721 analogue using ethyl-AMP and are still working on obtaining a full length Cryo EM PfAcAS structure. Meanwhile, they have started working on MMV-019721 optimization to increase the potency, solubility and metabolic stability without the structural information and the outcome suggests that this compound is ready to enter lead optimization (pharmacokinetics and potency studies).

Lauren B. Arendse (University of Cape Town, South Africa) shared her current work on *P. falciparum* kinases as antimalarial targets. Set of 84 human kinase inhibitors were screened against asexual blood stage parasite and kinobead study. Also, her team and colleagues used chemogenomics to explore more potential kinase inhibitors. ADP-Glo assay was used for validation. One compound showed polypharmacology and inhibited two *P. falciparum*kinases: *Pf*PI4K and *Pf*PKG. These kinases were found essential across the parasite life cycle, and therefore suitable for antimalarial targets. For instance, MMV030084 was found to inhibit liver stage, asexual blood stage, and male gametocyte of *P. falciparum*. Arendse proposed that polypharmacology, where several plasmodium kinases are inhibited by the same inhibitor, could be a potential opportunity in antimalarial development.

Christian Doerig (RMIT University, Australia) presented a new aspect of anti-infectives. Prof. Doerig and his team focuses on host-directed therapy for malaria, because this approach will reduce the ability of parasites to mutate to acquire drug resistance. In this approach, treatment of infectious diseases is focused on host cell factors required for parasites. Doerig's team employed a Kinexus antibody microarray for exploring host-cell phospho signalling responses to infections. This method has been validated in many infectious agents including Hepatitis C. His team then applied the same method to study the signalling response in malaria-infected erythrocytes, leading to identification of human MEK1, c-MET and B-Raf that are targeted by inhibitors. They were unable to raise resistant lines from such inhibitors, suggesting refractoriness to resistance, which is a desirable trait in antimalarial drugs. The future work is to elucidate the mechanism of actions of the characterized inhibitors.

Stuart A. Ralph (The University of Melbourne, Australia) presented work on apicoplast inhibitors and decreased hemoglobin digestion. Apicoplast inhibitors such as the antibiotics clindamycin, doxycycline, indolmycin, etc. cause delayed death in parasites due to isopentenyl pyrophosphate (IPP) deprivation. IPP is used in protein prenylation, which are on proteins that interact with membrane

proteins or vesicles. Using 3D Block-face SEM, Ralph's group found that indolmycin treatment leads to cytostomal invaginations that are abnormally long and winding, and disrupted digestive vacuoles (DV); this effect can be rescued by geranyl geranyl alcohol (GGOH). There is also a decrease in hemoglobin-derived peptides as measured by metabolomics. The working hypothesis is that antibiotics lead to defects in rab-based trafficking of the cytosome to the DV. K13 forms a ring/"collar" around cytostomes. In K13 knock sideways experiments (in which K13 is mislocalized), the cytostome collar is opened up. Treatment with doxycycline or clindamycin results in opening up of the collar similar to when K13 is mislocalized, as seen by microscopy. Hemoglobin metabolism is also similarly impaired when treated with these antibiotics or when K13 is mislocalized, as detected by mass spectrometry studies. Drug interaction analysis revealed antagonistic interaction between dihydroartemisinin and delayed death drugs (clindamycin, and doxycycline). It is suggested that this is because antibiotics reduce hemoglobin uptake due to the inability to traffic from the cytostome to the DV and thus there is lower amount of free heme to activate artemisinin.

Session 6: Reducing the burden of Malaria Part II

Philip Rosenthal (University of California, USA) gave an overview of key antimalarial drugs deployed in Africa for treatment (artemisinin-based combination therapy - ACT) and for prevention (sulfadoxine-pyrimethamine - SP). To look for antimalarial drug resistance, they performed clinical trials of antimalarial drug efficacy, *in vitro* drug sensitivity of fresh parasite isolates and resistance surveillance. Recent treatment efficacy of ACTs in Uganda highlighted efficacies of artemisinin-lumefantrine (AL), artesunate-amodiaquine (AS/AQ) with an improved efficacy of AS/AQ compared to AL. Ex-vivo drug susceptibility revealed a change in drug sensitivity in Uganda and transporter genotypes. He also reported evidence for artemisinin resistance in Rwanda and Uganda with K13 mutations. Regarding the decreasing resistance to aminoquinolines and increasing resistance to SP, he calls for a continued surveillance of change in drug sensitivity.

Elizabeth A. Ashley (Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Laos) talked about the increasing artemisinin resistance in Asia and Africa and the need to move towards new combinations treatments through triple artemisinin-based combinations or TACTs. This approach showed clinical relevance when treating uncomplicated *Plasmodium falciparum* malaria. It is presumed that a new non-artemisinin containing treatment will be available in 2027 talking about ganaplacide-lumefantrine as many challenges are rising. Among others, reluctance to adopt TACTs by most countries without evidence of efficacy in local context, huge increase in cost of drugs compared to the status quo. Many TACTs are under evaluation and implementation considerations such as safety and tolerability concerns and market positioning need to be addressed. TACTs are an option to integrate with other strategies to delay emergence of resistance to artemisinin or partner drugs in countries where it has not been reported.

Ric Price (Menzies School of Health Research, Australia) talked about novel approaches for the safe and effective radical cure of *P. vivax,* where the highest burden is found in children and in remote areas. Other challenges to the radical cure of *P. vivax* malaria include poor sensitivity of parasite diagnostics, variable risk of relapse, safety concerns, poor adherence, and lack of sustained financing. He then identified new tools and strategies for tackling *P. vivax* malaria including areas such as parasite diagnostics, treatment regimens, G6PD diagnostics, adherence to drug regimen and safety of drugs. Diagnosis of *P. vivax* requires experienced, trained microscopists and specific rapid diagnostic test (RDTs) kits. Primaquine (PQ) and Tafenoquine (TQ) are the main anti-hypnozoite drugs used for the treatment of *P. vivax* malaria. The combination of these drugs with/in ACTs show that PQ works better, and that high doses of PQ is a more efficient treatment. He also stated that the commonest cause of treatment failure is underdosage and poor adherence on the part of both patients and prescribers. Some patients don't complete the 14-day regimen for an acute febrile illness leading to relapse. Thus,

malaria surveillance and proper prescriptions are required. Finally, he mentioned G6PD deficiency and its associated haemolytic risk; and listed quantitative G6PD testing aside qualitative G6PD testing as a new alternative though costly.

Marielle Bouyou Akotet (University of Health Science, Gabon) started by stating the mission of Malaria National Control Program (MNCP) which is to provide the best interventions for the prevention and treatment of malaria with the view of eliminating malaria through co-ordinated efforts of several bodies and partners. She presented data on the trend of malaria cases and malaria related deaths nationwide and in Africa. She emphasized that there hasn't been any change in malaria prevalence and death during the period from 2015 – 2020. She mentioned the goals and some interventions already in place to control malaria such as the distribution of insecticide treated nets (ITNs), indoor residual spraying (IRS), and Sulfadoxine-pyrimethamine (IPTP-SP) coverage in some key malaria transmission sites. She attributed causes to the challenges confronting them to include patient behaviors such as not seeking care and self-medication with anti-malarial drugs, quality of case management, poor diagnosis, little or no data, poor access to remote sites, and involvement of different stakeholders. She then recommended the setting up of sentinel sites for malaria survey.

Lauriane Sollelis (University of Glasgow, UK) started her talk by mentioning that parasite adaptability enables survival and that there is natural selection for variation in sexual conversion rates across parasite populations and time. Sollelis and her team sought to find the genetic determinants that select for parasite transmission, and which may facilitate the spread of drug resistance in *Plasmodium falciparum*. Using a genome wide association study, they measured the expression of an essential gametocytogenesis gene, a*p2-g*, across tracking resistance to artemisinin collaboration (TRAC-1) samples. Their studies found two lncRNAs, Ben and Glen, that are genomic determinants of stage conversion in *P. falciparum*; and that the geographical distribution of high/low *ap2-g* expression alleles are associated with low and high transmission areas. Lastly, she showed that the spread of artemisinin resistance is associated with the presence of high *ap2-g* allele expression.

Meeting Wrap-up: Outcomes and Future Directions

After the last talk of session 6, the Chairperson, **Collin Sutherland** handed over to **Elizabeth Ashley**, one of the organizers of the symposium. She gave the vote of thanks and thanked everyone including the organizers, attendees both in person and virtual, the various enlightened speakers and poster presenters who shared their work and insights, as well as the sponsors of the event. **David A. Fidock** gave the closing remarks but not without appreciating his fellow organizers and the Keystone Organization who played a key role in the event. Finally, he asked attendants to fill the online survey as the feedback from the survey would help in decision making and policies for future events.

This report is brought to you by the MESA Correspondents Patricia Doumbe Belisse, Nutpakal Ketprasit and Samuel Blankson. Senior editorial support has been facilitated by the Chairs and Speakers of the session.

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