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ABSTRACTS

1 - ORAL PRESENTATIONS

OP-01

Towards malaria elimination: a randomized trial of high-dose ivermectin (IVERMAL)

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Ivermectin, an antiparasitic drug used for onchocerciasis and lymphatic filariasis control at single doses of 150-200 mcg/kg, also kills malaria mosquitoes feeding on recently treated individuals. However, the effect is short-lived (< 7 days). Ivermectin has a wide therapeutic index and doses of 2,000 mcg/kg are well tolerated. High-dose ivermectin could generate longer effect-durations required for malaria elimination. We conducted a randomized, double-blind, placebo-controlled trial comparing the safety, tolerability, and efficacy of 3 days ivermectin 0, 300, or 600 mcg/kg/day, co-administered with dihydroartemisinin-piperaquine, in randomly assigned (1:1:1) adults with uncomplicated malaria in Kenya. Patients' blood taken on post-treatment days 0, 2+4h (C_{max}), 7, 10, 14, 21, and 28, was fed to laboratory-reared *Anopheles gambiae s.s.;* mosquito survival was assessed daily for 28-days post-feeding. The effect of ivermectin on malaria transmission was modelled. Between July 2015 and May 2016, 141 patients were randomized. Compared to placebo, ivermectin was associated with higher 14-day-post-feeding mosquito mortality when fed on blood taken 7 days post-treatment (primary outcome) (600 mcg/kg/day: RR 2.26, 95% CI 1.93-2.65; HR 6.32 95% CI 4.61-8.67; 300 mcg/kg/day: RR 2.18, 95% CI 1.86-2.57, HR 4.21, 95% CI 3.06-5.79, $p < 0.001$ for all results). Mosquito mortality remained significantly increased 28 days post-treatment. High-dose ivermectin was well tolerated. Modelling predicted that adding high-dose ivermectin to mass drug administration with dihydroartemisinin-piperaquine enhances malaria prevalence reduction by 56%-61%. High-dose ivermectin is well tolerated and reduces mosquito survival for at least 28 days post-treatment, making it a promising new tool for malaria elimination.

OP-02

Antibodies to Pfs355, a novel early gametocyte protein, predict decreased *Plasmodium falciparum* gametocyte density in humans

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Anti-gametocyte specific immune responses may play an important role in regulating *Plasmodium falciparum* gametocyte density and transmission efficiency. This hypothesis provides the rationale for the pursuit of transmission blocking vaccines (TBVs) that target gametocytes in the human host. To identify novel anti-gametocyte TBV candidate antigens, we have interrogated the *P. falciparum* gametocyte stage proteome with our whole proteome differential screening method using sera and epidemiologic data previously collected from a treatment-reinfection study conducted in a holoendemic area of western Kenya. At the start of the high transmission season, we enrolled n=143 males aged 12-35 years, treated them with quinine and doxycycline, obtained a peripheral venous blood sample, and followed them with weekly blood films for 18 weeks. Using sera pooled from individuals with low gametocyte carriage as assessed on the weekly blood films versus sera pooled from individuals with high gametocyte carriage, we screened a *P. falciparum* gametocyte stage cDNA expression library. We identified eight parasite genes uniquely recognized by gametocyte-resistant but not by gametocyte-susceptible individuals. In generalized

estimating equation models, antibodies measured at the start of the high transmission season to one of these novel antigens, which we annotate as Pfs355, predict significantly lower gametocytemia measured over the 18-week transmission season (P = 0.021). When analyzed dichotomously, anti-Pfs355 responders have 31% lower gametocyte density compared to non-responders (P = 0.04) after controlling for potential confounders. In immunofluorescence assays, Pfs355 localizes to the erythrocyte membrane in early ring stage gametocytes, and to gametocyte specific structures in other gametocyte developmental stages. To further evaluate Pfs355 as a TBV candidate, we are currently examining the potential of anti-Pfs355 antibodies to arrest gametocyte development *in vitro*.

OP-03

Zoonotic malaria transmission in Rio de Janeiro Atlantic Forest

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Autochthonous malaria cases have been increasing in the Atlantic Forest (AF) coastal region of Brazil (Rio de Janeiro state). The cases have been diagnosed as *Plasmodium vivax*, based on the morphology of the parasites. As the *P.vivax-like* non-human primate malaria parasite species *Plasmodium simium* is locally enzootic, we performed a molecular epidemiological investigation to determine whether zoonotic malaria transmission was occurring. Blood samples of humans from 2015-16 presenting malaria as well as from local howler monkeys were examined. Additionally, sequencing of the parasite mitochondrial genome was applied. Of 28 human samples and three monkey's samples for which the parasite mitochondrial genome sequencing was successfully performed, all were found to contain the two single-nucleotide polymorphisms considered diagnostic for *P.simium*. Three additional human parasite isolates had successful full-length mitochondrial DNA sequencing done, with identical alignment to a *P.simium* reference monkey strain, and specificity verified against a large number of homologous *P.vivax* reference sequences from around the world. This study shows that these parasites are *P.simium*, a closely related parasite species whose natural hosts are non-human primates (NHP) native to the AF. Some, if not most, of the autochthonous cases previously diagnosed as *P.vivax* in the AF region are likely to have been *P.simium* acquired via mosquitoes infected from monkeys, thereby making this part of Brazil the site of a second global focus of zoonotic malaria. Thorough screening of the local NHP and anophelines is required to evaluate the extent of this newly recognized zoonotic threat to public health and malaria elimination in Brazil. This situation has immediate implications for public health in this region, and further and more profound consequences for the control and eventual elimination of malaria in Brazil.

OP-04

Repeat region of the circumsporozoite protein has a functional role in sporozoite motility

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The sporozoite stage of the malaria parasite, *Plasmodium*, utilizes a substrate based form of motility, gliding motility, to infect a mammalian host. Motility is critical at multiple stages of the infection process, but much of the molecular mechanisms involved are unknown. Circumsporozoite protein (CSP) is the major surface protein of sporozoites, completely coating its surface. The overall structure of CSP is conserved among all *Plasmodium* species and includes a central tandem-repeat flanked by an N-terminal domain and a C-terminal adhesion domain. Previous studies have elucidated the roles of the flanking domains but little is known about repeat region structure and function. We discovered that the repeat region is essential for parasite migration in the mosquito and mammalian hosts. As motility is required for this migration, we probed the role of the CSP repeat region in sporozoite motility utilizing *in vitro* assays. Gliding mutant parasites move at similar speeds as wildtype, however, a higher proportion of parasites are unable to engage in normal gliding motility and instead are found exhibiting abnormal gliding morphologies. These motility defects impact their infectivity *in vivo*, likely because they cannot traverse cells or enter the hepatocytes. Our data suggests that the repeat region has structural constraints and functional properties not previously appreciated and provides new insights into the sporozoite motility machinery.

OP-05

Complement-mediated inhibition of *Plasmodium falciparum* sporozoite infectivity by antibodies induced after chemoprophylaxis and sporozoite immunization in the controlled human malaria infection model

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Long-lasting and sterile protection against *Plasmodium falciparum* malaria can be achieved by exposure of malaria-naive volunteers under chemoprophylaxis to NF54-infected mosquitoes (CPS-immunization). While CPS-induced antibodies neutralize sporozoite infectivity *in vitro* and *in vivo*, antibody-mediated effector mechanisms are still poorly understood. Here, it was investigated for the first time whether the complement system contributes to specific antibody-mediated inhibition of homologous NF54 and heterologous NF135.C10 sporozoite infectivity. To this end, heat-inactivated sera collected before and after completed CPS-immunization in the presence of complement (untreated or heat-inactivated) were assessed for recognition of sporozoites, complement fixation, sporozoite lysis, and reduction of *in vitro* sporozoite infectivity in human hepatocyte traversal and invasion assays. CPS-immunization induced sporozoite-specific IgG ($p=0.001$) and IgM antibodies ($p<0.0001$). Complement fixation ($p<0.0001$), sporozoite lysis ($p=0.017$), antibody-mediated traversal ($p<0.0001$) and invasion inhibition ($p<0.0001$) by CPS-induced antibodies were significantly enhanced in the presence of complement. Complement-mediated invasion inhibition negatively correlated with cumulative parasitaemia ($p=0.013$). While IgG antibodies similarly recognized NF54 and NF135.C10 sporozoites, IgM binding to NF135.C10 sporozoites was reduced ($p=0.023$). Complement fixation, lysis or traversal inhibition of either NF54 or NF135.C10 sporozoites by CPS-induced antibodies did not differ, however, NF135.C10 invasion was more strongly inhibited in the presence of complement ($p=0.008$). Taken together, CPS-induced antibodies interact with the complement system, with complement further reducing homologous and heterologous sporozoite infectivity *in vitro*. The combined data highlight the importance of the complement pathway as a complementary immune effector mechanism in pre-erythrocytic immunity after whole-parasite immunization against *Plasmodium falciparum* malaria.

OP-06

Longitudinal clinical and molecular analysis of asymptomatic *Plasmodium falciparum* infection in Malawi

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In Malawi, asymptomatic *P. falciparum* infections are common and may drive transmission. The frequency, persistence, and clinical outcome of asymptomatic infections is unknown. Although school aged children (SAC) carry the majority of prevalent infections, we do not know if this is due to increased exposure to infection or a prolonged duration of asymptomatic infections. We do not know if asymptomatic infections progress to clinical disease and eventually prompt treatment. We characterize the age-specific dynamics of asymptomatic infections in a high-transmission setting and examine the association between asymptomatic infection and clinical disease. 120 participants with uncomplicated malaria (treated with artemether-lumefantrine) were enrolled in our longitudinal study. Subjects were one through 50 years of age. Participants were followed monthly for up to two years. Samples from all visits were tested for parasites (microscopy and qPCR). Genotyping with *msp* was used to identify heterogeneity of infecting parasites. Molecular force of infection was defined as the number of unique infecting genotypes/person/year. In the first half of the study, 1702 person-months of follow up time were analyzed. Asymptomatic infections were detected in 23% of visits. Asymptomatic infection was associated with increased time to next clinical malaria (HR 0.45, $p < 0.001$) in all ages. Overall, 785 incident infections were detected; 35% at a visit when no symptoms were reported. SAC are a distinct risk group, with a significantly higher molecular force of infection (IRR 2.4, $p < 0.001$) than other groups. Asymptomatic infection persisted up to 16-months. The mean duration of persistence of individual infections will be calculated by age.

Clinical malaria was more likely to be due to newly acquired infection (OR 4.6, 95%CI 2.5-8.5) than a persistent infection. Asymptomatic infections constitute a significant reservoir of *P. falciparum* in Malawi but clearance of asymptomatic infections may not lead to a decrease in clinical malaria.

OP-07

Protective anti-sporozoite antibodies inhibit motility of sporozoites in the skin

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Malaria infection begins with sporozoites deposited in the skin by infected mosquitoes. These injected sporozoites move in the skin to reach the blood vessels and enter the blood circulation that takes them to the liver where they invade hepatocytes and develop into exoerythrocytic stages. Using confocal microscopy to visualize fluorescent sporozoites injected in the skin we investigated the effect of antibodies on sporozoite motility. In normal sporozoite immunized mice, we observed a clear defect in parasite motility particularly affecting the speed and net displacement of parasites. In contrast, when using immunized B-cell KO mice this effect in sporozoites is not apparent, indicating that the anti-parasite effect is antibody dependent. Passive transfer of anti-CSP monoclonal antibodies recapitulate the effects observed in immunized mice and allowed us to further characterize the antibody effect on sporozoites. We found that in the presence of circulating antibodies a high percentage of parasites become non-motile. Among motile parasites they display a severely reduced speed and traveled shorter distances compared to parasites inoculated in naïve mice. We determined that these effects are dose and time dependent. Importantly, we observed that in the presence of antibodies there is a severe impairment of sporozoite migration and entry into blood vessels, thus indicating that the effect of antibodies in the skin represent the earliest manifestations of protective immunity against malaria sporozoites.

OP-08

The role of Exp2 in small molecule transport across the parasitophorous vacuole membrane of *Plasmodium falciparum*

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During red blood cell invasion, *Plasmodium falciparum* (Pf) surrounds itself with the parasitophorous vacuole membrane (PVM). While it is known that protein transport across the PVM is mediated by the *Plasmodium* translocon of exported proteins (PTEX), a previously described pore allowing small molecule (i.e. nutrient) permeation remains unidentified. Here we test the hypothesis that the putative pore-forming component of PTEX, EXP2, has an unprecedented second role in facilitating small molecule transport. Vacuole permeability was characterized using on-cell patch clamp of osmotically extruded parasites that remain in their vacuole. To correlate EXP2 expression with permeability, we used conditional EXP2 knockdown and overexpression lines and recorded the probability of finding the channel in the patch clamped vacuole. We found that the probability scaled with EXP2 content, implicating EXP2 in channel formation. Contribution of the PTEX complex to the formation of the pore was tested by interference with a different PTEX component, HSP101. We find that HSP101 interference does not reduce the probability of finding a channel. To directly implicate EXP2 with the pore, we truncated EXP2's highly charged C-terminal end, as this is likely to reduce the pores response to voltage, if EXP2 is part of the pore. Indeed, we find that gating of the truncated channel is significantly altered in a region around 40 mV. The data implicates EXP2 directly in small molecule transport across the PVM. We conclude that EXP2 itself is most likely the PVM pore. It remains to be shown if EXP2 is complexed with other proteins to form the ion permeation pathway.

OP-09

Single-cell RNA-sequencing reveals a transcriptional signature of sexual commitment in malaria parasites

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Plasmodium falciparum parasites have to balance continuous

asexual replication within red blood cells with differentiation into non-replicating sexual stages, called gametocytes. Commitment to either fate is already determined during the preceding cell cycle that begins with invasion by a single, asexually-committed merozoite and ends, 48-hours later, with a schizont releasing newly formed merozoites, all of which are committed to either continued asexual replication or differentiation into gametocytes. Sexual commitment requires activation of *ap2-g*, the transcriptional master regulator of sexual development, from its epigenetically silenced state during asexual replication. Expression of AP2-G during this "commitment cycle" poises gene expression in nascent merozoites to initiate sexual development through a hitherto unknown mechanism. In order to maintain a persistent infection, *ap2-g* expression is limited to a sub-population of parasites (1-30%, depending on genetic background and growth conditions). As sexually-committed schizonts comprise only a sub-population and are morphologically indistinguishable from their asexually-committed counterparts, defining their characteristic gene expression has been difficult using traditional, bulk transcriptome profiling. To determine the transcriptional changes induced by AP2-G within this sub- population, we applied highly-parallel, single-cell RNA sequencing (scRNA-seq) to malaria cultures undergoing sexual commitment. In this first application of scRNA-seq to eukaryotic pathogens, we surveyed over 19,000 single parasite transcriptomes from a conditional AP2-G knockdown (AP2-G-DD) line and NF54 wildtype parasites in multiple stages of development and found that sexually committed, AP2-G+ mature schizonts specifically up-regulate additional regulators of gene expression, including AP2 transcription factors, histone modifying enzymes, and regulators of nucleosome positioning. These epigenetic regulators likely act to poise the expression of genes necessary for initiation of gametocyte development in the subsequent cell cycle.

OP-10

Triple Artemisinin-based Combination Therapies (TACTs) for the treatment of uncomplicated falciparum malaria

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The spread of artemisinin (ART) resistance, and subsequent ACT partner drug resistance, threatens malaria control in the Greater Mekong Subregion (GMS) and beyond. The efficacies of DHA-piperaquine and artesunate-mefloquine have declined dramatically in the GMS. The spread of multidrug-resistant *P. falciparum* to Africa, where most of the world's malaria transmission, morbidity, and mortality occur, would be disastrous. Since new drugs are at least 5 years away, there is an urgent need to evaluate alternative treatments using existing drugs. A promising novel approach is the use of Triple ACTs (TACTs), which combine a short-acting artemisinin with two longer-acting partner drugs. TACTs can exploit fortuitous inverse relationships between susceptibility to paired partner drugs, such as amodiaquine and lumefantrine, or piperaquine and mefloquine. A large multinational study, the "Tracking Resistance to Artemisinin Collaboration II" (TRAC II) was initiated to map the current spread of resistance and assess the efficacy and safety of TACTs in 18 hospitals in 8 countries in Asia and Africa. In this presentation, we will present the near-final results of this large-scale initiative.

OP-11

The relative contribution of symptomatic and asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections to the infectious reservoir in a low-endemic setting in Ethiopia

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The majority of *P. vivax* and *P. falciparum* infections in low-endemic settings are asymptomatic but may be infectious to mosquitoes. The relative contribution to the infectious reservoir of these asymptomatic infections, that are often of

low parasite densities, compared to clinical malaria cases is currently unknown but important for malaria elimination scenarios. We assessed the infectivity of self-presenting symptomatic malaria (41) and microscopy (41) and PCR-detected (82) asymptomatic malaria-infected individuals using membrane feeding assays with locally maintained *Anopheles arabiensis* mosquitoes in Adama, Ethiopia. Malaria incidence and prevalence data allowed estimating their contribution to the infectious reservoir for malaria. Overall, 32.9%(28/85) and 14.0%(8/57) of *P. vivax* and *P. falciparum* infected individuals infected ≥1 mosquitoes, respectively. Mosquito infection rates for *P. vivax* were strongly associated with asexual parasite density ($\rho = 0.63$; $P < .001$) whilst the same was not observed for *P. falciparum. P. vivax* symptomatic individuals were more infectious to mosquitoes (infecting 43.9% mosquitoes, 307/699) compared to asymptomatic microscopy-confirmed (infecting 10.0% mosquitoes, 70/699; $P = .0052$) and PCR-confirmed malaria-infected individuals (infecting 0.8% mosquitoes, 6/763; $P < .001$). As a consequence of their prevalence in the population, asymptomatic microscopy-confirmed and asymptomatic PCR-confirmed malaria-infected individuals were responsible for 63% and 30% of the infectious reservoir for *P. vivax*. For *P. falciparum* the majority of mosquito infections were from asymptomatically infected individuals. In low-endemic settings that aim for malaria elimination, asymptomatic infections are highly prevalent and responsible for the majority of mosquito infections. Early diagnosis of infections to prevent asymptomatic parasite carriage may thus cut many onwards infectious events.

OP-12

Neurogenetic characterization of *Anopheles gambiae* mosquito repellents

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Anopheles mosquitoes, as vectors for malaria parasites, are the most dangerous animals to humans. Malaria alone caused 438000 deaths in 2015. Many mosquito-borne diseases have no successful vaccine or treatment. In addition to population control methods, the prevention of mosquito bites could be a particularly successful strategy to combat these diseases. Mosquitoes depend on their sense of smell (olfaction), in combination with other senses, to seek a host. Therefore, a mosquito's sense of smell is an ideal target to prevent them from biting humans. A special class of odorants (called spatial repellents) can actually prevent mosquitoes from approaching a host. These spatial repellents can thus be effective treatments to prevent mosquito biting. Surprisingly, the mode of action for most repellents is unknown. In addition, many of these repellents require high odorant concentrations (*i.e.,* DEET, IR3535, picaridin), dissolve plastics (DEET), and might pose health risks to humans. It is therefore vital to characterize how repellents work as a rationale approach to find new repellent alternatives. To reach this goal, we generated transgenic *Anopheles gambiae* mosquitoes in which a majority of their olfactory neurons fluoresce more brightly when activated by odors (genotype: *Orco-QF2, QUAS-GCaMP6f*). We used these new strains in antennal imaging experiments in order to understand how currently used repellents affect the mosquito olfactory system. We found two categories of repellents: 1) "activators": those that activate a subset of olfactory receptors (e.g. Lemongrass oil and Eugenol), and 2) "maskers": those that do not directly activate receptors but mask (or decrease) the response to other odorants (e.g. DEET, IR3535, and Picaridin). Next, we utilized a heterologous *Drosophila* system to identify which *Anopheles gambiae* Odorant Receptors are stimulated by "activator" repellents. Future efforts aimed at screening natural odorants for activation of these spatial repellent receptors could identify effective alternatives to currently used repellents.

2 - POSTER SESSION A

A-01

Effect of naturally-occurring *Wolbachia* in mosquitoes of the *Anopheles gambiae* complex from Mali on Plasmodium falciparum malaria transmission

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Wolbachia introduction into natural culicine populations is one of the most promising strategies to halt dengue transmission in endemic areas. Wolbachia has also been suggested to reduce Plasmodium infections in anophelines, but Anopheles mosquitoes have been thought to not harbor stable Wolbachia infections. However, a population of Wolbachia-infected Anopheles gambiae mosquitoes in Burkina Faso was recently reported. Here, we report the

identification of a new strain of Wolbachia (wAnga-Mali) circulating in A. gambiae and A. coluzzii mosquitoes from Mali. Sequencing of 16S rRNA suggested that wAnga-Mali is a member of Wolbachia supergroup A and that it carries sequence divergence from the Wolbachia strains identified in Burkina Faso. Using field collected mosquitoes, we observed that Plasmodium prevalence and intensity was reduced in mosquitoes carrying Wolbachia. To further study the impact of Wolbachia on Plasmodium transmission, we established a colony of Wolbachia-infected A. coluzzii. Following infection of mosquitoes with P. falciparum (NF54 strain), we observed a slight, but statistically significant, increase of oocyst infections in Wolbachia-infected mosquitoes. However, Wolbachia infection significantly reduced the prevalence and intensity of sporozoite infections. Together, our study shows that Wolbachia reduces sporozoite levels in the mosquito opening the possibility of exploring Wolbachia infections as a strategy to reduce malaria transmission.

A-02

A mosquito associated *Chromobacterium* causes lethality in *Anopheles gambiae* larvae through production of hydrogen cyanide

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Vector control strategies are an important component of preventing transmission of *Plasmodium* parasites and other vector borne pathogens. One potential source of novel biocontrol agents are bacteria with natural insecticidal properties. Previously, we have shown that *Chromobacterium species Panama* (*Csp P*), a bacterium found in association with *Aedes* mosquitoes in Panama, has insecticidal properties against *Anopheles gambiae* mosquitoes at multiple developmental stages. In the current work, we dissected the mechanism by which *Csp P* causes larval mortality when present in larval breeding water. We determined that *Csp P* produces a larvicidal factor that accumulates in the growth media and breeding water and that this factor persists after removal of live bacteria via filtration. Further, we determined that the larvicidal factor is less than 3kDa in size and is heat-stable and volatile. The genome sequence of *Csp P* revealed that the bacterium's genome encodes a hydrogen cyanide (HCN) synthase cassette, as is the case for other species of *Chromobacterium* and *Pseudomonas*. We then determined that hydrogen cyanide is produced by *Csp P* in culture, increasing during log phase of bacterial growth and peaking near the end of log phase. We also found that *Csp P* produces hydrogen cyanide when present in larval rearing water, at concentrations sufficient to kill larvae, and that all hydrogen cyanide evaporates by 48 hours after addition of bacteria. Lastly, by treating larval breeding water with the cyanide antidote hydroxocobalamin, we successfully eliminated larval mortality caused by *Csp P.* Taken together, these data strongly suggest that the larvicidal activity of *Csp P* is caused by production of hydrogen cyanide in the larval breeding water.

A-03

Exploring melanin-based *Anopheles gambiae* immune response to malaria parasite

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Malaria parasite transmission is dependent on the survival of *Plasmodium* during its cycle in the mosquito. The major bottleneck for parasite development occurs during traversal of the midgut epithelium, where it is exposed to human-blood derived factors, mosquito immune defenses, and gut microbiota. Indeed, melanin-based cellular immune response and midgut microbiota are responsible for the refractoriness of certain mosquito strains to *Plasmodium* infection. Several studies have suggested that parasite killing precedes the melanization step; nonetheless, molecular mechanisms that govern these processes are poorly understood. We hypothesize that key mosquito factors that associate with the parasite to mediate killing and melanization will be trapped in the melanotic capsules. Thus, *An. gambiae* L3-5 refractory line was infected with *P. berghei* to enrich the melanotic capsules for proteomics. Melanotic capsules were successfully recovered using a protocol for fungal melanin isolation. We identified a subset of *Anopheles* proteins associated with the melanin matrix by performing a pilot proteomic analysis using a filter-aided sample preparation (FASP) procedure followed by tandem MS/MS. In parallel, a confrontational assay between *Cryptococcus neoformans* (fungal biosensor) and mosquito midgut-derived bacteria was performed to detect melanin-precursors released by the bacteria. We identified dopaminergic bacterial strains that induced fungal melanization. Accumulative evidence has shown that catecholamines regulate the crosstalk between microbes and the immune system. Currently, we are validating *Anopheles* proteins implication in parasite killing,

encapsulation, and association with the parasite, as well as identifying melanin-precursors released by mosquito intestinal microbiota. This project will provide mechanistic insights on the vector-parasite interactions while enhancing our knowledge of mosquito vectorial capacity and malaria parasite transmission, possible contributing to the development of potential novel malaria control strategies to make these mosquitoes more resistance to *Plasmodium*.

A-04

A single nucleotide polymorphism in a *Plasmodium berghei* ApiAP2 transcription factor alters the development of host immunity

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The Apicomplexan Apetela2 (ApiAP2) family of transcription factors are major regulators of *Plasmodium* parasite gene expression. Twenty-seven members of this family have been identified since their discovery in 2005. However, our understanding of which ApiAP2 members regulate key features of *Plasmodium* biology is incomplete. Here, we describe the impact of a single nucleotide polymorphism (SNP) in the active site of the AP2 domain of PBANKA_011210 on the outcome of infection in mice. *Plasmodium berghei* Anka (*Pb*A), the only rodent parasite that causes experimental cerebral malaria (ECM), codes for phenylalanine at position 1823 whereas all other *P. berghei* parasite strains, none of which cause ECM in mice, including *Pb*NK65, have serine in this position. Using CRISPR, we modified the *Pb*NK65 parasite (*Pb*NK65-WT) to encode the *Pb*A SNP (*Pb*NK65-*Pb*A). The course of infection with *Pb*NK65-WT or *Pb*NK65-*Pb*A were similar and showed no ECM pathology indicating that the *Pb*A ApiAP2 SNP is not sufficient to cause ECM. However, mice infected with *Pb*NK65-*Pb*A had dramatically higher IFN-gamma and TNF alpha in their serum and they generated more persistent, stronger germinal center responses and produced higher titers of parasite-specific antibodies. When infected mice were treated with sub-therapeutic doses of the anti-malarial drug chloroquine, to lower the parasitemia, *Pb*NK65-*Pb*A-infected mice cleared the infection whereas mice infected with *Pb*NK65-WT were unable to control the infection resulting in severe anemia and death. Using protein binding microarrays (PBMs) to examine the DNA binding specificity of the mutant and WT AP2 DNA binding domain, we have identified that this single nucleotide polymorphism alters the sequence specifity of DNA motif recognized by PBANKA_011210. Furthermore, RNA-seq comparing *Pb*NK65-WT and *Pb*NK65-*Pb*A identified differential expression of over 40 genes that may collectively be responsible for the altered pathogenicity. These results suggest a novel role for ApiAP2 proteins in controlling host immunity to *Plasmodium* infections.

A-05

PTEX component EXP2 is crucial to transport across the *Plasmodium falciparum* parasitophorous vacuolar membrane

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Intraerythrocytic malaria parasites reside within a parasitophorous vacuolar membrane (PVM) generated during host cell invasion. Erythrocyte remodeling requires export of effector proteins across this barrier and depends on the *Plasmodium* translocon of exported proteins (PTEX). PTEX consists of three core proteins including the AAA+ ATPase chaperone HSP101 and two novel proteins known as PTEX150 and EXP2. EXP2 has been hypothesized to form a membrane-spanning channel but its contribution to parasite biology has remained obscure. To interrogate EXP2 function in *Plasmodium falciparum*, we employed the TetR-DOZI-aptamer system to achieve regulatable EXP2 translation. Knockdown of EXP2 revealed critical roles in protein export, small molecule transport and blood-stage parasite survival. Surprisingly, functional complementation studies with EXP2 mutants showed that a highly charged C-terminal stretch of >50 residues is dispensable for blood-stage function. Visualization of PTEX components in live parasites revealed a patchwork distribution within the PVM suggestive of discrete sites of export. However, split-GFP assays showed that PTEX components are freely accessible to non-exported proteins residing in the PV lumen, arguing against the existence of export-dedicated sub-compartments. The expression timing

of HSP101 and PTEX150 are closely mirrored, peaking early in the ring-stage, while EXP2 peaks in the trophozoite-stage. This corresponded with an increased ratio of EXP2 to HSP101 in the later part of the cycle as assessed by western blot and co-immunoprecipitation assays. This suggests substantial quantities of EXP2 exist outside of PTEX, which may be required for additional roles in small molecule transport. Our results demonstrate that EXP2 is necessary for translocation of molecules across the malaria parasite PVM and provide support for its proposed role as a PVM pore.

A-07

Patients' perception and satisfaction on quality of laboratory malaria diagnostic service in Amhara Regional State, Northwest Ethiopia

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Inappropriate perception and inadequate satisfaction of patients are significant challenges reported to affect the quality of laboratory malaria diagnostic services. A facility-based, cross-sectional study was conducted from November to December 2013 among 300 patients. Their level of satisfaction was measured using both pre-tested structured and open ended questionnaires. A 5-point Likert scales and their weighted average were used to categorize satisfaction level of the patients. Data were entered in Epi-Info version 3.5.3 and analyzed using SPSS version 20. Chi-square test was used to see the association between the outcome variable and independent and the strength of the association was identified using odds ratio in the binary logistic regression. In addition the open ended questionnaire findings were coded and analysed thematically. Over half (52.6%) of the patients were satisfied with the malaria diagnostic service with a 98.7% response rate. The majority (89.3%) of patients perceived they were well diagnosed in facing fever upon giving blood for laboratory malaria diagnosis within 30 min waiting time in most (62.5%) of the patients. Ethnicity, residence, knowing malaria diagnosis after consulting clinician, and time period to receive malaria result were the independent predictors for patient satisfaction ($p < 0.05$). The open ended questionnaire responses also revealed providing precise laboratory result timely, availability of the right treatment, presence of health professionals performing the laboratory test upon request in the health facility were among the major enabling factors for patients' satisfaction. The observed level of satisfaction in the current study though encouraging when compared with some previous studies conducted in eastern Ethiopia on general laboratory services, still it requires scale-up in the enhancement of malaria laboratory diagnostic service in the fight against malaria.

A-08

Defining the population structure of *Plasmodium falciparum* across the Democratic Republic of Congo using high-throughput molecular inversion probes

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A better understanding of the spread of malaria across space and time is critical for control and elimination. The spread can best be understood using genetic tools. Here, we have developed and validated a panel of molecular inversion probes for Plasmodium falciparum and applied it to dried blood spots obtained from the Democratic Republic of Congo (DRC). This approach allows highly-multiplexed genotyping of polyclonal isolates. Control experiments demonstrate accurate genotyping of 25 microsatellite loci in single reactions. Principal Component Analyses suggest that the vast majority of parasites are closely related genetically, but that there are genetic outliers close to the country's borders with Angola and Zambia. These findings suggest that parasite subpopulations may exist in border regions. Further work is in progress to develop larger MIP panels that will differentiate drug resistant parasites from sensitive parasites. These should help in predicting where and how such parasites may spread.

A-09

Initial Results of a National Screen of *Plasmodium vivax* Infections in Duffy negative Individuals in the Democratic Republic of the Congo

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Plasmodium vivax has long been assumed to absent from Sub-Saharan Africa in association with the near fixation of the Duffy-negative phenotype. However, recent evidence indicates that *P. vivax* is prevalent across the region. Here we present our work screening 8,812 children and 17,934 adults for *P. vivax* from the 2013-2014 Demographic and Health Survey, a population representative survey, in the Democratic Republic of the Congo (DRC). Initial results suggest that *P. vivax* is prevalent in the region and found within Duffy negative individuals. This result is consistent with the growing evidence that *P. vivax* is actively infecting Duffy-negative individuals in Africa. Analysis of risk factors for *P. vivax* infection are ongoing with an emphasis on mapping the prevalence distribution of *P. vivax* in the region. In addition, a subset of samples underwent hybrid selection and whole genome sequencing for population genetic analyses. Compared with other globally sourced isolates of *P. vivax*, the samples from the DRC are genetically distinct with a particularly high nucleotide diversity. Future molecular and sequencing work will be leveraged to perform demographic modeling and phylogenetic analyses to determine the infectious origin of these samples and better quantify their role in the evolutionary history of *P. vivax*.

A-10

Identification of endoplasmic reticulum-shaping proteins in Plasmodium parasites

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The endoplasmic reticulum (ER) of eukaryotic cells is an essential organelle with many critical functions. The ER consists of a network of tubules and sheets, whose formation is determined by a set of key proteins. ER function is closely tied to its shape. The relative ratio of these structural domains in the ER is modified in different cell types to fulfill specific functional requirements. For example, the ER of professional secretory cells such as pancreatic cells consist almost entirely of sheets while the ER of non-secretory cells such as neuronal axons have abundant tubular networks. Disruption of ER morphology, through mutations in these proteins, causes specific growth and developmental defects in diverse organisms. Specific classes of integral membrane proteins have been identified to shape the ER. To understand how the ER acquires its shape in *Plasmodium*, we identified *Plasmodium* homologs of key ER-shaping proteins. *Plasmodium* species encode three homologs of reticulons, a family of proteins required to form ER-tubules in higher eukaryotes. These proteins are highly conserved in all five human-infective species and the rodent parasites, *P. berghei* and *P. yoelii* suggesting that their function is likely to be conserved regardless of the mammalian host. We demonstrated that at least one protein forms tubules *in vitro*, and functionally substitutes for its yeast homolog in the cell. Parasites lacking the protein are severely attenuated in the asexual cycle and have striking reduction in mosquito infectivity. In addition, *Plasmodia* encode a single homolog of atlastin GTPases, that fuse together ER tubule membranes to form a tubular network. The *P. berghei* homolog rescues ER morphology in mammalian cells lacking endogenous atlastin GTPases. These data are the first demonstration that ER-shaping mechanisms in *Plasmodium* are likely to share common features with other eukaryotes and validate further functional analysis of *Plasmodium* homologs of ER-shaping proteins.

A-11

Measurement of Full Body Bioluminescent Signal Can Replace Traditional Techniques for Determining Parasitemia in Mice Infected with Luciferase-Expressing ANKA *P. berghei* Parasites

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Rodent malaria models are extensively used for malaria drug efficacy studies. Plasmodium berghei, which is the main species of rodent malaria, has been extensively used to assess antimalarial efficacy of candidate compounds. At the Walter Reed Army Institute of Research, we utilize a modified Thompson's Test, one of the most frequently used

rodent malaria models, to determine the schizonticidal efficacy of an antimalarial compound through its ability to eliminate parasitemia and keep mice parasite free for several weeks in ICR mice infected with $1X10^5$ asexual luciferase-expressing *P. berghei* parasites. In this study, we describe utilization of a novel bioluminescent *in vivo* imaging technique to replace time-consuming and expensive techniques that are routinely used to measure parasitemia in infected animals, such as Giemsa staining and flow cytometry measurements, with whole body bioluminescent signal measurements. This new method of determining parasitemia in mice will provide a simple, precise, and objective method of parasitemia assessment compared to other methods and will reduce to minimum false positive results that can lead to the premature removal of animals from study. Furthermore, from the animal welfare and the 3 R's point of view, the new methodology will also facilitate early removal of sick animals from study, sometimes well before the clinical signs of disease are present.

A-12

Genome-wide diversity of *Plasmodium falciparum* from spatially and temporally paired vectors and humans in northern Zambia.

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Plasmodium falciparum undergoes asexual reproduction within the human host, but reproduces sexually within its vector host, the *Anopheles* mosquito. In regions of moderate and high malaria transmission, infectious mosquito bites occur frequently and infections of humans are typically comprised of multiple parasite clones. Under these conditions, cross-fertilization between genetically distinct parasite gametes may occur more frequently than inbreeding within the mosquito, possibly contributing to the observed genetic diversity of the parasite population. Despite the important implications for disease transmission dynamics and malaria control, empirical studies on how parasite diversity in infected mosquitoes relates to the diversity of the parasite exiting in humans has yet to be undertaken. To gain insights into the role that mosquitoes plays in modulating parasite diversity, we conduct a comparative population genomic study of *P. falciparum* obtained from spatially and temporally paired vectors and humans in a high transmission setting in Nchelenge District, northern Zambia. First, we developed a multiplexed hybrid capture array to selectively enrich and deep sequence 24 *P. falciparum* whole genomes directly from field-caught *Anopheles funestus* mosquitoes and dried blood spot human samples. Our multiplexed hybrid capture array enabled a high capture efficiency (proportion of sequence reads mapping to *P. falciparum* 3D7) and the sequencing of ~67.5 % of *P. falciparum* genome with at least 5-fold coverage. Among the sequenced samples we identified thousands genome-wide single nucleotide polymorphisms (SNPs) enabling an unprecedented high-resolution study of parasite diversity, previously unavailable in Zambia. Principle coordinate and admixture analyses based on SNPs will provide detailed insight into fine spatial and temporal trends in parasite population diversity and degree of genetic relatedness of parasites circulating in humans and mosquitoes. This research will help clarify the role of mosquitoes in modulating parasite diversity at the population level and inform targeted vector control strategies aimed at disrupting malaria transmission.

A-13

Targeting *Plasmodium* sporozoite liver invasion with a phage display library.

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After inoculation by the bite of an infected mosquito, *Plasmodium sporozoites* enter the blood stream and infect the liver with unique specificity. To initiate productive infection of a vertebrate host, sporozoites must traverse the lining of the liver blood vessels (sinusoids), preferentially trough macrophage-like Kupffer cells, followed by infection of hepatocytes. Using a phage display library we previously selected peptides which structurally mimic (mimotope) a sporozoite ligand for Kupffer cell recognition, which further led identification of a sporozoite ligand (GAPDH) and the corresponding Kupffer cell receptor (CD68). Importantly, we showed that the mimotope peptide acts as an effective vaccine antigen that generates sterile protection. We used the same phage display library in search of *P. berghei* sporozoite receptor(s) of mouse hepatocyte invasion. The screen yielded hepatocyte-binding peptides that

competitively inhibit sporozoite invasion of hepatocytes *in vitro*. Antibodies against the selected peptides recognize a ~52 kDa sporozoite surface protein and antisera against one of the selected peptides inhibits *Plasmodium* liver invasion. Most likely, this peptide is a mimotope of a sporozoite ligand for hepatocyte invasion. This line of research may lead to the identification of a vaccine antigen targeting malaria liver invasion.

A-14

MitoNEETs: mitochondrial redox sensitive iron-sulfur cluster transfer proteins in malaria parasites

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MitoNEET is a homodimeric protein that is present on the outer mitochondrial membrane with the C-terminal domain facing the cytosol. It contains a CDGSH domain, which harbors a unique three-cysteine/one-histidine (**CCCH**) type of iron-sulfur (FeS) cluster binding geometry. Studies in mammalian cells have shown that MitoNEET transfers 2Fe-2S clusters in a redox sensitive manner. This protein is involved in a critical process to maintain the homeostasis of the cytosolic Iron-Sulfur Cluster Assembly (CIA) pathway and the functions of many FeS containing proteins in the cytosol and the nucleus. MitoNEET has been implicated in a spectrum of diseases including diabetes, cancer, and neurological disorders. Using bioinformatics search, we have found three mitoNEET-like orthologues in the *Plasmodium falciparum* genome, named as PfML1, 2 and 3. We have localized these proteins individually to the blood stage parasite's mitochondrion by immunofluorescence assay. Using the CRISPR/Cas9 approach, we knocked out PfML1 and PfML2; however, PfML3 was refractory to gene deletions after many attempts, suggesting that PfML3 is essential for malaria parasites. To test this further, we are generating a PfML3 conditional knockdown line with the aptamer-TetR-DOZI system to regulate the expression of PfML3. We expect to observe a lethal effect on the parasites upon PfML3 knockdown. We will then rescue the phenotypes by complementing a wild type PfML3 or a mutated PfML3 (**CCCH-AAAA)** to further verify the essentiality of the conserved domain of PfML3 protein. We are also making attempts to purify recombinant PfML3 protein expressed in *E. coli* to study the biochemical and biophysical properties of the protein.

A-15

Effect of *P. falciparum crt* single nucleotide polymorphism and *plasmepsin* 2-3 copy number increase on ex vivo piperaquine resistance in *P. falciparum* isolates from northwestern Cambodia, 2012-2015

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P. falciparum resistance to piperaquine (PPQ) emerged across western Cambodia over the last 5 years, leading to high rates of dihydroartemisinin-piperaquine (DHA-PPQ) treatment failure. Molecular correlates of PPQ resistance would allow timely surveillance, important for monitoring the efficacy of DHA-PPQ outside Southeast Asia and helping to determine if DHA-PPQ could be reintroduced in the region. We investigated the relationship of ex-vivo PPQ susceptibility amongst 162 Cambodian isolates to copy number variation of the *plasmepsin 2-3* gene and single nucleotide polymorphisms in the *Pfcrt* gene, including a *Pfcrt* F145I mutation recently implicated in DHA-PPQ treatment failure. Isolates were collected pre-treatment from two cohorts, the first enrolled in Dec 2012-Feb 2014, the second between Dec 2014-Sep 2015. Median PPQ IC_{90}, measured using an HRP2-based assay, rose from 104 nM (IQR: 65 – 368) in the earlier cohort to 837 nM (IQR: 113 - 12050) in the later cohort. Real-time PCR and whole genome sequencing of 78 isolates from the earlier cohort show that 38 (49%) have multiple copies of *plasmepsin 2-3*, 11 (14%) contain the F145I mutation, and 8 (10%) have both the mutation and increased copy number. When PPQ IC_{90} is dichotomized at a cut point of 200nM, increased copy number is associated with an odds of increased IC_{90} more than six times that of the odds in those with only one copy

(OR = 6.6, 95% CI = 2.2 - 22.8, p = 0.001). Preliminary SNP analysis suggests the presence of the F145I mutation is also associated with increased mean IC_{90} (6513 nM vs. 258 nM). This study confirms the previously described association between increased *plasmepsin 2-3* copy number and increased IC_{90}, and supports an additional role for the *crt* F145I mutation in PPQ resistance. Future analyses will define the independent and joint effects of these molecular correlates on PPQ resistance.

A-16

Chemo-proteomic discovery of lipases in *Plasmodium falciparum*

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During the development of the malaria parasite in the host RBC, there are important lipid catabolic processes which have not yet been biochemically defined. For example, the enzyme(s) responsible for catabolism of triacylglycerol stores in lipid droplets is presumably important for parasite development but has not been identified. As many lipases belong to the serine hydrolase superfamily, we have focused our lipase discovery efforts on this enzyme class. Using an activity-based probe strategy, we have profiled the serine hydrolase superfamily in the asexual erythrocyte stage of *P. falciparum*. Competition assays with class-specific lipase inhibitors has revealed a number of serine hydrolases with distinct putative lipase activities. Candidate lipases are currently being identified by affinity capture and mass spectrometry.

A-17

Reticulocyte conditioned media stimulates gametocytogenesis in *Plasmodium falciparum* culture

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Malaria infection by *Plasmodium falciparum* continues to afflict millions of people worldwide, with transmission maintained by the definitive host mosquito. Advances in medical treatment and drug therapies must be coupled with efforts to reduce transmission, which is dependent upon mosquito ingestion of the gametocyte stage of the parasite. These sexually committed stages develop from the asexual stages, yet the factors behind this transition are poorly understood. In the human host, immune status, age, and severe anemia correlate with prevalence of gametocytes, with this stage of the parasite sequestering in the reticulocyte-rich bone marrow. Difficult to study the influencing factors *in vivo*, *in vitro* studies have revealed that diffusible extracellular factors present in different media and cellular environments influence gametocytogenesis. Parasite conditioned media has been shown to increase gametocytogenesis, suggesting a novel form of quorum sensing. We hypothesize gametocytogenesis is induced through molecular factors found in parasite- or reticulocyte-rich environments. We have demonstrated that human induced pluripotent stem-cell derived erythrocyte media significantly influences gametocytogenesis in an NF54 strain *P. falciparum* infection. Both conditioned and non-conditioned terminal maturation media (TM media) significantly increased gametocyte numbers counted via blood film. We then adapted a quantitative luciferase assay specific for gametocytes to more efficiently quantify gametocytes in different environments. This assay supports the data from blood films, with significant increases in gametocyte numbers on days 4 to 6 in both conditioned and non-conditioned TM media. Current studies are focused on optimizing the adapted luciferase assay, and replicating parasite-conditioned media experiments. We are also working to understand how supplements of TM media and known quorum sensing molecules may influence gametocytogenesis. We plan to use size exclusion chromatography coupled with mass spectrometry and metabolomics to identify unique molecular triggers of gametocytogenesis.

A-18

Malaria profile in Vanga Health Zone, Democratic Republic of Congo and the impact of long lasting insecticide treated bed net distribution

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Malaria remains a topical and public health issue in tropical and inter-tropical areas, particularly in the Democratic

Republic of Congo (DRC) where it is among the leading causes of morbidity and mortality. Although its global impact is declining and the implementation of control measures including the distribution of long-lasting insecticide treated bed nets (LLIN), an upward trend is suspected in the DRC. Data on malaria transmission are therefore needed. Our objective was to determine the trend of malaria in relation with the distribution of LLIN in the VANGA health zone, from 2013 to 2016. This was a documentary study based on the National Sanitary Information System canvas of the Vanga ZS from 2013 to 2016. Data on the number of malaria cases and the distribution of the LLIN were taken. The number of cases of malaria increased markedly from 2013 to 2016 (from 9,053 cases to 36,402) two peaks in the number of cases of malaria were noted in each year (April-May and October-November). Around 167,000 LLINs were distributed on December 2015, distribution of LLIN had little influence on trends in the number of malaria cases. This results could be explain by the lack of utilization of LLIN or his bioefficacy against malaria vectors in this region. An other reason might be the improvement in the collection of data by the National Sanitary Information System during this period Although different means of control were in place against malaria, especially LLIN, we found that the malaria trend in the Vanga Health Zone from 2013 to 2016 is on the rise and the mass distribution of LLIN has only a minimal influence on the number of malaria cases.

A-19

Surprising Secretion Signals of *Asaia sp. SF2.1* for Malaria Control in Mosquitoes

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Plasmodium sp., the parasite that causes malaria, is transmitted by the *Anopheles sp.* mosquito vector. Symbiotic bacteria within the mosquito midgut can be transgenically modified to affect the mosquito's phenotype, otherwise known as paratransgenesis; this strategy can be used to engineer the bacteria to secrete anti-malarial effector molecules outside of the cell and into the mosquito midgut to combat the parasite. One such bacterial candidate is *Asaia sp.*, a gram-negative and rod-shaped bacteria that has been shown to colonize the midgut, ovaries, and salivary glands within the *Anopheles* mosquito. However, common secretion signals, such as the *E. coli* Type II OmpA and TolB leader peptides, as well as signals from closely-related species do not function in *Asaia*. A genetic library screen found only one native secretion signal that provided sufficient secretion of protein into the supernatant. Therefore, the *Asaia sp. SF2.1* genome was sequenced and used to predict Type II secreted proteins, and further processed using SignalP4.1 to identify the leader signals. These signals have been cloned into the plasmid pNB92, containing the PnptII constitutive promotor and the c-terminal domain of alkaline phosphatase lacking a secretion signal. These plasmids were transformed into the *Asaia sp. SF2.1* lab strain. Positive colonies were grown overnight to log phase and separated into the supernatant, lysate, and cell surface fractions. Secretion of alkaline phosphatase was tested by an ELISA assay for the abundance of protein into the different fractions. To ensure alkaline phosphatase is active when secreted, another multiwall plate assay using PNPP substrate was used. This is important because some of the antimalarials being used contain disulphide bonds, which are formed in the periplasm and important for proper protein folding and function. Ongoing research is being conducted to further test these secretion signals *in vivo* for sufficient secretion and *Plasmodium* repression.

A-20

A longitudinal study over three years leads to the identification of *Plasmodium vivax* infections in Duffy blood group negative children in Bandiagara, Mali

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Plasmodium vivax was thought to only infect the red blood cells of Duffy blood group positive people particularly in Asia and South America. In the last decade, *P. vivax* has appeared in Africa in areas such as Madagascar and Ethiopia, where Duffy positive and negative people live side-by-side. We sought evidence of *P. vivax* infections in a cohort of 300 children in Bandiagara, Mali, West Africa (a Sahelian area of Mali where people are primarily Duffy negative) from the beginning of one transmission season through a second season and into the beginning of a third season. We used quantitative PCR of blood samples dried onto filter paper to determine the prevalence of *P. vivax* and *P. falciparum*. We

observed the occurrence of *P. vivax* in 25 children in Bandiagara, Mali. Duffy negativity was confirmed in all 25 children by Sanger sequencing the single point mutation (T to C) in the GATA1 binding region 5' prime of the Duffy blood group antigen open reading frame. The prevalence of *P. vivax* infection was 2.0-2.5% at every time point (June 2009 to June 2010). While experiencing *P. vivax* infections, none of the children had a history of fever, chills, headache or muscle ache, which are symptoms that may be associated with malaria. The parasite densities were extremely low between 8 and 157 parasites per microliter. The present data indicate that at some time in the past, *P. vivax* has adapted to infect Duffy-negative people in Africa which could become a burden to sub-Saharan Africa in future. Hence, the evidence of *P. vivax* existence needs to be taken into consideration in designing malaria control and elimination strategies in Africa.

A-21

Characterizing global genetic diversity and copy number variation of VAR2CSA

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Pregnancy associated malaria (PAM) is mediated by the VAR2CSA protein, a member of the *Plasmodium falciparum* multicopy *var* gene family. The binding of VAR2CSA, found in the membrane of infected erythrocytes (IE), to chondroitin sulfate A (CSA) on syncytiotrophoblasts causes the sequestration of IE to the placenta of pregnant woman leading to adverse pregnancy and birth outcomes. A 1.6kb region spanning the ID1-DBL2X domains has found to be the minimal CSA binding domain, parts of which are used within the two vaccines currently in clinical trials, both of which incorporate only 1 strain each (FCR3 or 3D7). To further aid in developing other vaccines and help predict the effectiveness of current vaccines, we developed a novel haplotype reconstruction pipeline to call haplotypes from whole genome shotgun sequencing to call VAR2CSA haplotypes from publically available data from countries in Southeast Asia (n=6), Africa (n=8), and South America (n=1). We analyzed the global diversity by calling local haplotypes for the 7.5kb exon1 of VAR2CSA (n=754), the minimum binding domain (n=1094), and the regions spanned by the current vaccines (n=1094). Similar to previous studies of VAR2CSA diversity, though the diversity for VAR2CSA is extreme, we found most sequence variation to be shared between worldwide populations suggestive of balancing selection keeping the populations of VAR2CSA similar. We also found that the copy number variation of VAR2CSA to be similar between global populations with the exception of the 22 samples from South America, which were all found to have 1 copy of VAR2CSA. The global distribution of copy number was found to be 1 copy: ~82%, 2 copies: ~16%, and 3 copies: ~2%. Extreme, though globally shared, diversity suggests the most effective VAR2CSA based vaccine should incorporate several strains.

A-22

Driving mosquito refractoriness to *Plasmodium falciparum* with engineered symbiotic bacteria

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The huge burden of malaria in developing countries demands urgent development of novel approaches to fight this deadly disease. Whereas engineered symbiotic bacteria were shown to render mosquitoes resistant to the parasite, the challenge remains to find a way to effectively introduce such bacteria into mosquito populations. We describe a Serratia bacterium strain (AS1) isolated from Anopheles ovaries that stably colonizes the mosquito midgut, female ovaries and male accessory glands, and spreads rapidly through mosquito populations. Serratia AS1 was genetically engineered for secretion of anti-Plasmodium effector proteins. The resulting recombinant strains inhibit development of Plasmodium falciparum in mosquitoes. Furthermore, we also explore potential issues of Serratia application in the natural environment.

A-23

Transcriptome analysis of genes associated with pyrethroid resistance in South and Central American Anopheles albimanus

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Decades of unmanaged insecticide use and routine exposure to agrochemicals have left many populations of malaria vectors in the Americas resistant to multiple classes of insecticides, including pyrethroids. Using a transcriptome-wide approach, we characterized the mechanisms of deltamethrin and alpha-cypermethrin resistance in Anopheles albimanus from sites in Peru and Guatemala. Field collected An. albimanus were phenotyped as either deltamethrin or alpha-cypermethrin resistant using the CDC bottle bioassay. RNA from 1) field-collected resistant, 2) field-collected unexposed, and 3) a susceptible laboratory strain of An. albimanus was sequenced and analyzed using RNA-Seq. The expression profiles of the three groups were compared based on the current annotation of An. albimanus reference genome sequence. Several candidate genes associated with pyrethroid resistance in other malaria vectors were found to be over-expressed in resistant An. albimanus mosquitoes. The cytochrome P450 monooxygenase CYP9K1 was overexpressed in both Guatemala and Peru, relative to a susceptible laboratory colony (Sanarate), but to a much greater extent in Peru (14x) than Guatemala (2x). CYP6P5 was over-expressed in Peru (68x) but not in Guatemala. The results suggest different mechanisms may mediate pyrethroid resistance in different populations of An. albimanus. Differences were also noted in the voltage-gated sodium channel between Peruvian alpha-cypermethrin-resistant and deltamethrin-resistant samples. In deltamethrin resistant samples, the knockdown resistance mutation (kdr) variant alleles at position 1014 were rare, with approximately 5% frequency but showed approximately 15-30% frequency in the alpha-cypermethrin resistant samples. Validation of selected candidate genes and the kdr mutation as a resistance marker for alpha-cypermethrin will provide a key step for the development of mechanism-specific assays.

A-24

The type II NADH dehydrogenase of *Plasmodium falciparum* is dispensable and not likely to be an antimalarial drug target

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The battle against malaria has been substantially impeded by the recurring development of drug resistance in *P. falciparum*. Hence, unique or divergent pathways of the parasite have been one focus of the search for novel antimalarial drugs. The mitochondrion of the parasite is drastically distinct from its counterpart in the human host. It has been successfully exploited as a drug target to yield antimalarial compounds in clinical use, as well as in the development pipeline. The type II NADH dehydrogenase in *Plasmodium* is one potential drug target that has attracted much research effort in the last decade. Unlike the conventional multi-subunit Complex I "NADH dehydrogenase" present in mitochondria of human cells and other organisms, *Plasmodium* NDH2 is a single subunit, non-proton pumping, transmembrane protein that donates electrons to ubiquinone. The absence of a type II NADH dehydrogenase in human cells prompted researchers to believe that the malarial NDH2 might be a good drug target. Although NDH2 in *P. berghei* was genetically ablated a few years ago, in the absence of specific disruption of the *P. falciparum* PfNDH2 gene, drug development efforts focused on the *P. falciparum* NDH2 have continued, on the assumption that PfNDH2 is essential during asexual blood stages. Here, we have knocked out PfNDH2 in wildtype *P. falciparum* parasites via a CRISPR/Cas9 mediated genetic approach. The ΔPfNDH2 line does not exhibit any growth defects in the asexual stages. HDQ has been considered a PfNDH2 specific inhibitor. However, ΔPfNDH2 parasites were equally susceptible to HDQ as wildtype parasites, indicating that HDQ is targeting other pathways. Our results clearly show that PfNDH2 is not essential in asexual blood stage parasites, and therefore not a promising drug target.

A-25

Antibodies promote complement activation against *Plasmodium falciparum* sporozoites, providing a novel mechanism of anti-malarial immunity

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At present, we lack an in depth understanding of the immune mechanisms against *Plasmodium falciparum* sporozoites, and consequently lack reliable correlates of protective immunity. Without this fundamental knowledge, the development and evaluation of existing or candidate malaria vaccines is severely impaired. Antibodies to sporozoites and their major surface antigen, circumsporozoite protein (CSP), have been loosely associated with protection in studies of naturally acquired and vaccine-induced immunity, but it remains unclear how they function, and there is limited knowledge of key functional epitopes. Here we investigated the functional role of antibodies to CSP and sporozoites in activating the complement system via the antibody-dependent classical pathway. We have established that antibodies form malaria-exposed individuals can fix the complement protein, C1q, to recombinant CSP, which is an essential interaction that initiates the classical activation pathway, and leads to formation of the membrane attack complex. These C1q-fixing antibodies were strongly correlated with IgG1, IgG3 and IgM antibody types, and could recognise multiple CSP epitopes. Malaria-specific antibodies mediated C1q-fixation and subsequent classical activation on the surface of *P. falciparum* sporozoites, which consequently inhibited parasite migration through cells and could lead to cell death *in vitro*. Therefore complement activation may be an important antibody mechanism to inhibit sporozoite infection and pre-erythrocytic development. Effective C1q-fixing antibodies were poorly acquired though natural malaria exposure in children and only a minority of highly exposed adults had anti-CSP antibodies with strong complement-fixing activity. In summary, we identified a novel function of malaria-specific antibodies in activating the human complement system and consequently inhibiting sporozoite function and leading to cell death, which provides important insights for vaccine development and evaluation.

A-26

Developing Long-Term Malarial Chemoprophylactic Compound Releasing Implants

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Despite the successful development of chemoprophylactic compounds and multi-drug combination therapies, malaria infection remains a crucial health threat to U.S. Soldiers in malaria endemic areas. The successful prevention of malaria infection is highly dependent on compliance with a prescribed chemoprophylaxis regimen. The Experimental Therapeutics (ET) Branch at Walter Reed Army Institute of Research (WRAIR) is the U.S. Army's premier research program for the development of anti-malarial prophylaxis drugs. A current effort of ET, in scientific collaboration with the Southwest Research Institute and Titan Pharmaceuticals, is to develop long-term release implantable anti-malarial drug matrices. These implants provide continuous drug release with a non-fluctuating drug levels over an extended period from two to six months, and could potentially relieve deployed service members from adherence to a daily oral drug dosing schedule. EVA (ethylene-vinyl acetate) implants that contain piperaquine, a compound effective against blood stage parasites, were tested in the *Plasmodium berghei*-mouse model to characterize the pharmacokinetics (PK) profile and long-term prophylactic efficacy *in vivo.* The piperaquine formulated implant study showed the PK profile exhibited slow drug release for six weeks while maintaining stable plasma levels. Furthermore, the piperaquine implants after longer than eight weeks of implantation demonstrated sufficient suppression in early blood stage malaria and complete protection from infection of *Plasmodium berghei* parasites in mice. The development of long-acting prophylactic implants with greater potency and safety is a novel approach, and one that could greatly improve compliance of deployed service members in malaria endemic regions. Furthermore, the target products will support the multi-domain battlefield operational concept by allowing ground combat forces maneuver and perform in an uninterrupted manner in resource-constrained environments. These preliminary findings with piperaquine allow us to pursue a series of long-acting implants that include more regulatory-attractive FDA-approved anti-malarials, atovaquone/proguanil (Malarone®) and doxycycline, for follow-on *in vivo* preclinical studies.

A-27

Testing the hypothesis that a BAFF variant predisposing to multiple sclerosis determines differential immune responses to malaria

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Malaria has been one of the most powerful selective pressures in human history. Unfortunately, the systematic identification of targets of malaria selection has, until recently, been slow, and generally driven by prior knowledge that suggests candidate genes. By contrast, modern genomics can interrogate the entire genome for signatures of natural selection and provide new clues about the complex interplay of human hosts and pathogens. In a recent Genome-Wide-Association-Study (GWAS), using case-control samples from a Sardinian population, we discovered a variant in the *TNFSF13B* gene (that we called BAFF-Var) associated with increased risk of multiple sclerosis. The association signal was resolved to the causal variant, an insertion/deletion which creates an alternative polyadenylation site, generating a truncated 3′ UTR that escapes an inhibitory site for miRNA and RNA binding proteins (RBP), thus increasing BAFF production. Increased blood levels of soluble BAFF (sBAFF) results in increases in B lymphocytes and immunoglobulins and reduced levels of monocytes. The BAFF-Var variant shows significant evidence of strong positive selection, and based on evidence from animal models, we hypothesize that the selective pressure was most likely due to malaria. We are now performing functional studies using lysates from *Plasmodium falciparum*-infected erythrocytes to stimulate monocytes and PBMCs purified from Sardinian donors genotyped for BAFF-var (WT versus BAFF-var). Our aim is to identify differential *P. falciparum*-induced expression of proteins and regulatory transcripts between the two genotypes to gain insight into possible mechanisms by which the BAFF-Var variant might protect from malaria.

A-28

An examination of the persistence and immunogenicity of *Plasmodium* blood-stage parasites following commencement of chemoprophylaxis with delayed death antimalarial drugs.

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Malaria vaccine research has generally focused upon single parasite derived proteins for the development of a subunit vaccine. However, many of these proteins are highly variable and are unable to elicit protective responses to multiple strains. A vaccine approach utilising the whole parasite is advantageous in containing multiple parasite antigens, including those that are conserved between parasite strains. Adopting an infection and drug cure (IDC) immunisation approach, blood-stage whole parasites were administered alongside treatment with the delayed death causing antimalarial drugs, doxycycline and azithromycin. This approach was investigated in C57BL/6 and BALB/c strains of mice receiving IDCs with either *P. chabaudi* AS or *P. yoelii* YM. In both mouse strains, one IDC with *P. chabaudi* AS or *P. yoelii* YM elicited protection from homologous challenge. Protection was enhanced with a further two IDCs, compared to a single IDC. Heterologous protection was also observed in mice receiving three IDCs of *P. chabaudi*; however, there was minimal to no protection in the *P. yoelii* model. Cell mediated immune responses were observed in both models, while humoral mediated immunity was observed in the *P. yoelii* model. Results to date suggest protection is primarily mediated through a TH1 response, with the presence of IFN-gamma and IL-2 production being observed in both mouse models. Current work aims to further elucidate the mechanisms of immunity and investigate the use of a slow drug release to replace repeated treatment.

A-29

Infection rates in Anopheles darlingi mosquito populations under a scenario of malaria elimination using RADseq high throughput sequencing of mosquito DNA

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Infection rate by the malaria parasite in mosquito populations has been determined in studies of parasite transmission in different scenarios of malaria control. Techniques used to determine infection rate have been by dissection, PCR, qPCR and immuno-detection, with observed rates ranging from 0.1 to 18%. However, these techniques rely on single parasite markers, which are prone to false positive or negative results. In a parallel project, profiling the genetic makeup of Anopheles darlingi in a scenario of malaria elimination in a Brazilian Amazon village, RADseq DNA fragments of these mosquitoes were sequenced using high throughput sequencing technology. Taking advantage of the data, we determined the parasite infection rate of An. darlingi captured before (BT, before treatment) and after (PT, post-treatment) malaria elimination. Preliminary analyses of sequences obtained from mosquitoes BT indicated an infection rate of 19% (69 with Plasmodium sequences out of 363 mosquitoes specimens) while PT sequences gave as expected a lower rate of 6% (5 with Plasmodium sequences out of 82 mosquito specimens). Plasmodium sequences found included sequences from P. vivax and P. falciparum. These results indicate that the model of malaria elimination involving SIPT (Selective Intermittent Preventive Treatment), a treatment used in the malaria elimination program in the village, in which both symptomatic and asymptomatic patients are treated, is a sustainable form of malaria control at the transmission level. This study demonstrates the usefulness of large-scale genomic analyses in the study of transmission of diseases by invertebrate vectors. We dedicate this study in memoriam of Professor Luiz Hildebrando Pereira da Silva.

A-30

A PvDBP monoclonal antibody recognizes a conserved epitope in *Plasmodium falciparum* and *P. chabaudi* antigens

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The *Plasmodium falciparum* parasite has evolved numerous adaptations to survive in its human host. A key adaptation is the cytoadherence of infected erythrocytes (IE) to host tissues, allowing the parasite to evade the immune system and resulting in the significant pathologies associated with malaria. In pregnancy, *P. falciparum* expresses the protein VAR2CSA on the surface of IEs, which allows the parasite to adhere to chondroitin sulfate A (CSA) expressed on the placenta. In Africa, where *P. falciparum* is holoendemic, antibodies to VAR2CSA are acquired through successive exposure to *P. falciparum* in pregnancy and are able to protect the mother and child by blocking parasite adhesion to CSA. Recently, we discovered a novel mechanism for protection against malaria in pregnancy through exposure to a surface protein from *P. vivax* that is structurally related to VAR2CSA. We found that a monoclonal antibody (mAb) against the *P. vivax* protein, PvDBP, recognized VAR2CSA through shared DBL domains. Here, using live and fixed CS2 IEs, we show that there is partial overlap between the recognition sites of a polyclonal antibody against VAR2CSA and the PvDBP mAb. In addition, prior incubation of IE with the VAR2CSA antibody did not block recognition by the PvDBP mAb, suggesting there are epitopes, possibly cryptic, that are exclusively recognized by the mAb. We further show that cross-recognition extends beyond VAR2CSA. As demonstrated by flow cytometry, the PvDBP mAb recognized a significant proportion of unselected 3D7 IEs. Lastly, using ELISA and western blot methods, we found that the mAb recognized a recombinant protein from the murine malaria parasite *P. chabaudi*, which we identified based on sequence homology with PvDBP. These results suggest that there is a highly conserved epitope in proteins expressed by several species in the *Plasmodium* genus, which could offer important insight into malaria immunity and vaccine development.

A-32

Antibody in the Skin: Do Antibodies Have Their Greatest Impact at the Inoculation Site?

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Malaria-causing *Plasmodium* parasites are deposited into host skin as infected *Anopheles* mosquitoes search for blood. In order for *Plasmodium* to establish infection in the liver, sporozoites need to exit the inoculation site, which they do by moving in the skin to find blood vessels and enter the circulation. This stage of the *Plasmodium* lifecycle (the pre-erythrocytic stage), at which parasite numbers are the lowest, has been recognized as a bottleneck for the parasite. RTS,S, the only vaccine candidate to have shown efficacy in

Phase III clinical trials, targets the pre-erythrocytic stages of the parasite. Indeed further studies have shown that antibodies targeting the major surface protein of sporozoites (circumsporozoite protein or CSP) are critical for RTS,S-mediated immunity. We hypothesized that since sporozoites are extracellular for a significant period of time at the inoculation site, antibodies in the skin could contribute significantly to decreasing sporozoite infectivity. Using rodent malaria parasite *Plasmodium berghei*, we standardized the dose of sporozoites delivered intravenously and by mosquito bite that result in comparable liver infection. We then compared the efficacy of three different doses of a monoclonal antibody (mAb) specific for the *P. berghei* CSP repeats (50 µg, 25 µg, and 12.5 µg mAb 3D11; IgG1) in their ability to inhibit infection when sporozoites were inoculated intravenously versus by mosquito bite. Our data shows that all three concentrations of antibody have greater efficacy when sporozoites are inoculated by mosquito bite. These results have important implications for malaria vaccine development, and provide further insight into host-pathogen interactions in the skin.

A-33

Thrombocytosis in anemic population infected by *Plasmodium falciparum*

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During malaria disease, platelets bind to *Plasmodium*-infected erythrocytes and kill the parasites within. Although low platelet count has been associated with acute malaria infections, we investigated for platelets level in 92 clinically healthy but *Plasmodium falciparum*-infected people aged, 4-80 years in malaria-endemic population. Blood parasite was diagnosed by the thick film 10% Giemsa stained method and by nested PCR technique. An ABX Micros 60 automated cell counter was used for blood cells counts. All infected volunteers were treated with artemether/tumefantrine (20mg/120mg) drugs and after 18 days a second round of blood was collected. All participants inclusively had a uniform level of parasitemia of 16/µl of blood and *Plasmodium falciparum* was identified at 205 bp. There were no malaria parasites detected 18 days after treatment. In infection, thrombocytopenia affected 30.4% of the population; WBC, hemoglobin, and granulocytes had reduced values also but thrombocytosis was observed 18 days after treatment in 27.2% of the population. We observed a negative correlation between platelets level and hematocrit/red blood cells/ hemoglobin concentration before and after treatment. The use of aspirin in the treatment of malaria fever should be discouraged in malaria endemic area.

A-34

Spontaneous resistance to *Plasmodium falciparum* due to variation in glycophorin receptors located on red blood cells

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Hitherto, Africa remains the continent with the highest number of malaria infections. There were 212 million new cases of malaria worldwide in 2015 (range 148–304 million). The World Health Organization (WHO) African Region accounted for most global cases of malaria (90%), followed by the Southeast Asia Region (7%) and the Eastern Mediterranean Region (2%). Transmitted by mosquitoes, the most widespread malarial parasite in Africa is *Plasmodium falciparum*; it is also the most dangerous. A thorough examination of the human genome sampled across Ibadan North East Local Government, Nigeria shows the existence of receptor genes called glycophorins. The arrangement of this receptor genes to some extent determine the susceptibility of individuals to *P. falciparum.* As it is, high complexity means higher resistivity and otherwise. For the purpose of this study, glycophorins are divided into two types based on morphology: Glycophorin A and Glycophorin B, both on chrosome 4. Summarily, complex rearrangement of both glycophorins reduces susceptibility to *Plasmodium falciparum* in human carriers, hence go through mild fever complications is reduced. Finding that the glycophorin region of the genome has an important role in protecting people against malaria. An extensive research on how glycophorin morphology affects the severity of malaria fever will in the future motivate more research on specifically how *Plasmodium falciparum* attack RBCs.

A-35

A *Halobacterium* expression system for production of full-length *Plasmodium falciparum* circumsporozoite protein and other proteins for vaccine development

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The salt-loving archaeon *Halobacterium* sp. NRC-1 produce gas-filled buoyant protein nanoparticles, called gas vesicles or GVNPs. GVNPs are extremely stable, non-toxic, bioengineerable, and self-adjuvanting. We recently developed an expression system employing *Halobacterium* sp. NRC-1 for the production and display of antigenic proteins on GVNPs. Using our designer-vaccine platform, we were able to express antigenic proteins fused with the GVNP GvpC protein, which is localized on the GVNP external surface. Here we report on the production of full-length circumsporozoite protein (CSP) of *Plasmodium falciparum*, which is of interest for formulation of a protective malaria vaccines. We designed a codon-optimized gene and cloned it into the expression vector pDRK for production of the CSP protein alone, and as a fusion to the GvpC protein in the pSD expression vector for display on gas vesicle nanoparticles. In our system the entire CSP is expressed, including an N-terminal signal sequence and a putative GPI anchor sequence. These sequences contain additional epitopes recognized by CD8+ T cells that were lacking in previous constructs, and therefore may provide increased immunogenicity or immunostimulation. Additional studies are needed to determine the immunogenic effects of the full-length CSP produced in *Halobacterium*. The *Halobacterium* sp. expression system provides a novel approach for production of *P. falciparum* CSP and other proteins for vaccine development since *Halobacterium,* lacks LPS, is biocompatible, and antigens fused to GVNPs are extremely stable. Moreover, antigen-displaying GVNPs can be administered intradermally through the use of microneedles.

A-36

Generating whole-genome sequences from non-leukocyte depleted *Plasmodium falciparum* clinical samples

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The field of genomics offers great tools in understanding the evolution of drug resistance, disease transmission, and designing a broadly effective malaria vaccine. But this requires obtaining whole-genome sequencing data, which is difficult to obtain from dried blood spots and non-leukocyte depleted samples. We optimized selective whole-genome amplification approach to obtain high quality whole-genome sequences from *P. falciparum* clinical samples with low amounts of DNA. We show that selective whole-genome amplification approach efficiently amplifies only the parasite DNA, even in the presence of host DNA contamination. Our results also show that diluting and filtering the DNA before amplification greatly enhances the amplification process. This suggests that inhibitors from DNA extraction could be interfering with the amplification. We also observed that this optimized approach improves the percent of the parasite genome covered for a range of parasitemias from 500 parasites/uL to 10,000 parasites/uL in mock dried blood spot samples. Our initial results also show that we can obtain high-quality whole-genome sequences, with >90% of the parasite genome covered at 5x, from non-leukocyte depleted clinical samples like red blood cell pellets and cryopreserved parasites. Effective use of this approach on clinical samples will help overcome one of the major hurdles of malaria genomics and also help lessen the problem of leukocyte depleting samples in the field.

A-37

In vitro and *in vivo* anti-malarial activity of extracts from *Terminalia mantaly* (Combretacéae)

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Emerging drug resistance in *P. falciparum* and its rapid spread in endemic countries have made the quest for new antimalarial a research priority. In Cameroon, medicinal plants such as *T. mantaly* are used in traditional medicine for the treatment of malaria and have been playing an important role in the fight against malaria in rural community. However, their efficacy are still to be validated. This work aimed to investigate the *in vitro* and *in vivo* antimalarial potency of extracts from *T. mantaly*. Extracts from stem barks, leaves and roots of *T. mantaly* were macerated in water and methanol. The susceptibility of red blood cells to the extracts was performed using the MTT assay and the antiplasmodial activity was performed on the W2 strain of *Plasmodium falciparum*. Prior to the curative test, acute toxicity of the promising aqueous stem bark extract was assessed in mice at a dose of 2,000 mg/kg/bw. Mice infected

with *P. berghei* MRA 406 strain were treated with the promising extract at doses of 100, 200, 400mg/kg and their parasitemia were monitored. Extracts did not shown any cytotoxicity on erythrocytes at up to 1mg/ml. Out of the six extracts tested, two (aqueous extracts from stem barks and leaves) presented *in vitro* antiplasmodial activity with IC_{50} of 0.809 and 2.203 µg/ml respectively. The acute toxicity assay of the aqueous extract from stem bark revealed 50% lethal dose (LD50) higher than 2000mg/kg per body weight. The curative test showed an effective dose that reduce 50% of parasitemia (ED_{50}) of 69,50mg/kg with no significant effect on biochemical, hematological and histological parameters. The results from this investigation support the traditional usage of *T. mantaly* and suggest that stem bark of *T. mantaly* could be potential source of compounds with antimalarial activity. However, further investigations are needed to characterize active principles.

A-38

Identification of a Helix-Turn-Helix Protein Critical for Development of *P.falciparum* Gametocytes

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The malaria parasite genome has a reduced number of DNA binding proteins. Most of them are belong to the Apicomplexan AP2 (ApiAP2) family, a major class of transcriptional regulators that are found across all Apicomplexa. Earlier analyses suggested that the *P. falciparum* genome lacked proteins containing canonical DNA binding domains of the homeodomains, leucine zippers or basic Helix-loop-Helix motif families that are common among higher eukaryotes. We recently identified a non-ApiAP2 DNA binding protein (named PfHDP1) that possess a 60 amino acid DNA binding motif referred to as the homeodomain-like domain (HLD) and a conserved Helix-Turn-Helix (HTH) motif, that seems to be more closely related to bacteria and archaea TFs than to higher eukaryotes. *pfhdp1* is differentially expressed between asexual and sexual stages. Epitope tagging of the endogenous locus revealed nuclear localization in early stage gametocytes (I-III), and interestingly a re-localization to the peripheral of cytoplasm in late stage gametocytes (Stage IV). To investigate its function during the parasite life cycle we generated a Δ*pfhdp1* knockout strain. Δ*pfhdp1* parasites maintained normal asexual growth. However, we observed significant aberrant morphology and decreased viability during early sexual development. RNA-seq data also suggests a significant transcriptional change when comparing Stage I gametocytes of parental wildtype and Δpfhdp1 line. The data revealed differential expression in 351 genes in the Δpfhdp1 line early gametocytes, among which the multigene families, like *var* and *rifin*, were highly deregulated. Altogether, our results offer a preliminary view of *Pfhdp1* DNA binding specificity and take the first step inferring that modulates key regulatory networks during gametocyte development.

A-40

Sex predicts the efficacy of immunization with irradiated *Plasmodium* sporozoites in adult mice

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Females tend to develop more robust adaptive immune responses and experience a greater frequency and severity of adverse events relative to males following vaccination in both preclinical animal studies and human clinical trials. While there is currently no malaria vaccine available, several anti-sporozoite vaccines, including RTS,S/AS01 and attenuated sporozoite vaccines, are in clinical trials but the impact of sex on their efficacy remains uncharacterized. In this study, we sought to examine sex-based differences in the efficacy of malaria immunization. Using an established murine model, adult (10 weeks of age) male and female C3H mice were twice vaccinated via intravenous injection with irradiated transgenic *Plasmodium berghei* sporozoites expressing the *P. falciparum* CSP. Vaccine efficacy was then measured at 45 days post 2nd inoculation via mosquito bite or intradermal challenge and hepatic *P. berghei* rRNA load, CSP-specific antibody, and splenic as well as hepatic CD8+ T cells responses were quantified. Immunization with irradiated sporozoites resulted in greater protection against challenge in adult females, which was associated with greater anti-CSP-specific antibody production and hepatic CD8+ T cell responses in adult females than in adult males. Future studies will determine if these sex differences in vaccine response and efficacy are sex hormone dependent.

A-41

Capturing differential protein turnover dynamics in Artemisinin resistant *Plasmodium falciparum* using pulse-SILAC

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Malaria caused an estimated 438,000 deaths in 2015. Although the first-line antimalarial, artemisinins (ARTs), have contributed to decreased mortality rates, ART resistance is a major threat. Several lines of evidence indicate that artemisinin resistance is mediated, at least in part, by altered protein turnover dynamics. To investigate how ART-resistant parasites are capable of overcoming artemisinin-induced toxicity we developed a pulse-SILAC (stable isotope labeling amino acid in cell culture) approach that is capable of quantifying the rates of protein turnover across the proteome. We observe that following ART exposure, both sensitive and resistant lines have impaired protein turnover that affects all detectable proteins. However, ART-resistant lines begin recovering after 18 hours post drug exposure with increased turnover of proteins associated with protein folding, translation and response to oxidative stress. This difference is parasite recovery at 12-18 hours post-artemisinin treatment was independently validated with a firefly luciferase reporter assay. Here we present how this novel duplex pulse-SILAC approach can provide insight into investigating protein turnover dynamics across the intra-erythrocytic developmental cycle for artemisinin-resistant parasites. These findings indicate that long-lasting endoperoxides should overcome artemisinin resistance and we present our evidence for OZ439, an endoperoxide with enhanced pharmacokinetics which is capable of clearing artemisinin-resistant parasites.

3 - POSTER SESSION B

B-02

Biological characteristics and drug resistance profile of *Plasmodium falciparum*

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Malaria remains as an important public health problem worldwide. The global malaria control and eradication effort are challenged by the emergence of drug resistant malaria parasites. Although the emergence of drug resistant parasites have been reported from time to time, the biological characteristics and drug sensitivity profile of parasites remain unclear. Therefore, there is a need for a thorough research to fill the gap. An *in vitro* biological characterization and drug sensitivity profiling of both laboratory adapted strains and field isolates were conducted. We did an *in vitro* drug sensitivity profiling of parasites using four types of antimalaria drugs. We used chloroquine sensitive and resistant *falciparum* strains as quality control. A modified *in vitro* drug sensitivity assay was conducted on 3D7 and Dd_2 ring stage parasites using a high concentration of artesunate. In addition, an *in vitro* rosetting assay was also conducted using 3D7 lab strain and FVT201 field isolate. An *in vitro* drug sensitivity profiling of parasites showed statistically significant sensitivity difference($P<0.05$).The 3D7 strain was sensitive for chloroquine whereas the Dd_2, MKT116, ARS-272 and ARS-233 strains were resistance to chloroquine(IC_{50}>100nM). Both chloroquine resistance and sensitive strains were sensitive to artesunate(IC_{50}<10nM).The different pattern of resistance was observed for the field isolates, MKK183 and FVT201.The MKK183 isolate showed resistance to chloroquine (IC_{50}>100nM) and mefloquine (IC_{50}>30nM),whereas the FVT201 isolate showed resistance to mefloquine only (IC_{50}>30nM). All of the parasites were sensitive to lumefantrine and artesunate (IC_{50}<150nM) and (IC_{50}<10nM)respectively. However, the 3D7 strain showed significant level of sensitivity($P<0.05$) to lumefantrine when compared to FVT201 and Dd_2 strains. In the modified *in vitro* sensitivity assay, the parasites showed different sensitivity profile at the ring stage subpopulations. The ring stage parasites at 11hours and 16hours of age were sensitive than ring stage parasites at 6hours of age. There was also sensitivity difference between 3D7 and Dd_2 ring stage parasites at 100nM and 200nM concentration. We also did rosetting phenotyping assay *in vitro* using two *P. falciparum* strains. *P. falciparum* strains showed significant difference of rosetting rate *in vitro*($P<0.05$). Both laboratory adapted and field strains showed different drug sensitivity profile, and all parasites were sensitivity to both lumefantrine and artesunate. Ring stage parasites showed hypersensitivity profile to artesunate. The ring stage parasites at 6hours had reduced sensitivity to artesunate compared to late ring stage parasites. *P. falciparum* form rosetting *in vitro,* and there was different rosetting rate between falciparum strains.

B-03

Molecular analyses of the synchronicity of *Plasmodium vivax* polyclonal infections

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P. vivax infections are often polyclonal and typically display asynchronous blood-stage parasites, including rings, trophozoites and gametocytes. Our understanding of the dynamics of these polyclonal infections is limited and, for example, it remains unclear whether all clones contribute to all stages at a given time. This information is important to better understand and control vivax malaria as it influences both disease transmission (whether all clones can be transmitted at any given time) and possible resistance to antimalarial drugs (as some stages are more susceptible than others). Here, we describe a new genomic approach that allows to accurately characterize the relative contribution of each clone to the different developmental stages. We designed 8 primer pairs targeting polymorphic regions of genes specifically expressed during different developmental stages including gametocytes. We then amplified paired genomic DNA and cDNA from 23 *P. vivax* infected blood samples from Cambodia and, after next-generation sequencing, determined the relative allele frequency of each SNP polymorphic with an infection. While the allelic frequencies determined from genomic DNA provide a robust description of the entire *P. vivax* parasite population circulating in blood, the corresponding frequencies determined from cDNA indicate which clones are present at specific stages.

B-04

Population analysis of non-falciparum malaria on the China-Myanmar border

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Outside of Africa, *Plasmodium vivax* (PV) is the most common malarial pathogen. Many areas have strived for eradication in recent years, including the Greater Mekong Subregion (GMS), an area with a particularly high PV burden. Out of the six GMS countries, Myanmar has the highest documented Malaria burden, but very little is known about specific malaria populations in Myanmar, or spread of PV to and from nearby countries. Due to high diversity and rapid evolution it's increasingly apparent that malaria eradication should be addressed in a regional way. Regional population analysis is, therefore, an important public health tool for monitoring pathogen spread and evolution. Unfortunately, in the past population analysis for PV in Myanmar, and on its Chinese border, has been limited by sample availability among other factors. Using whole genome sequencing data from PV field isolates, we've addressed variation in China-Myanmar border malaria on the whole-genome-level as well as within individual genes of functional interest. Continuing analysis will elaborate on these results and relate them back to existing GMS data to guide local malaria eradication programs.

B-05

The good, the bad and the loop: epitope mapping of *Plasmodium falciparum* Pfs47 reveals a new malaria transmission-blocking target

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Malaria is a life-threatening disease caused by *Plasmodium falciparum* parasites transmitted by anopheline mosquitoes. Reducing the rate of disease transmission is one of the key steps required for malaria control and eradication. Malaria transmission-blocking vaccines rely on functional antibodies that interact with proteins present on the surface of sexual/sporogonic stages of *Plasmodium* and disrupt vector-parasite interactions. *P. falciparum* evolved a strategy to evade the mosquito immune system responses through the Pfs47, a female gametocyte specific three domain surface protein. Studies testing the potential of Pfs47 as a transmission blocking target showed a moderate transmission blocking activity, but monoclonal antibodies obtained after immunization could only recognize domains 1 and 3 of Pfs47. Antibodies generated against Pfs47 domain 2 showed high transmission blocking capacity but its activity decreased after boosting immunizations. Epitope mapping of Pfs47 domain 2 with monoclonal antibodies recognized an immunodominant region that increases transmission (bad epitope) and region that would confer transmission blocking capacity (good epitope). Policlonal antibodies generated against the good epitope showed robust and reproducible transmission blocking (above 90%) when using *A. gambiae* and *A. stephensi*. The biological effect of these antibodies will be discussed.

B-07

Drone-based high-resolution mapping of malaria vectors breeding sites in the Peruvian-Brazilian Amazon

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We proposed the use of drones to collect high-resolution imagery (~0.1 meters/pixel) of *Anopheles darlingi* breeding sites in the Peruvian and Brazilian Amazon, using conventional and multispectral imagery. The objectives of this study were to evaluate the feasibility of high-resolution mapping in Amazonian areas and to test if there were differences in the spectral signature of water bodies with or without *An. darlingi* larvae. Drone surveys were carried out in four communities in the Peruvian Amazon and in Mâncio-Lima, Brazil. At each site, the drone was flown to an altitude of 100m, which assures a ground sampling distance (GSD) of 0.1 meter/pixel. Simultaneously, mosquito larvae sampling was performed. The imagery was processed in PhotoscanPro to construct orthomosaics (georeferenced mosaic of overlapped images which includes correction for topographic distortions) for each community. Between 382 and 1785 images were used to build the orthomosaic in each community. The use of drones to perform surveillance of malaria vector habitats related to seasonality and/or anthropogenic activities, as well as obtaining high-resolution images, has been demonstrated to be feasible. However, and because it is a relatively new methodology, some adjustments will be needed, such as improving photo aligning, specifically in some Peruvian sites. Ongoing image classification procedures and statistical analyses will address if it is possible to differentiate positive and negative (to *An. darlingi*) breeding sites.

B-08

Simulating within-vector generation of the malaria parasite diversity

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Plasmodium falciparum, the most virulent human malaria parasite, undergoes asexual reproduction within the human host, but reproduces sexually within its vector host, the *Anopheles* mosquito. Consequently, the mosquito stage of the parasite life cycle provides an opportunity to create genetically novel parasites in multiply-infected mosquitoes, potentially increasing parasite population diversity. Despite the important implications for disease transmission and malaria control, a quantitative mapping of how parasite diversity entering a mosquito relates to diversity of the parasite exiting, has not been undertaken. To examine the role that vector biology plays in modulating parasite diversity, we develop a two-part model framework that estimates the diversity as a consequence of different bottlenecks and expansion events occurring during the vector-stage of the parasite life cycle. For the underlying framework, we develop a stochastic model of within-vector *P. falciparum* parasite dynamics and go on to simulate the dynamics of two parasite subpopulations, emulating multiply infected mosquitoes. We show that incorporating stochasticity is essential to capture the extensive variation in parasite dynamics, particularly in the presence of multiple parasites. In particular, unlike deterministic models, which always predict the most fit parasites to produce the most sporozoites, we find that occasionally only parasites with lower fitness survive to the sporozoite stage. This has important implications for onward transmission. The second part of our framework includes a model of sequence diversity generation resulting from recombination and reassortment between parasites within a mosquito. Our two-part model framework shows diversity increases with the possibility for recombination and proliferation in the formation of sporozoites. Furthermore, when we begin with two parasite subpopulations in the initial gametocyte population, the probability of transmitting more than two unique parasites from mosquito to human is over 50% for a wide range of initial gametocyte densities.

B-09

The role of transcription factor T-bet in B cell mediated responses to *Plasmodium* infection

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Malaria is a global health concern which affects over 200 million individuals worldwide. Although the immune system rapidly responds to *Plasmodium* infection with specific antibodies, natural antibodies fail to establish long term protection, often leading to repetitive infections and chronicity. Recent studies showed that in a chronic malaria setting both B cells and T cells induce expression of T-bet transcription factor. It is not completely understood whether this is a part of the specific host defense or a result of disease pathogenesis. Here using transgenic mouse models, we show that global knockout of T-bet or IFN-g similarly reduces survival of mice compared to WT upon infection with *Plasmodium chabaudi*, therefore suggesting a protective role for T-bet. To determine the specific role T-bet plays in B cell responses to *Plasmodium* we first identified that T-bet expression in mouse B cells are triggered by dual stimulation of IFN-g and B cell receptor. Similarly using Tbet-zsgreen transgenic mice we showed that conditions specific to *Plasmodium chabaudi* infection drives T-bet expression in multiple B cell subsets in a time dependent fashion. However, no such induction is shown when mice are immunized with T-dependent protein antigens adjuvanted with Alum. On the other hand, bone marrow chimeric animals reconstituted with equal amounts of congenically labeled WT and T-bet KO bone marrow cells showed that for most B cell subsets the lack of T-bet does not change the ratio of WT and KO cells upon *Plasmodium* infection indicating that T-bet expression may not be equally critical for all B cell responses to infection. These results demonstrate the need for further investigation on the role of T-bet transcription factor in development of humoral responses against *Plasmodium*.

B-10

A point-of-care assay to detect antimalarial drugs from finger stick blood samples

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Artemisinin-based combination therapies (ACTs) have begun to fail as first-line therapies for the treatment of *Plasmodium falciparum* malaria in Southeast Asia. Preventing the spread of drug-resistant parasites to Africa is a top priority for global malaria elimination campaigns. A low-cost, field-based assay to detect slow-clearing ACT partner drugs from patient blood samples would allow us to track antimalarial drug use, monitor drug compliance, and could serve as an indicator of previously failed treatment and spreading resistance. The goal of this research is to develop a rapid, colorimetric assay to detect several slow-clearing ACT drug compounds from finger stick blood samples. In order to do this, we aim to identify aptamers that bind and differentiate between small molecule partner drugs. Aptamers are single stranded DNA molecules selected from a pool of random DNA sequences for their binding affinity to various targets. They can be selected in vitro and their binding can be tailored for specificity and sensitivity under varying conditions. We are using a capture-SELEX method to identify aptamers that, when dye-tagged in a structure switching sensor format, will allow for a micromolar limit of drug detection. Our current focus is to optimize SELEX protocols to improve aptamer sensitivity and specificity.

B-11

Integrative genomic approaches to study *in vivo* host and parasite transcriptome in malaria

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Genomic research in malaria has been approached mostly from the perspective of either host or pathogen, but rarely both jointly. Given the interplay between pathogens and host factors, it is logic to expect that both host and pathogen interact at multiple levels. Thus, there is a need to complement current mapping studies performing GWAS on the host and sequencing of the pathogen to study the complex architecture of host-pathogen interactions. Here we present the preliminary results of an effort to develop an innovative integrative approach to achieve this goal, demonstrate the feasibility of performing joint RNASeq profiling of host and parasite transcriptome and show an application of the approach using peripheral blood samples

from a pediatric cohort in Burkina Faso. The results highlight the potential of joint host-parasite genomic profiling to enable novel applications in epidemiological malaria genomic research.

B-12

Evaluating three years of a targeted IRS campaign in a high transmission area of northern Zambia

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Evidence-based approaches are needed to meet malaria control and elimination goals in endemic countries. The World Health Organization has identified vector control as a key strategy to reduce transmission, however limited resources and surveillance capacity remain a challenge in regions with the greatest need. An increase in malaria cases has been reported in Luapula Province, northern Zambia since 2007 despite consistent reductions nationwide. In response, a large-scale indoor residual spraying (IRS) campaign was conducted using novel methodology to target high-transmission areas through household satellite mapping and health center malaria reports. Annual targeted spraying was done prior to the rainy season in 11 districts in Luapula Province starting in 2014 using the organophosphate insecticide Actellic. A multi-year evaluation of the IRS campaign was conducted in Nchelenge District, Luapula Province using active malaria surveillance data collected by the Southern Africa ICEMR project. From April 2012-July 2017, 3,527 participants from 1,080 households participated in cross-sectional surveys in sprayed and unsprayed areas, with an overall parasite prevalence by RDT of 50%. Fifty five percent of households within IRS-targeted areas reported being sprayed with Actellic. In multivariate regression analyses controlling for demographics, weather, and geographic factors, there was a 25% reduction in rainy season malaria prevalence by RDT within the IRS-targeted area compared with the same geographical area pre-intervention (P<0.001) but no decrease in dry season malaria prevalence. However, malaria prevalence in adjacent unsprayed areas increased each year throughout the period of surveillance, and a difference-in-differences analysis to compare pre- vs. post-intervention malaria prevalence between sprayed and unsprayed areas revealed a significant effect of IRS for both rainy and dry seasons (P=0.02 for each). Targeted IRS using Actellic had a moderate but measurable impact on parasite prevalence in Nchelenge District, but more intensive interventions are needed to achieve Zambia's malaria elimination goals.

B-13

Longitudinal analysis of *Plasmodium falciparum*-specific atypical and classical memory B cell responses to natural malaria infection in children and adults

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In naturally acquired immunity to malaria, antibodies that reliably protect are only acquired after years of repeated *Plasmodium falciparum* (*Pf*) infections. We have shown in Mali that this inefficiency in humoral immunity to malaria is associated with an expansion of $CD21^{lo}CD27^{-}$ 'atypical' MBCs that are isotype-switched and somatically hypermutated, but that exhibit markedly reduced B cell receptor (BCR) signaling and effector function. It remains unclear, whether atypical MBC expansion is *Pf*-specific or represents a global expansion as a result of chronic immune activation from repeated *Pf* infections and/or co-infections that are common in malaria-endemic areas. Using B cell probe staining we recently identified an expanded population of *Pf*-specific MBCs in malaria-exposed individuals in Mali. Using a similar B cell probe approach, we are tracking *Pf*AMA1/*Pf*MSP1-specific classical and atypical MBCs in a longitudinal cohort study of children and adults in Mali. Using B cell probes specific for influenza, we will determine the relative role of *Pf* in driving atypical MBC expansion and also compare the magnitude and kinetics of *Pf*- and influenza-specific atypical and classical MBC responses from ages 3 months to 25 years at well-defined time points before, during and after acute *Pf* infection. In addition, we will single cell sort antigen-specific atypical and classical MBCs to compare their BCR features (VH gene diversity and somatic hypermutation rates), and

the affinity and function of expressed monoclonal antibodies. This analysis should provide important new insights into the mechanisms underlying the delayed acquisition of naturally acquired immunity to malaria in children.

B-14

Mitochondrial population structure of *Anopheles funestus* in southern and central Africa

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Malaria control and eradication faces many challenges; among these is *Anopheles funestus*, one of three major malaria vectors in sub-Saharan Africa. *An. funestus* poses a significant threat because of its expansive distribution and high rates of insecticide resistance. However, relatively little is known about the population structure and dynamics of *An. funestus* compared to other major malaria vectors such as *An. gambiae* and *An. arabiensis*. In this study, individual samples (N=44) from geographically distant sites in Zambia, Democratic Republic of the Congo, and Tanzania were subject to whole genome sequencing for determining the degree of population structure of *An. funestus* in Southern Africa, and for the development of genome-wide markers suitable for population genetic studies at the spatial scale of this region. A reliable set of single nucleotide polymorphism markers will allow for high-throughput and cost-effective population genomic analyses at the spatial scale required by this study. Preliminary assessment suggests that more gene flow than expected exists between populations from Zambia and DRC, however a more detailed analysis is required. Robust estimate of gene flow between populations, especially related to insecticide resistance genes, could be used to assess efficacy of vector control. Moreover, association of whole-genome-based genetic clusters in relation to our discovery of sympatric *An. funestus* mitochondrial Clade I and Clade II in northern Zambia could illuminate if mitochondrial clades are relevant to mating structure within the *An. funestus* complex. Additional structure within the complex, if related to ecological or behavioral traits, would have implications for vector control strategies.

B-15

Multi-stages antiplasmodial activity of purified fractions from *Aspergillus niger*, an endophytic fungi, from *T. catappa*

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New efficient drugs for the effective management and control of malaria are urgently needed. In view of the vast repertoire of natural bioactive products associated with endohytes with potential applications in medicine, the present work was designed to assess the bioguided fractionation of extract from *Aspergillus niger*, an endophytic fungus isolated from a Cameroonian Antimalarial plant *Terminalia catappa* with the aim to discover potent compounds against the malaria parasites. *Aspergillus niger* extract with IC_{50} 2.25-6.69 µg/mL displayed promising antiplasmodial potencies against both Chloroquine sensitive *Pf3D7* and resistant *PfINDO* strains and were large scale fermented (6L). Twelve grams of *Aspergillus niger*'s crude extract from large scale fermentation were fractionated by two successive rounds of silica gel chromatography followed by RPHPLC. Antiplasmodial potency was found to increase at successive bio-guided steps of chromatography from IC_{50} 4.03 µg/mL (crude extract) to 1.44 µg/mL (silica gel 1) to 0.59 µg/mL (silica gel 2) to 0.09µg/mL (RPHPLC). The resistance indices of purified molecules/fractions ranged from 0.30 to 3.2 while the selectivity indices were in the range of 7.75 to 120.3. Fractions 18 and 59 with IC_{100} concentration showed complete inhibition of the *Pf3D7* ring stage growth only 12hours after incubation. Against *Pf3D7* trophzoite stage, fractions 18 and 59 showed complete inhibition of the parasite growth after 36 hours incubation with significant modification of the morphology of malaria parasite after 12 and 24hours incubation. While against schizont stage, fractions 18 and 59 showed complete inhibition of the parasite growth after 24 hours incubation with inhibition of the transition of schizont to ring after 12 hours incubation. The study of the ROS mediated action of these fractions showed no significant production of H_2O_2 after 6hours incubation with trophozoite stages when compared to the

control. Our investigations showed that endophytic fungus *A. niger* from Cameroonian medicinal plants have the potential to provide novel pharmacophores against the malaria parasite. Further studies are ongoing to isolate/characterize such potent entities from these purified fractions.

B-16

Interrogation of the seasonal microbiome of *Anopheles coluzzii* in Mali

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The poorly understood mechanisms of the seasonal maintenance of *Anopheles* spp. mosquitoes through the dry season in Africa remain a critical gap in our knowledge of *Plasmodium* disease transmission. While it is thought that adult mosquitoes remain in a dormant state throughout this seven-month dry season, the nature of this state remains unknown and has largely not been recapitulated in laboratory settings. To elucidate possible life history traits allowing for this phenotype, the spatiotemporal change in the microbiome of mosquitoes in the dry and wet seasons in Mali was analyzed by sequencing the 16S ribosome bacterial region in mosquitoes collected from two locations with varying water availability. These locations were a village near the Niger River with year-round water sources (N'Gabakoro, "Riparian"), and an area closer to the Sahara with highly seasonal breeding sites (Thierola Area, "Sahelian"). The 16S bacterial data consisted of 2057 unique sequence variants in 426 genera across 184 families. With these, we found compositional differences that were seasonally and spatially linked. Counter to our initial hypothesis, there was a more pronounced seasonal difference in the Riparian bacterial microbiome than the Sahelian area. These major seasonal shifts were in *Ralstonia, Sphingorhabdus,* and *Duganella* spp. bacteria that are usually soil and water-associated, indicating that these changes may be from bacteria acquired in the larval environment, rather than during adulthood. Additionally, through cytochrome B analysis we found a greater heterogeneity in host choice of *An. coluzzii* independent of season in the Thierola area compared to N'Gabakoro (77.5% vs. 94.6% anthropophily, respectively), which may indicate a relaxation of anthropophily in some locations. This study indicates there is limited evidence for strong seasonal microbiome abundance variation in areas where aestivation is likely to be occurring, though there may be qualitative biomarkers for seasonality based on presence/absence of bacterial genera.

B-17

Identifying adulticidal agents produced by field-collected entomopathogenic *Chromobacterium* species

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Through their transmission of the malaria parasite *Plasmodium*, *Anopheles* mosquitoes are culpable for the deaths of over a half million people annually. Despite ongoing efforts to develop an effective vaccine, vector control remains the most viable approach to preventing human malaria infections. However, continued emergence of mosquito resistance to commonly used insecticides fuels ongoing efforts to identify and develop new mosquitocidal agents, particularly from natural products. The past success of mosquito control products developed from the soil-dwelling larvicidal bacteria *Bacillus sp* led us to survey diverse environments for additional bacterial isolates that demonstrate insecticidal activity. The *Chromobacterium* genus, another soil-resident group of microbes, has been previously shown to exert insecticidal (larvicidal and adulticidal) activity, but the molecular basis of this activity has not yet been characterized. We have identified a novel *Chromobacterium, C.* species *Panama* (*Csp_P*) as having adulticidal effects when live bacterial cultures are fed to female *An. gambiae* mosquitoes. We have also collected several uncharacterized *Chromobacterium* species from the field which may have insecticidal properties. Furthermore, we have identified that the adulticidal agent appears to be secreted into the supernatant of stationary cultures in the case of *Csp_P*. Continued investigation into this effect will take a fractionation as well as comparative genomics approach to determine the identity of the insecticidal agent or agents produced by *Chromobacterium.* Natural products derived from these bacteria may ultimately be leveraged into new tools for vector control and reduction of the global burden of malaria.

B-18

***Polyalthia suaveolens* as a potential source of inhibitors of ring stage from *Plasmodium falciparum* chloroquine resistant K1 strain**

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Malaria is a one of the deadliest diseases and represents a serious public health in Sub-Saharan Africa which is the most affected region with 92 % of the deaths recorded in 2015. *P. falciparum* is most dangerous specie responsible of 99 % of death in tropical and subtropical regions. The currently prescribed treatments particularly the Artemisinin-based Combination Therapies (ACTs) are threatened by emerging resistance of *P. falciparum* strains. Given these limitations, there is a motivated search for new drugs against *Plasmodium* parasites. Nowadays, exploration of medicinal plants is an evidence to develop the new antimalarials components. Particularly, *Polyalthia suaveolens* (Annonaceae family) is known for their wealth in active molecules (alkaloids, phenols and acetogenins). The antiplasmodial effect of extracts, fractions and subfractions against *P. falciparum* Chloroquine resistant (PfK1) strain was determined using SYBR green florescence assay. The promising fraction was assessed for cytotoxicity against Human Foreskin Fibroblast (HFF) cells. The methanol sub-fraction of P. suaveolens [PStw(Ace)] showed the highest potency with IC_{50} of 3.24 µg/mL. Sub-fraction PS8 from PStw(Ace) was the most active with IC_{50} of 4.42 µg/ml. Overall, the results achieved showed, the PStw(Ace) fraction, more interestingly might be of interest to identify exactly the active site in genetic sequence of *P. falciparum* parasite for the future development of an antimalarial phytodrug.

B-19

Differences in the brain vasculature: implications for cerebral malaria

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Cerebral malaria (CM) is a severe complication of malaria with mortality rates of up to 30%. It affects mostly children in sub-Saharan Africa where it causes neurological impairment. A hallmark of CM is the sequestration of *Plasmodium falciparum*-infected red blood cells (Pf-IRBC) in the brain microvasculature via binding of *Plasmodium falciparum*-encoded erythrocyte membrane protein-1 to the host endothelium via receptors such as intercellular adhesion molecule-1 and endothelial protein C receptor (EPCR). The pathogenesis of CM is still not well understood and its comprehension will be helpful in the development of appropriate adjunctive interventions to prevent neurological sequelae. Sequestration of Pf-IRBC in CM is observed in both the white matter (WM) and gray matter (GM) of the brain. Importantly, the pathology of CM in the WM is predominantly hemorrhagic, whereas this is not observed in the GM. In this study, we hypothesized that the difference in CM pathology as observed in the GM versus WM of the brain is due to differences in the protein receptors expression profile of their respective vasculature. Microvessels from different brain areas were isolated and immune stained for CD31 (endothelial cell marker), glucose transporter type 1 (GLUT-1), EPCR, and alkaline phosphatase (AP). Overall, these microvessels differed in their expression of EPCR, GLUT-1 and AP. This difference may be due to their different cellular environments. Specific vessels in the various brain areas may therefore differ in their susceptibility for the sequestration of Pf-IRBC and/or lead to differing host endothelial responses. In CM, this may explain the differential pathologies of the GM vs WM in CM patients. Knowing that the brain microvasculature differs in the expression of some receptors/transporters will lead to a better understanding of CM pathology and may have implications for other neurological diseases involving vessel pathologies, including multiple sclerosis and stroke.

B-20

Serosurveillance to inform malaria elimination programs in South-East Myanmar

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Malaria prevalence in Myanmar has declined dramatically over the past decade. Plasmodium cases, however, are still highest in Myanmar compared to other countries within the Greater Mekong region. The presence of artemisinin-resistant P. falciparum in the region prompted the WHO to recommend total malaria elimination by 2030. Accurate surveillance of Plasmodium transmission in Myanmar is therefore imperative to achieving this goal. The objective of this study was to explore the use of serosurveillance to identify populations at high risk of infection in Southeast Myanmar, and to longitudinally observe changes in the immune response to Plasmodium spp. in a region that has recently achieved a marked decline in malaria transmission (> 90% reduction in disease incidence between 2005 - 2014 in some provinces). We performed a longitudinal study of 114 villages across South-East Myanmar from April 2015 to June 2016. RDTs and ELISA's were performed on > 15,000 participant dried blood spots to determine current infection and exposure levels. Prevalence of Plasmodium spp. infection by RDT was extremely low (<1%), however, serology revealed high levels of IgG specific for P. falciparum schizont extract. IgG seroprevalence was greater in high-risk populations (migrant workers (41%) and forest dwellers (50 %)) compared to village residents (36%). IgG responses were sustained (>40% seroprevalence) throughout the 14 months of the study. Through measuring serological responses to malaria, we have revealed that antibodies are being maintained in a pre-elimination setting despite recent large reductions in malaria incidence. Antibodies were also capable of identifying high-risk populations, and the maintenance of high IgG levels over time may be indicative of an undetected parasite reservoir in the region. Combined with molecular diagnosis, serosurveillance could prove to be an important surveillance tool to inform the ambitious target of malaria elimination by 2030 in the Greater Mekong region.

B-21

Direct cost of severe malaria management in children under 15 years in the Democratic Republic of Congo

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As a major public health problem, malaria also affects the household economy in low-income countries, including the DRC. Per Capita Gross Domestic Product was 489,9$ in 2016. Several partners are committed to support malaria control by making available malaria inputs to reduce the economic and social burden of this endemic in the DRC. The aim of this study was to evaluate the direct cost of severe malaria management in children under 15 years. The study was carry out in 3 General Hospitals, Tshikaji general Hospital in rural zone Kinshasa provincial General in the dowtown of Capital of DR Congo and Ndjili General Hospital in suburban part of Kinshasa. More than 200 severe malaria cases in each hospital were analyzed in considering differents costs paid by families for the children, The average direct cost of severe malaria management was respectively $ US114$ ($ 54-$ 314), $ US121 $($ 65- $ 380) and $ US 335 ($ 169- $ 864) In Tshikaji Hospital, in Ndjili and in Kinshasa Provincial Hospital. In Tshikaji, Sanru, a NGO, will cover half of the costs. In despite this reduction the cost seems excessive for this rural population. Considering that a Congolese lives with less than 3 dollars a day. The direct cost of managing severe malaria would be unaffordable to the population and would be a factor of impoverishment. A total subsidy of severe malaria in children would be an approach for better care and a measure to reduce the economic burden of malaria.

B-22

Towards malaria elimination in Laos: a mixed methods study on health-seeking behaviors among different ethnic groups living in malaria endemic areas.

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Malaria has been affecting mankind for centuries. Even with the advent of medical technology in the past centuries, we

have unsuccessfully attempted to put a stop once and for all to this debilitating disease. However, past failures in malaria control and eradication hinted that biomedical approach alone, which was no doubt necessary, was never sufficient in the fight against malaria. This study is divided into three parts. The first part attempts to critically look at the malaria phenomenon through philosophical and anthropological lens and examine why mankind's fight against malaria has not always been successful. The second part zooms in on an issue related to malaria elimination in the Greater Mekong Subregion (GMS): the usage of primaquine and glucose-6-phosphate dehydrogenase deficiency (G6PDd). We conducted a systematic review on the clinical symptoms of G6PDd in the GMS. The major clinical symptom of G6PDd in the GMS was hemolysis induced not only by primaquine but also by other medicines and infections. Many malaria endemic areas in the GMS are remote and difficult to access. If hemolysis occurs, this might result in the loss of life. Therefore, our results highlighted the necessity to screen for a patient's G6PD status before primaquine prescription to reduce the burden of treatment at local settings where key resources are limited. For the third part, we plan to conduct an ethnographic mixed methods study to understand the health-seeking behaviors of the ethnic minorities in Lao PDR in the context of malaria.

B-23

Translation of poly-adenosine tracks is a stumbling block for most eukaryotes but not for malaria parasites

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The unicellular Apicomplexan parasite *Plasmodium falciparum* causes the most severe malaria symptoms in humans. In recent years, the health burden of malaria has been decreasing due to efficient prevention and control treatments. Nevertheless, there are still 200 million cases of infection resulting in around 429 000 deaths per year. Genome sequencing has laid the foundation for significant biological advances by exposing some surprising genomic information about this parasite. One interesting revelation from the genomic data is that *Plasmodium* exhibits an astonishing amount of AT richness with multiple polyadeninosine (polyA) stretches. In most eukaryotes, polyA tracks in gene coding sequences act as negative gene regulation motifs, facilitated through ribosomal stalling and frameshifting, resulting in mRNA and protein degradation. However, *Plasmodium* species are an intriguing exception to this rule. The percentage of polyA-carrying transcripts in the *Plasmodium falciparum* genome exceeds 50%, compared to a median of 2% in tested eukaryotic genomes. According to our data, polyA tracks are efficiently expressed in *P. falciparum,* but the stretches of AAG had down regulation of protein expression, again completely opposite than in other eukaryotes that we have tested. By understanding this process, we will begin to understand the fundamental differences between *Plasmodium* translation and all other characterized eukaryotes. Further, we have dissected components of *P. falciparum* translational machinery and mRNA surveillance pathways. We find that in comparison to human cells, absence of classical No-Go decay (NGD) pathway as well as specific features of *Plasmodium* ribosomes plays a role in polyA track translation. Our analysis of RACK1 and Pelo protein in human cells indicates a role for this protein in both NGD and non-stop decay (NSD) mechanisms. The presence of Pelo and lack of ribosome-associated *P. falciparum* RACK1 protein compared to the other eukaryotes suggest alternate mechanisms for targeting NSD and NGD substrates in malaria parasites.

B-24

Investigating the kinetics of transgenic *Plasmodium falciparum* HRP2 protein produced by *P. berghei* in a novel murine model

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Improved diagnostics for subclinical malaria are needed, as these cases act as transmission reservoirs but are currently difficult to detect. One way to better diagnose these cases is to increase the sensitivity of the malaria rapid diagnostic test (RDT) in detecting the presence of the *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2). To develop more sensitive diagnostic tools, a deeper understanding of PfHRP2 protein dynamics, localization, and kinetics *in vivo* both during and post infection is needed. Using a mouse model, we determined the average plasma half life of recombinant PfHRP2 protein to be ~2.1 hours following intraperitoneal injection, with a total clearance time of 4 days. Field studies have shown that PfHRP2 can persist in circulation for 1-4 weeks post parasite clearance, presenting an additional challenge for extending the limit of assay detection for PfHRP2. We established a novel murine model using a transgenic *P. berghei* parasite expressing PfHRP2 to study this phenomenon. We demonstrated that the

transgenic parasite produces PfHPR2 in the erythrocyte and exports it into the erythrocyte cytoplasm, similar to *P. falciparum*. In our model, PfHRP2 persists for 5-7 days in plasma and 8-9 days in erythrocytes following the start of artemisinin-based combination therapy. The half-life of PfHRP2 during infection was also significantly longer in the erythrocytes compared to the plasma. Due to the significantly extended persistence of PfHRP2 in erythrocytes compared to plasma, we hypothesized that persistence could result from slow clearance of PfHRP2 from the red blood cells, through the process of erythrocyte pitting, a consequence of the increased rigidity of infected cells. Preliminary immunofluorescence data has corroborated our pitting hypothesis, which we will explore further. In conclusion, we developed a novel murine model to investigate PfHRP2 kinetics and dynamics *in vivo* to eventually develop more sensitive and powerful diagnostics.

B-25

Amplicon Deep Sequencing of *Plasmodium falciparum* Suggests Cross-Border Malaria Transmission between Zambia and the Democratic Republic of Congo

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In Zambia, the national goal to eliminate malaria by 2020 is threatened by potential carriage of parasites across borders in human or mosquito hosts. Cross-border malaria transmission was evaluated using parasite genetics between Nchelenge District, Luapula Province (malaria RDT$^+$ prevalence 30-50%) in northwest Zambia and two villages, Kilwa and Kashobwe, across the border from Nchelenge District in the Haut-Katanga Province of the Democratic Republic of Congo (DRC) (malaria RDT$^+$ prevalence 47%). Randomly selected households in Nchelenge, Kilwa, and Kashobwe were visited in June and July 2016 and consenting individuals provided a dried blood spot (DBS) for detection of *Plasmodium falciparum* by PCR and genetic analysis. Among the DBS which tested positive for *P. falciparum* by PCR, 41 samples from Nchelenge, 39 from DRC, and 15 positive controls (spiked with known concentrations and varying ratios of laboratory *P. falciparum* strains NF54, 3d7, and 7g8) were extracted and prepared for amplicon deep sequencing at the CSP, AMA1, and var2csa, loci. Three hundred base pair paired end sequence reads generated on the Illumina MiSeq platform were stitched using Flash, filtered for quality control, and clustered by haplotype using SeekDeep. Discriminatory analysis of principal components (DAPC) revealed both shared diversity and population partitioning between the four sampling locations. Parasites sampled within the same country were not more genetically similar than parasites sampled from different countries. Overall, our findings are consistent with high levels of gene flow between populations on either side of the border. This research will inform regional control programs on the need to coordinate malaria elimination efforts, particularly along border regions.

B-26

Upregulation of gametocytogenesis in artemisinin-induced dormant forms of *Plasmodium falciparum*

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The deadliest form of human malaria is primarily caused by the protozoan parasite *Plasmodium falciparum.* These parasites establish pathogenicity in the human host with a very low number of sexual forms or gametocytes, which are transmitted by mosquitoes. Several studies have reported that exposing artemisinin sensitive *P. falciparum* rings to dihydroartemisinin (DHA) results in dormancy, and the artemisinin induced dormant (AID) forms recovered into normal growth stages after 5-20 days. In this study, we tested artemisinin resistant *P. falciparum* parasites for the development of AID forms and their recovery. Interestingly, we found that exposure of an asynchronous culture of artemisinin resistant *P. falciparum* IPC 5202, a line carrying a mutation in the *PfK13* gene, which is linked to artemisinin resistance, results in dormancy. Both the ring and late stages of these AID forms recovered after 10-15 days. Furthermore, 70-80% of the recovered dormant forms developed into sexual forms or gametocytes after 20-30 days, which is almost 7-8 times higher rate of conversion of asexual to sexual forms or the malaria transmissible forms. In contrast, only early ring forms of artemisinin sensitive parasites recovered slowly, and additional exposure of these parasites to artemisinin results in complete clearance within 4 days. This is contrast to the resistant parasites exposed to a second

dose of artemisinin which resulted in a very high rate of dormancy and recovered into sexual forms or gametocytes. The effect of DHA on the viability, development of AID forms, recovery, gametocytogenesis of various *P. falciparum* K13 mutants will be discussed.

B-27

Using MRI to elucidate the mechanism of murine cerebral malaria rescue by inhibition of glutamine metabolism

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We have previously demonstrated the efficacy of the glutaminase inhibitor, 6-diazo-5-oxo-L-norleucine (DON), as a possible adjunctive therapy to mitigate the clinical severity of human cerebral malaria (HCM). The pathology of HCM is heterogeneous, but generally characterized by the accumulation of infected red blood cells on the brain vascular endothelium, cerebral microhemorrhages, compromised blood-brain barrier, and brain swelling. These changes manifest in severe neurologic symptoms including headache, fever, ataxia, seizures, retinopathy, and coma. When susceptible C57BL/6 mice are infected with *Plasmodium berghei* ANKA (*Pb*A) parasites, they develop experimental cerebral malaria (ECM), which approximates the main characteristics of HCM. Studies in these mice found that treatment with DON or with a next generation glutaminase inhibitor, JHU-083, even in late stage ECM, rescued animals and increased survival while allowing for recovery of blood-brain barrier dysfunction and brain swelling. Previously, Seydel *et al.* (*NEJM* 2015) used magnetic resonance imaging (MRI) to characterize HCM pathology and correlate it to disease severity in children in Malawi. They observed increased brain volume in children that died from HCM that was uncommon in children that survived HCM. We performed MRI studies in the mouse model to determine if the disease course effectively recapitulates that of HCM, based on morphological changes in MRI. Preliminary studies have demonstrated that brain dysfunction initiates in the olfactory bulb and migrates rostral to caudal as the disease progresses. Herein, we utilize MRI to demonstrate recovery with JHU-083 treatment to evaluate the drug for possible clinical trials in HCM. Furthermore, we utilize immuno-histochemistry to visualize the role of immune response in brain tissue of children who succumbed to CM and compare those changes to brain tissue of animals with ECM both before and after treatment.

B-29

Evaluating the efficiency of reactive case detection to achieve malaria elimination in rural southern Zambia using follow-up household visits and parasite genotyping

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Targeted interventions to identify and treat the asymptomatic reservoir have been implemented in areas approaching malaria elimination. Reactive case detection is currently conducted in Southern Province, Zambia to enhance surveillance and clear the asymptomatic reservoir. After an index case is confirmed with malaria by a rapid diagnostic test (RDT), household members and neighbors residing within 140-meters are tested with an RDT and treated with ACT if positive. The efficiency of this strategy to detect and treat *P. falciparum* infections was evaluated in the catchment area of Macha Hospital in Southern Province. A subset of index cases was evaluated by a study team who administered a questionnaire, performed an RDT, and collected a blood sample on filter paper for detection of *P. falciparum* DNA by qPCR. As part of the study, the screening radius was extended to 250-meters and follow-up visits were performed 30 and 90 days after the initial visit. From March 2016-January 2017, 139 households with 935 residents participated. Parasite prevalence and transitions between RDT and qPCR positivity were compared between residents of index and neighboring households over the follow-up. Parasite genetic relatedness between persistent infections within individuals and new infections within households were analyzed using a *P. falciparum* 24 SNP molecular barcode. Overall, parasite prevalence was higher in index households (1.4% by RDT, 6.9% by qPCR) compared to neighboring households (0.7% by RDT, 2.7% by qPCR). Parasite prevalence by RDT and qPCR decreased over follow-up visits but *P. falciparum* infection persisted and was not eliminated in study households. Persistent infections were detected by qPCR among those negative by RDT. Identical molecular barcodes were detected among persistent infections within individuals and among newly identified infections within

households. The efficiency of reactive case detection in this setting is limited by the low sensitivity of the RDT and continued focal transmission after the intervention. Focal drug administration in the index household may be a more efficient strategy to achieve elimination.

B-30

Control of *Anopheles albimanus* mosquitoes: improving vector control with a RNAi based strategy

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Tropical diseases are a long standing companion for humanity. The complexity of the vectors and parasites life cycles requires deep understanding of their biology and behavior in order to design effective tools to reduce and eventually eliminate transmission. Guatemala is one of the many Latin American countries with active transmission of Malaria, and *Anopheles albimanus* is the main vector in the zone. One of the strategies with probably more probabilities to succeed is the Sterile Insect Technique (SIT), and one of the key points to be improved is the fitness of the sterile males. In the 1970s, a successful evaluation of a chemosterilization method was performed in El Salvador for *Anopheles albimanus*, the primary vector of malaria in Central America. If instead of radiation, an RNAi-rearing of the larvae achieves the production of sterile males, that would improve the fitness and reduce the safety hazards of radiation. We propose to develop a new generation of mosquito sterilization tools based on the oral delivery of bacteria expressing dsRNA specific for spermatogenesis genes expressed during larval development. We selected the *An. albimanus* homologues of *boule* and *zpg*, for their importance in meiosis and sperm development. We studied their expression during the life cycle of the mosquito. We designed a protocol for oral delivery of dsRNA produced by bacteria. We also developed a protocol to evaluate male sterility in the laboratory. After testing several gene fragments, we obtained stable inducible inserts of ~200 bp for both genes. We are currently designing long dsRNA and hairpin-dsRNA, to test silencing efficiencies with alternative bacterial expression strategies. We will use the tools and protocols thus far developed to feed larvae with bacteria expressing dsRNA and evaluate the silencing effects on fecundity.

B-31

***Plasmodium* exploits the host's vesicular pathways in the liver stage**

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To successfully complete its life cycle in the human host after being transmitted by mosquitoes, Plasmodium parasites must first invade hepatocytes. Within these cells, parasites replicate extensively to form blood-infectious forms that are released into the bloodstream. Despite clear exploitation of host cell resources during infection, little is known about the host factors that are essential for parasite development in hepatocytes. To identify these host factors, we executed a high-throughput siRNA screen of the human druggable genome. Interestingly, we discovered that the host vesicular transport pathway is critical in hepatic development of Plasmodium parasites. Gene suppression or chemical inhibition of the vesicular transport pathway significantly reduces Plasmodium berghei parasite load in hepatocytes. Furthermore, phenotypic analyses upon genetic or pharmacological disruption indicate that host trafficking components are key modulators of parasite growth. These results suggest that Plasmodium parasites co-opt the host hepatocytes' trafficking network, and implicate this pathway in the establishment of a successful infection.

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Using single-cell transcriptomics to elucidate sexual commitment and differentiation in *Plasmodium falciparum*

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Sexual reproduction is an obligate step in the *Plasmodium falciparum* life cycle, with mature gametocytes being the only form of the parasite capable of human-to-mosquito transmission. During the red blood cell stage of human infection, less than five percent of *P. falciparum* parasites commit to a sexual fate. This occurs in a 3:1 ratio of females to males that mature through five distinct gametocyte stages over 10-12 days before being transmitted to the mosquito. Previous studies utilizing *Plasmodium berghei* indicate that male and female gene expression is highly distinct. But in *P. falciparum*, although over 300 genes are predicted to be gametocyte specific, only a few genes have been postulated to be male or female specific. Since these

markers are expressed at late stages and their gender-specific expression is debated, separating male and female populations remains technically challenging. Additionally, it is not known what determines male/female commitment. Furthermore, there is a large amount of heterogeneity in the parasite population, especially at early gametocyte stages. To overcome these challenges, we have developed an unbiased single-cell approach to determine which transcripts are temporally expressed in males versus females. Using microfluidic technology, we have isolated over 300 single parasites at synchronized asexual and sexual stages to compare the expression of 90 conserved gametocyte-specific genes. These analyses have identified gender-specific gene expression for mid-to-late stage gametocytes, including two male-specific candidate genes. These validated male-specific genes are being knocked out using CRISPR-Cas9 technology and will be tested for their roles in male gametocyte development and gamete exflagellation.

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Modelling the epidemiological implications and explanations of discordances between microscopy and rapid diagnostic tests

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Rapid diagnostic tests (RDTs) have transformed malaria diagnosis. The most widely used *P. falciparum* RDTs detect histidine-rich protein 2 (PfHRP2), however false-negative RDTs due to *pfhrp2* gene deletions have been reported in a number of countries.[1] The use of PfHRP2-based RDTs has been suggested to select for *pfhrp2*-deleted parasites,[2] which would result in an increasing number of false-negative RDT results and an increase in the number of missed clinical cases. Using a previously developed individual-based model of malaria transmission that tracks *pfhrp2*-deleted parasites and individual infection histories, we model the potential impact of false-negative test results on clinical incidence. Using recorded discordances between microscopy-based diagnosis and diagnosis by RDTs from Demographic and Health Surveys to estimate the rate of false-negative RDT results, we estimate the *pfhrp2*-deletion frequency at first-administrative regions in 18 countries in sub-Saharan Africa. These estimates are subsequently used to map the estimated missed clinical incidence due to false-negative RDT results resulting from *pfhrp2* gene deletion. We conclude by conducting sensitivity analysis, exploring the impact of additionally conducted microscopy-based diagnosis, non-adherence to RDT results and additional sources of false-negative test results not due to *pfhrp2*-deletion. Our findings indicate that although *pfhrp2*-deletions could result in untreated clinical cases, there are a number of alternative explanations for elevated false-negative RDT results. Genetic surveillance efforts are thus needed to further our understanding of the observed RDT false negative.

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Interactions between *Plasmodium* parasites and *Anopheles* salivary glands

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Plasmodium parasites cause malaria, a mosquito-borne disease with hundreds of millions of infections that lead to ~500,000 deaths each year. Female mosquitoes acquire parasites in blood meals taken from previously infected individuals. *Plasmodium* undergoes reproduction and development in and on the midgut. Sporozoites are then released into the mosquito hemocoel, where they locate and specifically invade the salivary glands (SGs). During subsequent blood meals, the SGs represent the gateway for *Plasmodium* transmission to a new host. We sought to characterize interactions between sporozoites and SG cells using confocal immunofluorescence microscopy to better understand this stage of malaria transmission. *Plasmodium berghei*- (either ANKA or GFP-tagged ANKA) infected *Anopheles stephensi* SGs were dissected at time points between 18 and 30 days post-infection and stained for features including SG secreted saliva proteins, cytoskeletal markers, organelle markers, dyes (lipids, chitin/O-GlcNAcylation), and antibodies to parasite-expressed GFP, *P. berghei* circumsporozoite protein (CSP), Thrombospondin-repeat associated protein (TRAP; invasion protein), or acyl carrier protein (apicoplast marker). Using this strategy on a large sample size, we confirmed many of the described features of invaded SGs, including distal lateral lobe-focused invasion, SG cell stress and/or death, possible SG basal membrane repair after invasion, parasite bundling, and the presence of transient parasitophorous vacuoles. We have further uncovered novel preliminary information regarding SG infections, such as sites of subcellular SG localization of parasite CSP and TRAP, very few parasites in the salivary

duct (in the absence of a second blood meal), emergence from the parasitophorous vacuole, parasite maturation in the SG, cell traversal strategies, parasite orientation in bundles, and the effects of infection on SG structure/function. Interactions between SG cells and the parasites are likely to impact parasite transmission rates. Thus, a better understanding of these interactions will inform future strategies to block parasite transmission at the level of the SGs.