Impact of expanding seasonal malaria chemoprevention across new geographical areas and to children under 10 years – a modeling approach

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Seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) is recommended as an additional intervention against \textit{Plasmodium falciparum} malaria for children under the age of 5 years in the Sahel sub-region of Africa, where over 60% of clinical malaria cases occur during the 3 – 4-month rainy season and about 90% of the mortality and morbidity occurs among children under the age of 5 years. The aim of SMC is to prevent illness by maintaining therapeutic antimalarial drug concentrations in the blood throughout the malaria season. Randomized control trials have shown SMC to be effective, safe, relatively cheap and typically reduces clinical malaria episodes by approximately 75%. This study aims to quantify the potential burden reduction achievable by expanding SMC across new geographical areas and expanding the SMC age range to children under 10 years, using derived pharmacological properties of SP-AQ. We developed a computational model framework in EMOD, an agent-based stochastic model, that integrates the population dynamics of malaria transmission with pharmacokinetic and pharmacodynamics models to create simulated clinical trials of antimalarial drugs. The model outputs are fitted to clinical trial outcomes of AQ, SP-AQ and artesunate-AQ in regions where these drugs are used as curative treatment of malaria, to obtain the best fit pharmacodynamics parameters of SP and AQ that quantify the therapeutic effectiveness, i.e., the curative and prophylactic properties, of SP-AQ. The fitted model can be used to describe the impact of imperfect adherence on the success of SMC, the role a novel single-dose SMC drug could play in increasing SMC impact, as well as improve implementation of SMC by providing standard strategies and individual approaches to existing and new SMC regions.
Artemisinin and its derivatives (ARTs) underpin the most effective treatments for uncomplicated Plasmodium falciparum malaria. However, resistance to ARTs is becoming increasingly prevalent and the cellular mechanism of resistance requires further elucidation. Untargeted metabolomics and peptidomics analysis of PfKelch13-mutant P. falciparum revealed a down-regulation of haemoglobin digestion, and an increased abundance of glutathione (GSH) and its precursor gamma-glutamyl cysteine in ART-resistant parasites. We propose that these differences enhance the ability of ART-resistant parasites to ameliorate ART-induced free radical damage, as glutathione conjugation has been shown to function as a detoxification mechanism for many drugs that act via the generation of reactive intermediates. This study investigated the role of glutathione in PfKelch13-mediated ART resistance in P. falciparum parasites using metabolomics, and tested whether inhibition of this detoxification system can be a strategy for modulation of resistance to ART. ART-resistant isolates were incubated with buthionine sulfoximine (a gamma-glutamylcysteine synthetase inhibitor) in order to inhibit glutathione production, resulting in increased ART ring-stage activity in the ART-resistant parasites (40% survival decreased to 18%, p < 0.05) to a level of activity comparable to sensitive isolates. On the contrary, pre-incubation of ART-sensitive parasites with the glutathione precursor, N-acetylcysteine, was sufficient to reduce ART ring-stage activity to a level comparable to the resistant parasites. A targeted quantitative LCMS method for glutathione was then established to obtain a quantitative relationship between intracellular glutathione concentration and susceptibility to ART. In conclusion, we have identified altered glutathione metabolism as a major contributing factor to the mechanism of PfKelch13-mediated ART resistance. Furthermore, the in vitro ART resistance phenotype can be reversed by inhibiting this detoxification system.

Metabolomics identifies glutathione production as critical to the mechanism of artemisinin resistance in malaria parasites.

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Macha, in Choma District in southern Zambia, is working toward a national goal to eliminate malaria by 2021. Malaria prevalence in Macha decreased from 9% in 2008 to 1% in 2013 after insecticide-treated bed net (ITN) campaigns, however residual malaria transmission still occurs with yearly infection prevalence from 1-3% under active case surveillance. As vector control strategies are successfully implemented, identification of the vectors of residual transmission remains challenging. While ITNs reduce the threat from predominately endophagic and endophilic vectors, other opportunistic foraging species may contribute as secondary vectors in residual transmission settings. In 2013, the Zambian government implemented a reactive test-and-treat strategy in Choma District to pursue their goal of elimination. When an index case was identified positive for P. falciparum by rapid diagnostic test (RDT) at a local health facility, a healthcare worker visited their household and every household within 140 meters. In this study, the radius was extended to 250 meters and all individuals within the radius were screened for P. falciparum using a RDT and treated with ACT if positive. This procedure was repeated 30 and 90 days after the initial visit. Additionally, entomological samples were also collected by placing CDC light traps indoors next to a person sleeping under a bed net and outdoors near animal pens in the index and one neighboring household. Each anopheline was morphologically and molecularly identified to species level by PCR, analyzed for recent blood meal content via PCR, and evaluated for sporozoite prevalence using a P. falciparum ELISA. A known secondary vector in this area, Anopheles squamosus, was captured throughout all three years of this study. An analysis was performed to identify risk factors for the presence of this mosquito with the intention to recommend potential interventions that will target this residual malaria vector.

Characterizing risk factors of the residual transmission vector Anopheles squamosus in an elimination setting in Choma District, Zambia

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Plasmodium ovale accounts for a disproportionate number of travel-related malaria cases. It is also understudied in part due to a reliance on clinical samples. We collected a P. ovale curtisi parasite isolate from a clinical case in western Thailand and performed RNA-seq analysis on the blood stage transcriptional data, taking advantage of both de novo assembly and alignment-based techniques. We detected transcripts for 6628 out of 7280 annotated genes. For those lacking evidence of expression, the vast majority belonged to the PIR and STP1 gene families. We identified new splicing patterns for over 2500 genes, and mapped at least one untranslated region for over half of all annotated genes. Our analysis also detected a notable presence of anti-sense transcripts for over 10% of P. ovale curtisi genes. This transcriptomic analysis provides new insights into the blood-stage biology of this neglected parasite.

OP-06
Asymptomatic Plasmodium falciparum and Plasmodium vivax infections under different Transmission Intensities: Who contributes to Transmission?

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Understanding epidemiological variables affecting gametocyte carriage, and thus human-to-vector transmission potential and density is essential to design interventions that most effectively reduce malaria human-to-mosquito transmission. Plasmodium falciparum and Plasmodium vivax parasites and gametocytes were quantified by qPCR and RT-qPCR assays in 5 cross-sectional surveys involving 16,493 individuals in Brazil, Thailand, Papua New Guinea, and Solomon Islands. The proportion of infections with detectable gametocytes ranged from 44-94% for P. falciparum and from 23-72% for P. vivax. Blood-stage parasite density was the most important predictor of the probability to detect gametocytes. In moderate transmission settings (prevalence >5%), parasite density decreased with age and 63-84% of the cumulative gametocyte density was found in children <6 years. In low transmission settings (prevalence <5%), no age trends in gametocyte carriage were evident. Per survey, 37-100% of all individuals positive for gametocytes by RT-qPCR were positive by light microscopy for asexual stages or gametocytes (overall: P. falciparum 17B/348, P. vivax 235/398). 66-70% of the cumulative gametocyte density was found in light microscopy positive samples. Interventions to reduce human-to-mosquito malaria transmission in moderate-high endemicity settings will have the greatest impact when children are targeted. In contrast, all age groups need to be targeted in low endemicity settings to achieve elimination. Detection of infections by light microscopy is a valuable tool to identify asymptomatic blood stage infections that likely contribute most to ongoing transmission.

OP-07
The association between Plasmodium falciparum infection in the first six months of life and subsequent infection among children under 24 months in Malawi, 2016-2018

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In high transmission settings, infants under six months comprise up to 10% of malaria-related hospitalizations in children under five years old. Despite the prevalence of malaria infection and disease in this age group, the epidemiology is not well understood and incidence is rarely assessed. In the first six months of life, infants may either have increased immunity to infection due to presence of maternal antibodies or increased susceptibility due to exposure in utero. The impact of early malaria infection on subsequent disease is unclear. We aimed to determine if *P. falciparum* infection in the first six months of life alters the risk of subsequent clinical malaria up to 24 months of age. To address this question, infants in Malawi were followed at two sites from birth until 24 months of age. Study participants were scheduled for clinic visits at three-month intervals and asked to visit the clinic between appointments in the event of illness. RDTs were performed at all scheduled appointments and if malaria signs and symptoms were present at sick visits. Positive RDTs were confirmed by microscopy. Preliminary data from one study site included 94 participants with a mean follow-up time of 23.48 (SD: 6.54) months and a mean of 2.37 (SD: 4.30) infections. In the first six months of follow-up, 27 infections were reported for an incidence rate of 5.1 *P. falciparum* infections per 100 person-months of observation. In the following 18 months, there were 207 infections for an incidence rate of 44.6 infections per 100 person-months. A Poisson model, adjusted for season of birth, indicates that for every infection occurring before six months, an additional 1.73 (95% CI 0.97, 3.07) infections may be observed between six and 24 months, suggesting early infection is associated with increased risk of subsequent infection. These results might be due to increased exposure to *P. falciparum* or an impact on immunity among infants who are infected in the first six months of life. Analysis of our final analysis will include data from both health centers and covariates for bed net use and village to assess exposure.

**OP-08**

*Anopheles* innate immune factors CTL4 and CTLMA2: Solution structure, glycan specificity and phenol oxidase inhibitory activity


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Malaria, the world’s most devastating parasitic disease, is transmitted between humans by mosquitoes of the *Anopheles* genus. *An. gambiae* is the principal malaria vector in Sub-Saharan Africa. The C-type lectins CTL4 and CTLMA2 cooperatively influence *Plasmodium* infection in the malaria vector *Anopheles*. Here we report the purification and biochemical characterization of CTL4 and CTLMA2 from *An. gambiae* and *An. albimanus*. CTL4 and CTLMA2 are known to form a disulfide-bridged heterodimer via an N-terminal tri-cysteine CXCPC motif. We demonstrate in vitro that CTL4 and CTLMA2 intermolecular disulfide formation is promiscuous within this motif. Furthermore, CTL4 and CTLMA2 form higher oligomeric states at physiological pH. Both lectins bind specific sugars, including glycosaminoglycan motifs with β1-3/β1-4 linkages between glucose, galactose and their respective hexosamines. Small-angle x-ray scattering data supports a compact heterodimer between the CTL domains. Recombinant CTL4/CTLMA2 is functional in vivo, reversing the enhancement of phenoloxidase activity in dsCTL4-treated mosquitoes. We propose these molecular features underline a common function for CTL4/CTLMA2 in mosquitoes, with species and strain-specific variation in degrees of activity in response to *Plasmodium* infection.

**Measurement of Plasmodium falciparum- and P. vivax-specific antibody profiles on protein microarrays from dried blood spots**

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Sensitive and field-deployable detection tools are needed for malaria surveillance and elimination in low-prevalence settings. An ideal such tool would not only measure parasite prevalence, but also estimate recent aggregate malaria exposure, providing a more robust characterization of malaria transmission in a population. Serological biomarkers are promising targets for malaria surveillance because they can indicate cumulative recent exposure and are easily integrated into existing point-of-care platforms. Serological responses to *P. falciparum* and *P. vivax* antigens, identified and measured using protein microarrays, may serve as useful tools for identifying antibody biomarkers of exposure. Typically, serum samples are probed on protein microarrays to measure antibody responses to malaria antigens. The complexity of sample processing and cold chain requirement limit the utility of this method in remote hard-to-reach
POSTER PRESENTATIONS

**PP-01**

The Effects of pH and Temperature Parameters of Water on Abundance of Anopheles Mosquito Larvae in Different Breeding Sites of Kapiri Mposhi District of Zambia.

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Malaria is a vector borne disease a major cause of morbidity and mortality in Zambia. It is transmitted by *Anopheles* mosquitoes. There is paucity of evidence on the effects of physicochemical parameters on larvae in Kapiri mposhi district of Zambia. The study aimed at assessing the effects of physicochemical parameters on *Anopheles* larvae abundance in different breeding sites. The district was divided into four zones and surveyed for dams, rivers, swamps, marshlands and temporal water ponds. Temperature and pH measurement of water were taken on a weekly basis using a multi parameter meter (explorer GLX Pasco). 450 *Anopheles* larvae were collected and 73.6% (n=331) emerged adults, sibling species were identified using quantitative Polymerase Chain Reaction (qPCR). The abundance of An. gambiae s.s was significantly the highest (70.9% n=223), An. arabiensis Paton (29.1% =92), An. funestus (0%) and 5.2% non-amplified. 31.8% of An. gambiae s.s was found in temporal water ponds with an average pH and temperature of 6.37 and 26.9°C, respectively. Temporal water ponds had 24.6% An. arabiensis Paton with temperature and pH of 26.9°C and 6.37. The dams and rivers had no Anopheles siblings. pH has a positive coefficient correlation (r=0.114, p=0.05) while temperature has negative correlation (r=-0.373, p=0.05). Therefore, pH and temperature affects the abundance of the *Anopheles* mosquito larvae in breeding sites of Kapiri Mposhi district.

**PP-02**

Malaria in Nigeria, West Africa: An Overview

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Malaria caused by species of the parasite Plasmodium is holo-endemic in Nigeria, West Africa. More than 90% of the Nigerian population is at risk to this deadly infection with over 100 million cases yearly in the last decade. Reported cases of death has been estimated to be about 300,000 per year in tropical Nigeria. The poor standard of living in the rural Nigerian community is one of the causes of protracted infection leading to malaria disease. Species of Plasmodium responsible for malaria in Nigeria include vivax, malariae, falciparum and ovale. According to the WHO World Malaria Report, 2017, Nigeria contributed to 27% of global malaria burden in 2016 and accounts for 26% of global estimated malaria deaths. The Nigeria Government has recently reached an agreement with the United States Public Health on the eradication of malaria in Nigeria. The proposed Fiscal Year (FY) United States President’s Malaria Initiative (PMI) budget for Nigeria was 65 million dollars. Control strategies on the eradication of malaria in Nigeria include: Insecticide – treated nets, indoor residual spraying, seasonal malaria chemo-prevention, case management, pharmaceutical management. Surveillance, monitoring and evaluation reports have also been helpful in the control of this endemic disease in Nigeria, West Africa.

**PP-03**

The importance of copy number variation in metabolic insecticide resistance in *Anopheles gambiae*

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Insecticide resistance is a threat to the success in malaria control due to intensified use of the few available insecticides. Genetic variations in the *Anopheles gambiae* genome are important in increased resistance as evidenced in target site mutations. However, genetic variations such as copy number variations (CNVs) have been poorly studied and their role poorly understood in metabolic resistance. The aim of this study was to identify metabolic resistance genes that exhibit copy number variation and their significance in...
metabolic resistance. Pyrethroid phenotyped samples from Malawi (59) and Kenya (354) were screened for the presence of a specific Cyp6aa2 duplication known to exist in East Africa (Cyp6aa2-Dup1) with PCR. CNV discovery was done using the Anopheles gambiae 1000 genomes phase 3 data from Uganda and Tanzania, focusing on known metabolic resistance genes; Cyp6aa2, Gste2 and Cyp9k1. A high frequency of Cyp6aa2-Dup1 was found in both dead and alive phenotypes. A total of 177 (43 %) individuals had the duplication, 137 (33 %) did not have a duplication and 99 (24 %) were dropouts. The Cyp6aa2-Dup1 duplication was significantly associated with the resistance phenotypes. The CNV discovery revealed high CNV frequencies in the candidate genes screened. Cyp6aa2-Dup1, so far only known to exist as a duplication, was found to exist as a triplication in the Tanzania data, and a new duplication was found on Gste2 in 2 individuals from Uganda. These results have shown an importance of gene duplication in pyrethroid resistance, as well as high frequency of CNVs in the three metabolic resistance genes. More work should be done to functionally validate Cyp6aa2 in Anopheles gambiae and understand its role in insecticide resistance and as a diagnostic marker in metabolic resistance.

Transcriptome-wide in vivo mRNA target identification of RNA-binding proteins essential for P. falciparum sexual development

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Inappropriate perception and inadequate satisfaction of health workers pose significant challenges on malaria diagnostic services in the fight against malaria. Such information however is a gap in most rural health facilities. This study assessed Health Professionals perception and their satisfaction towards malaria quality diagnostic service in Northern Ethiopia. A cross-sectional facility based study was conducted in 2013 among 136 participants (110 clinicians and 26 laboratory professionals) engaged in malaria management. Level of perception and satisfaction was measured using a validated structured questionnaire. The structured data were entered using Epi-Info version 3.5.3 and analyzed by SPSS version 20. The finding indicated that about 61% (67/110) of the clinicians and 50% (23/26) of laboratory professionals were satisfied with the quality of work. Those clinicians who request laboratory malaria diagnosis based on sound clinical judgment were more satisfied (AOR=3.12, 95%CI=1.06-9.13) than their counterparts while those who trust laboratory malaria diagnostic result as just reliable were 68.0% (AOR=0.32, 95%CI=0.13-0.83) less likely to be satisfied than the referent groups. Laboratory professionals with no limiting factors for laboratory diagnosis were 30.6 times (AOR=30.6, 95%CI=1.83-511.8) more satisfied on the service compared with those who had constraints in their health facility. The level of satisfaction of health professionals in the current study was not encouraging and is lower than some previous
studies conducted in the country. Thus, targeting the identified limiting factors are crucial steps to consider in the fight against malaria.

PP-o6
Mitochondrial acetyl-coA biosynthesis is essential during the red blood stages of human malaria parasite Plasmodium falciparum.

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Acetyl-coA is a central molecule in carbon metabolism. Malaria parasites have three major pathways to make acetyl-CoA that are located different subcellular compartments: the apicoplast, mitochondrion and cytosol. Both the apicoplast and mitochondrial pathways use the pyruvate derived from glycolysis and enzymatically generate acetyl-coA using an organelle-specific pyruvate dehydrogenase enzyme. The cytosolic pathway relies on millimolar levels of exogenous acetate and its conversion to acetyl-coA using a cytosolic acetyl-coA synthetase enzyme. The apicoplast pathway is not essential during the blood stages, leaving the mitochondrial source of acetyl-coA a potentially important and vital source. In the present study, we generated a series of deletion mutants of important enzymes in the mitochondrial acetyl-coA biosynthetic pathway and showed that mitochondrial acetyl-coA biosynthesis is essential for the survival of the parasite. Unexpectedly, we found that two mitochondrial enzymes are capable of making acetyl-coA (Pyruvate Dehydrogenase and alpha-Ketoglutarate Dehydrogenase); deletion of both enzymes is required to kill blood stage parasites unless exogenous acetate is provided. Both enzymes rely on lipic acid as a cofactor and we were able to simultaneously block the activity of both enzymes, and the production of acetyl-coA by genetically targeting lipic acid metabolism. We studied the production of acetyl-coA in wild type and gene-knockout parasites using 13C-labeled glucose, acetate or glutamine and showed how these different carbon sources are metabolized in different metabolic scenarios. These results provide novel insights into parasite metabolism and identify an essential product of the mitochondrion.

PP-o7
The ecdysone receptor regulates vitellogenesis in Anopheles arabiensis female mosquitoes

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Anopheles arabiensis, one of the main malaria vectors in South Africa, is not efficiently controlled by the insecticide-based strategies used locally because of its increasing resistance, and exophilic/exophagic behaviour. Interestingly, several reports suggest that transmission-blocking vaccines targeting mosquito genes, or genetically modified mosquitoes could offer an avenue for An. arabiensis control. These techniques target genes that are essential for the mosquito vectorial capacity. In this context, one candidate of interest is the Ecdysone Receptor (EcR), as it was shown to regulate immunity, blood feeding, vitellogenesis, fecundity, and Plasmodium development in mosquitoes. Currently, no functional characterization of EcR in An. arabiensis has been reported. This study hypothesized that in An. arabiensis, EcR regulates vector density by controlling the expression of key effectors of vitellogenesis. To test this hypothesis, real-time-PCR and in silico analyses were used to isolate EcR from An. arabiensis. Then, EcR expression at different time points during vitellogenesis (12 hours post blood meal [hPBM], 24 hPBM, 36 hPBM, and 48 hPBM) was quantified with qPCR. Finally, we used RNA interference to investigate how silencing EcR affects the expression of four Yolk Proteins Precursors (YPPs): Vitellogenin (Vg), Lipophorin (Lp), Vitellogenin Carboxypeptidase (VCP), and Cathepsin b-like protease (VCB). Phylogenetic analyses revealed that An. arabiensis EcR orthologue is, as expected, closer to that of An. gambiae, as supported by a bootstrap value of 100%. During vitellogenesis, EcR expression increased from 12 to 24 hPBM and then declined from 36 to 48 hPBM, similar to findings in Aedes aegypti, the vector of dengue and Zika viruses. Finally, silencing EcR affected the expression of Vg, VCP, and VCB, while the expression of Lp remained unchanged. This was the first study to characterize the expression and function of EcR in vitellogenesis in a South African mosquito vector.

PP-o8
Diagnosis of asymptomatic malaria infections: PCR method as gold standard over ultrasensitive rapid diagnostic tests

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Malaria is a leading cause of morbidity and mortality in many developing countries including Bangladesh. Recent trends of low number of the cases in Chittagong Hill Tracts (Hyper endemic area of Bangladesh) underscore the need to give attention to the elimination of malaria from Bangladesh.
While dealing with the malaria elimination program that has already been adopted by NMEP, Bangladesh, it is important to pay attention to the asymptomatic malaria infections. The objective of this study was to identify the individuals with asymptomatic malaria and its relation to age, sex, and seasonal variations. A cross-sectional study was conducted from September, 2018 to May, 2019 in Kuhalong and Rajbila Union of Bandarban to diagnose the asymptomatic-malaria individuals using Nested PCR and to evaluate the sensitivity of Ultrasensitive RDT for field application as well. The sensitivity and specificity of the Us-RDT was compared with that of the gold standard PCR. Among 300 samples, 9 samples were confirmed to be positive by PCR. Both *Plasmodium falciparum* and *Plasmodium vivax* were the dominant among the *Plasmodium* species. In terms of sex and age, the females (66.67%) and the adolescent (44.44%), respectively, were harboring more infections compared to other study groups and also the rate was higher in September (44.44%). The Ultrasensitive RDT had the sensitivity of 33.33% (95% CI; 9.04%-69.08%) and the specificity of 90.3% (95% CI; 86.24%-93.40%). PCR has always been the gold-standard for the diagnosis of asymptomatic malaria. Moreover, the study findings suggest that the US-RDT, though convenient and user-friendly, still cannot be used as a tool for the detection of asymptomatic cases. Nevertheless, for field level and mass surveillance of asymptomatic cases, there is no other better alternative than US-RDT and hence, it imposes that the sensitivity and specificity of this technique must be improved to meet the diagnostic requirement.

**ABO blood group and mortality in children with severe malarial anemia due to *Plasmodium falciparum***

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Previous studies of ABO blood group and clinical outcomes in patients with *Plasmodium falciparum* infection suggest a protective effect of blood group type O. To assess the possible relationship between ABO blood group and survival in children with severe malarial anemia due to *P. falciparum* infection, we combined data from two observational studies of severe malaria conducted in two district-level hospitals in Zambia. Study participants were children ≤12 years admitted to the hospital with a diagnosis of malaria with severe anemia, defined as hemoglobin concentration ≤5 g/dl. For comparison, we included children with severe anemia due to a cause other than malaria. We examined associations between ABO blood group and mortality in unadjusted and adjusted logistic regression models. We identified 384 children with severe malarial anemia and 45 children with severe anemia due to another cause. Other causes included sickle cell disease, malnutrition, renal disease, and infections other than malaria. Among children with severe malarial anemia, blood group type O was the most prevalent (43%) followed by type A (28%), type B (23%) and type AB (5%). The median age was 23 months (interquartile range [IQR]: 12-36) and 52% were girls. The median hemoglobin concentration was 3.8 g/dl (IQR: 3.1-4.3 g/dl). Most of the children (86%) received blood transfusion and the case fatality ratio was 13%. Children with other severe anemia were similar across all characteristics. Adjusted for age, sex, hemoglobin concentration, and blood transfusion there was no statistically significant association between ABO blood group and mortality in children with severe malarial anemia, or in children with severe anemia due to other causes. Higher hemoglobin concentration and receipt of a blood transfusion were associated with improved survival (OR 1.6, 95% CI: 1.1-2.3, p<0.02 per g/dl increase in hemoglobin; OR 3.3, 95% CI: 1.5-7.4, p<0.01 for blood transfusion). Although previous studies demonstrated a relationship between ABO blood group and malaria-related outcomes, we did not find an association between blood type and mortality in Zambian children with severe malarial anemia, or severe anemia due to another cause. Further studies that assess the association between ABO blood group and malaria may lend insight into the pathophysiology of malaria caused by *P. falciparum*.

**Development of multi-epitope driven subunit vaccine in secretory and membrane protein of *Plasmodium falciparum* to convey protection against placental malaria infection**

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Placental malaria is the severe health concern for a long time which targets the pregnant woman regardless of previous acquired immunity. This severe pathological condition is mediated by the VAR2CSA, a Plasmodium antigen that interacts with the placental chondroitin sulfate-A (CSA). As per the WHO reports, the malarial infection causes huge mortality all around the world and is incomparable with any
other infectious diseases. The absence of effective treatment options and increasing drug resistance to the available therapeutics like artemisinin and other derivatives demand an efficient alternative to overcome this crisis death. We performed the literature survey and sorted the *Plasmodium falciparum* secretory and membrane proteins including VARzCSA to design multi-epitope subunit vaccine using an adjuvant, B-cell- and T-cell epitopes including cytotoxic T-lymphocytes (CTL) and helper T-lymphocytes (HTL) epitopes. Every helper HTL epitope was IFN-γ positive and IL-4 non-inducer. The physicochemical properties, allergenicity, and antigenicity of designed vaccine were analyzed for the safety concern. Homology modeling and refinement were performed to obtain the functional tertiary structure of vaccine protein followed by its molecular docking with the toll-like receptor-4 (TLR-4) immune receptor. Molecular dynamics simulation was performed to check the interaction and stability of the receptor-ligand complex. Multi epitope subunit vaccine of 704 amino acid residues was designed having its entire component as mentioned above including CTL, HTL, B-cell epitopes, adjuvant and linkers. Immunoinformatics evaluation revealed the immunogenic and stable nature of designed subunit vaccine while, its experimental validation may confirm its ability to enhance the maternal immunity against placental malaria infection. This way, we designed the multi-epitope subunit vaccine to serve the maternal health living in the global endemic zone.

**Achievements of Liver-Humanized Mice in the Elimination of Malaria**

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Although *P. falciparum* can be cultured in vitro, several aspects of malaria infections can only be addressed by in vivo research. The complex mechanisms of malaria transmission through mosquito bites, the multiple tissue barriers crossed by the parasite, and the different cell types that are infected during its life cycle make it impossible to study all aspects in one in vitro system. The development of liver-humanized mouse models has made it possible to study *P. falciparum* liver-stage infection in vivo. Humanization of the mouse bloodstream is achievable by frequent injections of human red blood cells (hRBCs) and is currently the only system with which to study human malaria blood-stage infections in a small animal model. The transfer of human antibodies generated by vaccine trials into liver-humanized mice has enabled researchers to determine their efficacy in preventing infection of either the liver, or transition to blood-stage. Infection of the liver-humanized mouse model with *P. vivax* has allowed researchers to study the development of hypnozoites and test novel drugs to kill the hibernating parasites. The current liver-humanized mouse models lack an adaptive immune system. This is knocked-out to prevent host vs graft disease. As a further enhancement, we are developing a liver-humanized mouse model, co-engrafted with a human immune system. This will allow our collaborators to study pathogen-host interactions between not only human hepatocytes, but also the activation of human immune cells.

**Dual Site and Mechanism of Action of Artemisinin Antimalarials**

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The delayed clearance of *Plasmodium falciparum* parasites following artemisinin combination therapy is characterized by a ring stage-resistance to artemisinins measured by elevated drug pulse concentrations to inhibit ring stages. The trophozoite stages remain sensitive to low nanomolar drug. The phenotype is closely associated with the Kelch13 genotypic changes at numerous amino acids. Here we investigated for parasitidal activity and mechanism of action on heme crystallization the heme-artemisinin adduct metabolite, previously thought to be inactive after heme or iron cleavage of the endoperoxide bridge. Heme-artemisinin adduct was synthesized in reducing conditions, column purified and validated by mass spectrometry with sharp peaks at m/z 838 and 898, with the absence of m/z 282 of free artemisinin. Observations of beta-hemin crystal growth inhibition in the presence of ART and H-ART by time-resolved *in situ* atomic force microscopy (AFM) revealed that the addition of non-activated ART had no effect on the surface features and the kinetics of layer nucleation and growth. In contrast, heme artemisinin adduct demonstrated near irreversible heme crystal growth inhibition by absorbing at growth sites to prevent subsequent addition. Biologic validation of exogenous heme-artemisinin adduct effect on parasites showed 50% inhibition in the 10 to 70 nMolar range varying by artemisinin or artesunate as the heme adduct in both resistant Kelch13 mutant and sensitive parasites. After a 6 hour exogenous 700 nM H-ART pulse of trophozoites, followed by extensively SDS/ bicarbonate/water washes to purify hemoxin, H-ART is detected with hemoxin heme by a mass spectrometry at m/zs 838. The previously thought inactive heme-artemisinin adduct kills parasites when exogenous in culture medium. Artemisinins work in the
parasite cytoplasm at ring stages and in the cytoplasm and digestive vacuole at the trophozoite stage via suicide activation by iron or heme to generate radicals which damage bystander proteins. In the digestive vacuole there is an additional quinoline-like mechanism of heme crystal inhibition by the heme-artemisinin adduct.

Dormancy and recovery of the arrest in Plasmodium falciparum isolates against Artemisinin-based Combination Therapy

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Dormancy is a phenomenon that Plasmodium falciparum use to tolerate the action of Artemisinin-based Combined Therapy (ACT). This study proposed to evaluate dormancy of clinical and reference isolates of P. falciparum against first-line regimens for malaria: artemether-lumefantrine and artesunate mefloquine by dormancy and flow cytometry assays. P. falciparum isolates were tested against dihydroartemisinin (DHA: 4 - 1,000 nM), artesunate (AS: 0.1 - 100 nM), lumefantrine (LMF: 3.1 - 200 nM), and mefloquine (MFQ: 0.2 - 1,000 nM) by ex-vivo and in vitro assays. The recrudescence in vitro of these isolates was accompanied in the dormancy assay. The viability of the reference isolate was assessed by flow cytometry using Rhodamine 123 and DAPI. In the dormancy assay, schizonts were observed in the P. falciparum reference isolate NF54 and in the clinical isolate S-01/15 after pressure with 62.5 nM, 250 nM and 1,000 nM DHA. The recovery period ranged from 4 to 40 days. For LMF, dormancy only was observed in NF54, in the days 7 and 12 after exposure to 66.6 nM and 200 nM of the drug, respectively. Schizonts were seen in the P. falciparum clinical isolate FS-08/15 after pressure with 200 nM of AS, right after incubation period of the ex-vivo assay, with no dormancy of trophozoites in the dormancy assay. In flow cytometry assay, viable young trophozoites of P. falciparum labeled with DAPI and Rhodamine 123 were observed at the maximum concentrations of DHA (1,000nM) and LMF (200nM). Our results suggest that the dormancy is not presented in all clinical isolates of P. falciparum. Flow cytometry was able to confirm the parasite viability accurately.

FLIM and Imaging Analysis of Plasmodium falciparum Exposed to Artemisinin

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Plasmodium falciparum recrudescence after 3 – 7 days artemisinin monotherapy is well known. Recrudescence is also observed in vitro when early ring stage parasites are exposed to the drug. Parasites after recrudescence are just as sensitive to artemisinin as before the initial drug treatment, indicating no change of drug response phenotype. Microscopic observation of a delay in the intraerythrocytic cycle with arrested development of the ring-stage parasites suggested cell dormancy may provide a means to escape artemisinin toxicity. Here, we apply Fluorescence Activated Cell Sorting (FACS), Fluorescence Lifetime Imaging (FLIM), and super-resolution AiryScan microscopy to compare ring-stage parasites exposed to dihydroartemisinin (DHA) or to control medium with dimethylsulfoxide (DMSO, DHA vehicle). Cell populations were isolated by FACS in each sample through staining with a DNA dye, SYBR Green, and a mitochondrial potential marker, MitoTracker Deep Red FM. The putative dormant parasite fraction was isolated based on staining of mitochondrial potential and was verified by re-culturing experiments. FLIM of intrinsic reduced nicotinamide adenine dinucleotide (NADH) was used to quantify the metabolic state and AiryScan microscopy was used to visualize parasite structure. DHA-treated parasites showed a collapse of the nucleus and mitochondria as well as a lower mean lifetime, whereas parasites in control medium showed these two organelles apart from one other. After standardizing this method, other antimalarials will be tested to see if these changes are uniquely associated with DHA exposure. Further characterization of this phenomenon may provide insights on prevention of clinical recrudescence.

Malian children with severe malaria exhibit distinct PfEMP1 antibody profiles that differ by blood type

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1The Johns Hopkins Malaria Research Institute 5th Annual Future of Malaria Research Symposium, November 18th, 2019
Severe malaria caused by *Plasmodium falciparum* primarily affects children in sub-Saharan Africa. Severe malaria pathogenesis is poorly understood but likely involves deep tissue sequestration and rosetting of infected erythrocytes. ABO blood type and *P. falciparum* erythrocyte membrane protein-1 ( PfEMP1) variant surface antigen expression appear to play critical roles in rosetting. Depending on blood type, parasites infecting subjects may express certain subsets of PfEMP1, such as DBLα1 domains, associated with severe disease pathogenesis. For each blood type, we predicted that Malian children with severe malaria would demonstrate a unique PfEMP1 serological profile that reflects the lack of antibody responses to PfEMP1 potentially involved in pathogenesis. We also predicted that severe malaria cases would have less recognition of and lowered seroreactivity to a subset of PfEMP1 protein fragments than controls with uncomplicated malaria. We probed a custom protein microarray populated with reference strain and field-derived PfEMP1 associated with severe malaria pathogenesis with sera from a 2000-2003 Malian severe malaria case-control study. Our findings suggest that sera from children with severe malaria differed in the number of PfEMP1 recognized and/or antibody recognition depending on the blood type: sera from blood type A severe malaria cases (n=27) recognized 91.6% of PfEMP1 fragments, sera from blood type AB cases (n=7) recognized 23.8% of PfEMP1 protein fragments, and sera from blood type O cases (n=13) recognized 81.6% of PfEMP1 protein fragments. In addition, sera from blood types A, AB, and O severe malaria cases reacted less intensely to 67, 12, and 34 PfEMP1 fragments, respectively, than uncomplicated malaria sera. Analyses investigating the differential serological responses to DBLα1-PfEMP1s are currently ongoing. These findings may inform our understanding of severe malaria pathogenesis and the design of vaccines to protect individuals from severe malaria.

**PP-16**

Lactic acid supplemented media stimulates gametocytogenesis in *Plasmodium falciparum* culture

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**PP-17**

Bacterial Suppression of Malaria Transmission by Mosquitoes

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The intolerable burden of malaria demands the urgent development of novel approaches to fight this deadly disease. Previously, we have shown that engineered mosquito symbiotic bacteria can render mosquitoes resistant...
to the parasite. However, translation of these findings to the field faces major regulatory barriers. Here we describe two non-modified symbiotic bacteria – *Delftia* sp. and *Pseudomonas* sp. These bacteria were originally isolated from mosquitoes that had lost the ability to sustain the development of *Plasmodium falciparum* parasites. While *Pseudomonas* easily colonizes the mosquito and is transmitted vertically, it is a poor inhibitor of *Plasmodium* development. *Delftia* on the other hand, is a potent inhibitor of *Plasmodium* development in mosquitoes. Further study showed *Delftia* secretes some molecules below 3 KD which 100% inhibit ookinete and oocyst of *Plasmodium falciparum* in mosquito midgut. This bacterium does not impose a fitness load: it does not affect mosquito survival, blood feeding behavior, fertility or fecundity. *Delftia* and its secreted molecules show promise for use in the control of malaria.

### PP-18

**Relating Larval and Adult Mosquito Salivary Gland Biology to Develop New Strategies to Block Disease Transmission**

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Mosquitoes are a dire threat to human health, accounting for hundreds of millions of infections and over 1 million deaths annually. The pathogens involved must travel through the adult female mosquito salivary glands (SGs) to be injected into the next human host during a future blood meal. This feature makes the SGs an ideal target for transmission blocking interventions. The adult SGs form in the pupa from a population of cells located in the larval salivary duct bud, while the embryonic/larval SGs are destroyed. Little is known about cell architecture and secretion in a larval mosquito SG, how the adult SG acquires its elaborate shape, and what genetic regulation is at work in larval and adult mosquito SGs. We developed a robust protocol for fixing and staining larval SG tissue (using dyes and antibodies) and applied this method to over 1000 SGs, imaging many of these samples using confocal microscopy. A comparison of larval and adult SGs morphology and gene expression has also been conducted. As with the adult mosquito SGs, the larval SGs have unusual and interesting morphologies that are likely linked to their function. We have learned about the localization of key cellular components and have gathered evidence supporting the presence of extracellular vesicles in larval saliva. We have further determined the relatedness in gene expression profiles between larval and adult mosquito SGs, as well as larval mosquito and fruit fly SGs. This work helps to describe the formation and regulation of an organ with a heavy impact on human health: mosquito salivary glands. This initial characterization of the larval SG and the adult precursors lays the foundation for exploring new strategies for preventing the spread of mosquito-borne diseases.

### PP-19

**Challenges, opportunities and updates: A focus on the 2019 Eastern Burma Retrospective Mortality Survey**

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Improving data collection in low-income settings is needed to support health equity and disease prevention efforts. Almost no literature exists in the South Asian context describing challenges encountered during household surveys capturing disease outcomes and determinants. We discuss: (1) challenges and opportunities associated with collection and use of health, healthcare and malaria-specific data from the Eastern Burma Retrospective Mortality Survey (EBRMS) in Myanmar and (2) share progress to date on the 2019 EBRMS. To explore these issues, a qualitative review of the notes on feedback questionnaires from EBRMS field staff at the Burma Medical Association (BMA) and Health Information System Working Group (HISWIG) was undertaken. Opportunities and challenges preceding and during the 2019 data collection are highlighted. Challenges included inaccessibility of village sites due to security problems from nearby armed conflict; uncertainty in handling responses to questions on sensitive topics; and the implications of a lengthy questionnaire on participation and data quality. Despite these limitations, EBRMS reports essential information, including those related to emergent infectious disease and malaria among displaced populations in ethnic states. This information is not available from government service providers or other health organizations. The 2013 EBRMS found malaria was the primary reported cause of death across all age groups and 63.6% of respondents reported using a bed net as a preventative measure against malaria. The 2019 EBRMS has improved its comparability to international demographic surveys by including new indicators measuring income, health beliefs, and potential emerging health issues that can further describe disease patterns. Populations in remote and conflict-affected regions of eastern Burma continue to face critical health and health equity challenges. Continued efforts are essential to systematically measure the nature and impact of these challenges. By anticipating survey-related difficulties, researchers in LMICs are more likely to be able to implement strategies to minimise their effects.

### PP-20

**Mutant Plasmodium with delayed growth during the liver stage development induces better immunity than its wild**
type counterpart in a Chemoprophylaxis Vaccination regimen

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Chemoprophylaxis Vaccination (CVac) is a relatively new and promising vaccination approach against malaria. Using whole organism sporozoites combined with Chloroquine treatment, CVac provides better sterilizing immunity, with two-log less sporozoites than the Radiation Attenuated Sporozoite (RAS) vaccine. We have engineered a Plasmodium mutant named PbATG8-OE (Plasmodium berghei overexpressing ATG8) that exhibits a severe growth delay in hepatocytes, thus exposing liver stage antigens to the immune system longer than WT parasites. We hypothesize that PbATG8-OE parasites would be more sensitive to a chemophrophylaxis treatment, hence an ideal antigenic constituent of a CVac regimen. Comparing the vaccine potential of P. berghei WT and PbATG8-OE using a CVac regimen, we show that PbATG8-OE provides superior memory response (100%) than WT parasites (60%) and confers better long-term protection (40%vs. 20% for WT). PbATG8-OE-CVac generates a comparable antibody-response against sporozoites and liver forms as WT-CVac. Interestingly, the protection elicited by PbATG8-OE-CVac is CD8-T cell-dependent and IFN-g is playing a minor role. We are currently investigating the protective mechanism in mice infected with PbATG8-OE-CVac, which would lead to the discovery of new antigens and memory effectors and may help develop better malaria vaccine.

Subclinical Plasmodium falciparum infection among children and adults residing in a high malaria transmission community

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Chronic, asymptomatic, and subpatent malaria are overlapping subclinical syndromes classified according to the extent or duration of parasitemia and the presence or absence of parasite-attributable pathology. Subclinical malaria is incompletely characterized in terms of its natural history, clinical consequences, and epidemiological contribution. We investigated demographic and clinical features of subclinical malaria to assess potential risk factors and elucidate its relevance to malaria control. Longitudinal and cross-sectional surveys were conducted between 2013 and 2017 in adults and children residing in a high malaria transmission area of northern Zambia (n=2,249). Plasmodium falciparum parasitemia was measured by polymerase chain reaction (PCR), microscopy, and rapid diagnostic test (RDT). Demographic, clinical data (temperature, hemoglobin, self-reported symptoms), bed net use, indoor residual spraying, and recent antimalarial treatment were recorded. Parasite prevalence was 31% by microscopy, 53% by RDT, and 55% by PCR. Participants were stratified according to PCR, microscopy, and RDT results: patent (+/+/+), subpatent (+/-/+), recent (-/-/+), and no (-/-/-) parasitemia. Among PCR-positive individuals, 66% (n=816) had subclinical malaria defined as temperature <38°C and no self-reported fever, chills, headache, vomiting, diarrhea, or cough within the prior 48 hours. Participants with patent (n=592) or recent (n=183) parasitemia tended to be younger (median age = 10 y, IQR 5-20) than those with subpatent (n=253) or no parasitemia (n=762) (25 y, IQR 10-41). Participants with patent or recent parasitemia were more likely to have anemia compared to no parasitemia (OR=1.8, 95%CI 1.5, 2.2). Those with no parasitemia were more likely to report sleeping under a bed net compared to those with patent, subpatent or recent parasitemia (OR=2.5, 95%CI 1.7, 2.5). This study identified a high prevalence of subclinical malaria in a holoendemic area. Characterization of this diverse group will inform public health and clinical approaches to malaria control in similar transmission settings.

Severe Malaria Surveillance in a Rural District Hospital in Northern Zambia

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Severe malaria surveillance is routinely performed in Zambia by hospital-based Health Management Information System (HMIS) personnel. HMIS surveillance relies on information aggregated from ward registers. Supplementation of HMIS data with hospital- and patient-level data from additional
sources may provide greater resolution of severe malaria clinical epidemiology. To assess hospital-based methods of severe malaria surveillance, we conducted a single center study that evaluated four data collection approaches over the period June 2017 to March 2019. Aggregate data were collected from HMIS records and central pharmacy artesunate inventories. Individual-level data were collected from artesunate administration records and laboratory blood transfusion logbooks. Means and standard deviations of cases per month were calculated and compared using Student’s t test and one-way analysis of variance. The HMIS monthly case estimate (79 ± 40) was systematically greater than estimates based on artesunate inventory (56 ± 38), artesunate treatment courses (54 ± 32), and pediatric blood transfusions (45 ± 18) (P=0.02). Age was similar between artesunate-treated patients and transfused patients (median 24 months, interquartile range 14-43) and similar across years of surveillance. Partial or complete blood product stockouts were recorded for 95 days over the 667-day surveillance period (44%). Accurate accounting of severe malaria case burden can help focus resources in a timely and effective manner. Shifts over time in case volume, age distribution, and allocation of blood transfusion may reveal underlying changes in local malaria epidemiology. Alternative data sources can supplement existing register-based HMIS surveillance.

PP-23

Small molecule screen of epigenetic inhibitors on Plasmodium falciparum blood stage replication and gametocyte maturation

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The regulation of gene expression by histone modifications is critical for parasite survival in both asexual and sexual blood stages of P. falciparum, and changes substantially as parasites differentiate from one to the other. This makes inhibitors targeting these pathways intriguing in terms of their potential as multi-stage active anti-malarials. Therefore, we screened two cherry-picked libraries of small molecule epigenetic inhibitors from Selleckchem (142 compounds) and Cayman Chemicals (139 compounds) for their effect on asexual blood stage replication and gametocyte development. These compounds include inhibitors of epigenetic writers, readers, and erasers known in mammalian systems, including histone deacetylases (HDAC), histone demethylases (HDM), histone methyl transferases (HMT) and histone acetyl transferases (HAT). We identified 74 epigenetic inhibitors with EC50s below 10 µM for inhibition of gametocyte development, of which 27 compounds retained their >90% inhibitory effect at 1 µM. Asexual stage growth was blocked >90% by 73 compounds at 10 µM, of which 30 compounds maintained their inhibitory effect at 1 µM. Dose-response curves were determined for compounds with potent activity at 1 µM, with several that had EC50 concentrations in the low nano-molar range against both asexual and sexual blood stages. These include inhibitors previously identified to be active against P. falciparum.
Plasmodium falciparum blood stages as well as several that had not previously been tested. Additionally, we identified multiple compounds that displayed higher activity against gametocyte maturation compared to asexual replication. The emergence of drug resistance in *P. falciparum*, the most virulent human malaria parasite, necessitates the development of novel anti-malarial drugs. These findings provide support for the development of new classes of antimalarials that target the parasite’s epigenetic regulation machinery.

**PP-25**

*Measuring Gene Expression to Evaluate the Effect of Artemether-Lumefantrine on Parasitemia and Gametocytemia in Zambian Children with Uncomplicated Plasmodium falciparum Malaria*

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Artemisinin combination therapy (ACT) is currently the treatment of choice for uncomplicated *P. falciparum* malaria. Although ACTs are known to be highly effective against asexual parasites, the in vivo effect of ACTs on various stages of gametocytes is incompletely characterized. Samples from 75 participants were selected from a therapeutic efficacy study of 100 children from Nchelenge, Zambia with uncomplicated *P. falciparum* malaria treated with artemether-lumefantrine (AL) and followed for five weeks. Dried blood spots (DBS) were collected from each participant every 6 hours for the first 48 hours, at 72 hours, and weekly; in this study, RNA was extracted from samples collected up to the second week of follow-up. This RNA was used for quantitative real-time PCR (qPCR) assays that quantify three stage specific transcripts: Pfbsbp2, Pfss16, and Pfss25. Pfbsbp2 is expressed by ring-stage asexual parasites, while Pfss16 and Pfss25 are gametocyte-specific markers. Pfss16 is most highly expressed by early gametocytes but is expressed at a lower level throughout the remainder of gametocyte development in both male and female gametocytes, and Pfss25 is highly expressed by stage V female gametocytes. We were able to successfully extract RNA and amplify transcripts present in quantities as low as 2.5 copy/μl for Pfbsbp2 and 1.25 copies/μl for Pfss16 and Pfss25. In this cohort, it was found that treatment with AL was associated with an 80-fold reduction of Pfbsbp2 copy numbers within the first 36 hours. Despite this reduction in copy number, 75% of the participants were positive for Pfbsbp2 by qPCR after 2 weeks. 17% of samples were positive for Pfss16 and/or Pfss25 after 2 weeks, but Pfss16 and Pfss25 copy numbers were differentially affected by treatment, consistent with stage-dependent variations in drug susceptibility to AL. Additional studies are needed to evaluate the epidemiological importance of such lowly expressed transcripts, to determine if the sustained prevalence of transcripts during the follow-up period is due to persistent parasitemia, reinfection, or recrudescence, and to determine the impact of drug treatment beyond two weeks.

**PP-26**

*Perceptions on the efficacy and benefits of the IRS programmes by beneficiaries has a bearing on spraying coverage*

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Two deltamethrin formulations were sprayed in inside houses in Pfungwe district, Zimbabwe, for malaria control purposes. A deltamethrin product containing 5% wettable powder (WP) as active ingredient, was sprayed in 1 899/2 157 (88%) of the rooms and another deltamethrin product containing 2.5% active ingredient (Suspension Concentrate) (SC) was sprayed in 1 790/1 892 (91.4%) of the rooms. Room coverage was not significantly different between the 2 insecticides (P=0.097). Room coverage was not associated with the level of perception on whether problems were being encountered by house holders when spraying was being conducted (no problems reported by 89.6% respondents in SC villages and 91.4% in WP villages), but by the level of knowledge pertaining to the spraying exercise mastered during the initial phase (95.8% respondents knew in SC villages than 91.4% in WP villages). Householders opened their doors for spraying when they observed other public health pests dying from their neighbor’s house (72.9% and 70.3% said so in deltamethrin SC and WP villages respectively). Percent locked rooms ranged from 0.7 - 9.3% and 3.4 - 14.4% in deltamethrin SC and deltamethrin WP sprayed villages respectively, although the results were not significantly different (P=0.22). The residual effect of both formulations was 6 months both on mud walls and thatched roofs. In conclusion, perceptions of householders on the level of product effectiveness influences spraying coverage and efforts should be made to increase program uptake.

**PP-28**

*Molecular characterization of recombinant Plasmodium falciparum PHISTb proteins as potential targets of*
naturally acquired immunity against malaria transmission in humans

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Plasmodium falciparum is known to cause the deadliest form of malaria in sub-Saharan Africa. Upon infection, remodeling of infected host red blood cells (iRBCs) by the parasite-exported surface proteins occurs to provide a favorable niche for parasite development and maturation. Some of these molecules include products of the multicopy family of Plasmodium Helical Interspersed Sub-telomeric (PHIST) proteins, whose role in malaria pathogenesis largely remains unknown at present. However, in a recent transcriptome analysis of clinical isolates, some members of the PHISTb gene family were associated with parasite adaptations to malaria transmission intensity and gametocyte development. It was further established that PHISTb proteins could serve as targets of naturally acquired immunity to malaria in individuals from three different geographical sites in Kenya and The Gambia with varying malaria transmission intensities. To characterize the possibility of these proteins as potential vaccine targets, recombinant PHISTb proteins were expressed in bacteria followed by ELISA-based evaluation of antibody responses using immune sera from malaria-exposed individuals. Our findings show that children and adults from malaria-endemic region recognized PHISTb proteins (i.e. PF3D7_0532400, PF3D7_1401600, and PF3D7_1102500), providing a clinical evidence for the role of PHISTb antigens in immune response against P. falciparum infection. Antibody responses against the three recombinant PHISTb antigens were however variable. An association study of antibody responses to the different PHISTb antigens with age revealed no correlation between the age and antibody responses to PF3D7_1102500 and PF3D7_1401600 (p<0.507 and p<0.15, respectively, CI=95%), but a significant correlation to PF3D7_0532400 (p<0.009). Furthermore, there was strong correlation of antibody responses to both the schizont extract and PF3D7_0532400 (p<0.0001), equivalent to those against PF3D7_1102500 and PF3D7_1401600 (p<0.0001). Collectively, these findings empirically provide evidence of recombinant PHISTb antigens as potential targets of naturally-acquired immunity against malaria in humans and possible serological markers to P. falciparum infection aimed at contributing to malaria control through vaccine development.

PP-30
Polymorphism of exon-20 in the voltage-gated sodium channel gene of Anopheles gambiae s.s. populations from wetlands across the Cameroon volcanic line

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The Effect of Malaria/HIV/TB Triple Infection on Malaria Parasitemia among patients attending the Limbe Regional Hospital

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Malaria, Tuberculosis (TB) and Human Immune deficiency virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) are 3 of the world’s most common and serious infectious diseases that underlie development in low and middle-income countries. These infections are not only associated with poverty, but also occur in the same geographic zone and have major public health implications. Hence there is likelihood for co-infections of these 3 infections. This was a hospital based cross sectional study in which 400 participants were randomly selected. Venous blood samples were collected from the study participants. Malaria diagnosis was carried out using polymerase chain reaction and positive samples were quantified using microscopy, HIV diagnosis was done using Uni-Gold rapid test kits and SD Bioline. TB was diagnosed using culture. The prevalence of malaria occurring as mono, co and triple infections was 23.3% (malaria), (1.5%) TB/ malaria, (21.3%) HIV/ malaria and HIV/TB/malaria (2.5%). The mean parasite density in single malaria infection was 410 ± 2515.5 parasites/µl. When this was compared with the parasite density in co and triple infections, those with TB/HIV/ malaria triple infection had the highest mean parasitemia (461.1 ± 295.0 parasites/µl). The lowest mean parasite density (271.0 ± 198.7 parasites/µl) was observed in patients co-infected with TB/malaria. However the difference in the mean parasite density was not statistically significant (p=0.329). Hence, there may be need to set up guidelines for the management of malaria in people with co-infections of HIV and TB.
Establishing the extent, geographical distribution and mechanisms of insecticide resistance in malaria vectors is a prerequisite for resistance management. Here, we report a widespread distribution of knockdown resistance (kdr) in the major vector of human malaria, Anopheles gambiae sensu stricto (s.s) across wetlands of the Cameroon volcanic line (CVL). Female Anopheles mosquitoes were collected throughout seven wetlands located in five volcanic massifs in Cameroon. The species composition, Plasmodium infection rate and molecular bases of the kdr resistance were then analyzed. Mosquito collection revealed a predominance of An. gambiae s.s. (including An. coluzzii and An. gambiae) mosquitoes with a low hybrid rate (1.1%) suggesting rare occurrence of hybridization. Overall, An. gambiae s.s. specimens were the second Plasmodium vector (0.72 % infection) and exhibited an entomological inoculation rate ranged between 0.7 to 2.24 infected bites per human per month. Both 1014S and 1014F kdr alleles were found in An. gambiae s.s. with overall frequencies of 8.9% and 97% respectively. Analysis of a 500bp region of exon-20 downstream the kdr locus revealed a total of 18 polymorphic sites. The number of haplotypes varies from 4 to 11 per wetlands, with an overall 0.802 haplotype diversity. The mean number of nucleotide differences was estimated at 2.30. Additionally, no signature of selection was observed on the voltage-gated sodium channel gene. The extensive distribution of knockdown resistance in CVL An. gambiae s.s. populations represents a challenge to vector control. Moreover, the mosaic of genetic events found in wetlands across the CVL of the situation throughout the country. This highlights the importance of evaluating the spatial and temporal evolution of kdr alleles for a better management of insecticide resistance.

Thrombocytopenia and whole blood transfusion in children with severe falciparum malaria

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Severe malarial anemia due to Plasmodium falciparum is often accompanied by thrombocytopenia. Treatment includes transfusion of whole blood, which contains erythrocytes, platelets, and other blood components. To assess the effect of whole blood transfusion on survival in children with severe falciparum malaria and to examine the potential association of thrombocytopenia with malaria mortality and transfusion response, we analyzed a retrospective cohort of 842 hospitalized children in Zambia with severe malarial anemia (703 transfused, 139 not transfused due to stock-out or other reason). Severe malarial anemia was defined as a positive rapid diagnostic test or blood smear in combination with an admission hemoglobin concentration ≤5 g/dL. Mortality was 13% (94/703) in the transfused group and 24% (34/139) in the non-transfused group. Kaplan-Meier survival estimates stratified by transfusion status and thrombocytopenia (150,000/μL threshold) showed increased mortality in children with thrombocytopenia who did not undergo transfusion, with no differences in mortality among the other transfused and non-transfused groups (log-rank test P=0.0001). Effect modification analysis by Cox proportional hazards regression adjusted for age, sex, hemoglobin concentration, blood group type, and eosinophilia showed a significant interaction between platelet count and transfusion status (P<0.028). Children with thrombocytopenia who were transfused and died had little or no post-transfusion increase in platelets, in contrast to those who survived. Freshness of transfused whole blood, construed from expiration dates, correlated with greater platelet recovery and improved survival. The role of platelets in malaria pathophysiology is complex and incompletely understood; prior studies describe preferential binding of platelets to parasitized erythrocytes and direct parasitocidal activity, whereas others detailed deleterious effects in malaria involving the central nervous system vasculature. These findings point to a potential clinical role for platelet-directed transfusion strategies to improve survival in children with severe falciparum malaria, which should be further assessed in randomized interventional studies.

Determining spatial heterogeneity of potential Anopheles breeding and blood-meal sites in Choma District, Zambia through image mining of remote sensing data

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Modeling local transmission and conducting geostatistical analysis of malaria transmission dynamics require location data for several types of sites: aquatic habitats potentially hosting *Anopheles* mosquito larvae, and households where human hosts reside are both too numerous and expensive to manually geolocate. Thus, we sought to mine satellite imagery of Macha, Zambia and the surrounding area for these sites of interest with GIS techniques and software. We utilized ArcGIS software to conduct our analyses and develop our model and for this required two different approaches to harvest our two types of spatial coordinates because of the high-resolution necessary to resolve small features. The classification stage of the aquatic-habitats/breeding site model consisted of a Principle Components Analysis (PCA), manual training set supervised Maximum Likelihood Classification (MLC). The classification stage of the household/blood-meal model applied pansharpening to the panchromatic raster followed by a 25-class unsupervised ISODATA classifier, which was reclassified to a binary raster with analyst generated class selection. Both models shared techniques for feature extraction once we had reached a binary raster; which consisted of a transformation from raster to polygon, then filtering the polygonal shape based on empirical data. After transformation and geolocation of coordinates, an accuracy assessment of our models resulted in enumeration and coordinates for 3,702 aquatic habitat locations and 27,548 host household locations. An accuracy assessment using at 350 km² test region gave the aquatic habitat geolocation model an F1 score: 0.9181, and the blood-meal/household geolocation model an F1 score: 0.8547. We were able to map the observed household and aquatic habitat distribution and densities across the 2400 km² study area as well as infer spatial relationship to potential malaria transmission sites from these data. These findings support the use of GIS software and spatial modeling to inform new microscale agent-based models for policy or intervention assessment.

**Manual dissection of mosquito salivary glands with shortened training timelines**

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*Sanaria Inc., Rockville, Maryland*

Sanaria’s product portfolio is based on the only proven approach in humans, of inducing robust and long-lasting protective efficacy against *Plasmodium falciparum* (Pf) malaria by sporozoite (SPZ)-based vaccines. All products use the stage of malaria parasite that is transmitted from mosquitoes to humans. Therefore, in Sanaria’s manufacturing process, the mosquito behaves as a bioreactor, from which parasites are then extracted by microdissection of the insects’ salivary glands ensuring several thousand-fold purification away from irrelevant mosquito constituents. The extraction of salivary glands by microdissection of mosquitoes involves four distinct steps performed by skilled personnel a) mosquito alignment b) decapitation c) gland extrusion and d) gland collection. In earlier iterations all steps of dissection were performed by individual trained disectors, whereas in late 2015, altered configurations led to a 2 to 3-fold increase in efficiency and throughput of fully manual dissection. In addition, ergonomic set up of the work stations reduced dissector fatigue and allowed longer working hours. Sanaria is currently in the process of scaling up production to meet the needs of upcoming Phase 3 clinical trials. Over the course of ten months, we have implemented a new training program that brings on part-time dissectors to participate in GMP dissection for manufacturing, selecting 22 dissectors from a pool of 85 applicants. In the past eight months, we have trained five cohorts of dissectors, fully qualifying two cohorts. A concentrated training schedule and consistent effort by the part-time dissectors has resulted in a significant decrease in training time, from seven to three months. We will discuss the structure of the training program and its impact on the efficiency and flow of our manufacturing process.

**Non-*Plasmodium falciparum* malaria parasite infections in symptomatic and asymptomatic adolescents in Nigeria**

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Malaria is caused by species of the genus *Plasmodium*. The ranges of these species overlap, and mixed species infections of the same host are common. There is currently little known concerning the extent to which interaction between coinfecting parasite species impact on the pathogenicity and transmission dynamics. In malaria endemic regions a certain percentage of the population will harbor parasites in their blood without succumbing to the symptoms. These asymptomatic malaria parasite carriers do not seek antimalarial treatment and so infections are likely to persist for long periods, moreover individuals may still transmit the parasite to mosquitoes, thus constituting a silent infectious reservoir. In order to gauge the level of asymptomatic...
carriage amongst adolescents in a highly endemic area, and to identify the risk factors associated with such carriage, we conducted a cross sectional survey of 1032 adolescents (aged 11-17) from eight schools located in Ibadan region, South West Nigeria. Blood film and filter paper samples were prepared for microscopic examination and for DNA. The prevalence of asymptomatic and symptomatic malaria was determined using microscopy, Rapid diagnostic tests and PCR. Species typing was performed using PCR targeting the mitochondria COXIII gene. We found a higher rate of mixed infections and a higher prevalence of P. ovale and P. malariae than usually observed in asymptomatic individuals. Interestingly, the symptomatic age mates inhabited single infections predominantly P. falciparum. We are currently investigating the hypothesis that the presence of non-falciparum species in mixed infections is responsible for protection against symptomatic P. falciparum infection.

**PP-35**

**A 7-year trend of malaria at primary health facilities in Northwest Ethiopia**

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Malaria is a severe parasitic disease that can progress to complications of the nervous system, respiratory distress, renal problems, metabolic acidosis and hypoglycemia which can result in death in case of delay or absence of appropriate treatment. The aim of this trend analysis was to assess the prevalence and the impact of malaria over the seasons and years. A cross-sectional retrospective study was conducted at two health centres Gorgora and Chuaht in Dembia district. The data was collected from lab log books routinely diagnosed and registered for seven years. A systematic sampling technique was used by taking patient results from lab log books during the first ten days of every month. Data was entered directly into EpiData Entry software version 3.1 and analysed with SPSS software version 20. Moreover, a chi-square test with a level of significance set at less than 5% was used. In the study a total of 11879 clients 6724 (56.6) of whom were males participated. The overall malaria prevalence in the last seven years was 21.8% and the dominant parasite was Plasmodium falciparum which accounted 16.5% of the cases. In the analysis of the seven years, October and September in which the prevalence of malaria was 32.6% and 27.2% respectively, constituted the peak months. High malaria prevalence was observed on autumn (September to November) season and the least was observed in winter (December to February) with 17.8% cumulative prevalence (p<0.001). Malaria attack showed a significant variability among different age groups, and the age group 15-29 was the most affected (p<0.001). There was also a significant differences in malaria prevalence between the two sexes and males were more affected than females

Drug resistance against malaria parasite is increasing day by day. To keep this background in the mind we find out the formulation which can help to reduce the drug resistance against malaria parasite. So the study was designed to prepare a combination therapy for malaria treatment in in vivo model of malaria parasite *Plasmodium berghei*, the study has been carried out in the Department of Medical Parasitology Post Graduates Institute of Medical Education and Research Chandigarh India. Total 36 mice were used in this study. Total six groups liver tissues were processed for the immunohistochemistry. *Plasmodium berghei* infected mice were treated with different formulations in mice model BALB/c. Parasitemia, Histopathology and, Immunohistochemistry (IHC), Flow cytometry analysis (FACS) was assessed and Transmission Electron microscopy (TEM) of different organ was done. Antimalarial effects of nanocapsules in combination with synthetic peptide and the *bLf* loaded nanocapsules were assessed. The comparison was done with the help of Drug Chloroquine. The tissues were prepared in the Histopathology department with the help of technical staff sectioning was done by microtome. The tissues preparation for TEM was performed in the TEM tissue processing lab. The tissues of intestine, liver, kidney were processed. Antigen and antibody complex was observed in the Positive control groups as well as treated with the drugs. The internalization of the Nanocapsules has been observed with the help of TEM of Intestinal tissues. The group treated with the help of combination therapy of *bLf* and synthetic peptides has shown maximum inhibition of parasite. The further the results were confirmed with the

**PP-36**

**Antimalarial effects of nanocapsules of synthetic peptides and bovine Lactoferrin combination in in-vivo model of Plasmodium berghei**

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Drug resistance against malaria parasite is increasing day by day. To keep this background in the mind we find out the formulation which can helpful to reduce the drug resistance against malaria parasite. So the study was designed to prepare a combination therapy for malaria treatment in in vivo model of malaria parasite *Plasmodium berghei*, the study has been carried out in the Department of Medical Parasitology Post Graduates Institute of Medical Education and Research Chandigarh India. Total 36 mice were used in this study. Total six groups liver tissues were processed for the immunohistochemistry. *Plasmodium berghei* infected mice were treated with different formulations in mice model BALB/c. Parasitemia, Histopathology and, Immunohistochemistry (IHC), Flow cytometry analysis (FACS) was assessed and Transmission Electron microscopy (TEM) of different organ was done. Antimalarial effects of nanocapsules in combination with synthetic peptide and the *bLf* loaded nanocapsules were assessed. The comparison was done with the help of Drug Chloroquine. The tissues were prepared in the Histopathology department with the help of technical staff sectioning was done by microtome. The tissues preparation for TEM was performed in the TEM tissue processing lab. The tissues of intestine, liver, kidney were processed. Antigen and antibody complex was observed in the Positive control groups as well as treated with the drugs. The internalization of the Nanocapsules has been observed with the help of TEM of Intestinal tissues. The group treated with the help of combination therapy of *bLf* and synthetic peptides has shown maximum inhibition of parasite. The further the results were confirmed with the
help of FACS analysis and DAPI staining the overall parasite reduction was noticed. Further the results were confirmed by the FACS analysis and DAPI staining of the infected RBCs from all the groups. So the present study revealed antimalarial effects of the combination therapy of Synthetic peptides and blf loaded nanocapsules. This is the first study which confers the best combination therapy of protein and synthetic peptides for malaria parasite inhibition in the rodent model. The study reveals that the combination therapy along with the peptides and protein is providing the best treatment for the rodent malaria infection. This will be the future medicine for the treatment of human malaria parasite. For the best results in the human’s parasite more studies are required in the higher vertebrate host and in cell lines also to check the best mechanism of these formulations. Moreover the material used in this formulation is biodegradable. It is safe and don’t required any toxicity study. It will be the future of antimalarial compounds. With the help of this study we can design the other molecules for the best results.

Factors Associated with Asymptomatic Malaria among People Living with HIV Following Discontinuation of Cotrimoxazole Prophylaxis at Kitgum Hospital, Northern Uganda

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Factors associated with malaria parasitaemia in patients living with HIV following discontinuation of cotrimoxazole prophylaxis at the Kitgum hospital HIV clinic. This was a cross sectional study conducted between March and April 2019 at the Kitgum hospital HIV clinic. We consecutively enrolled 599 participants aged 18 years and above and living with HIV attending the clinic. A pre-tested questionnaire was administered to the participants, and a standardized physical exam was conducted. All participants provided a finger-prick thick blood smear which was stained with Giemsa and used to assess for the presence of malaria parasites. Factors associated with malaria parasitaemia among those who had been discontinued on cotrimoxazole were assessed using binary logistic regression. Of the 599 participants enrolled, 452 (75.5%) had stopped cotrimoxazole prophylaxis for at least 3 months. The overall prevalence of malaria parasitaemia was 4.5% (95% confidence interval [CI] 3.0% - 6.5%). There was a significant difference in malaria parasitaemia prevalence among participants who had stopped cotrimoxazole (5.5% [95% CI 3.6% - 8.1%]) compared to participants on cotrimoxazole prophylaxis (1.4% [95% CI 0.17 - 4.83], p=0.034). Factors associated with malaria parasitaemia included increasing duration following the discontinuation of prophylaxis (adjusted odds ratio [aOR] 1.79, 95%CI 1.225 - 2.621, p = 0.003), CD4 count greater than 250 cells/ul (aOR 0.17, 95%CI 0.071 - 0.426) and bed net use the night prior to survey (aOR 0.31, 95%CI 0.105 - 0.906) were found to be significantly associated with malaria parasitaemia. People living with HIV who had stopped cotrimoxazole prophylaxis for at least 3 months had a significantly higher prevalence of malaria parasitaemia compared to those on prophylaxis. Increased duration was associated with increasing odds of having parasites while IRS, bed-net use and high CD4 count were associated with reduced odds of having malaria parasites. These results suggest that malaria preventive methods including IRS and bed-net use should be promoted in HIV positive patients in whom cotrimoxazole prophylaxis is being discontinued so that they are protected from malaria.

Screening Pathogen Box Compounds for Activity Against Plasmodium falciparum Motility

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As the malaria parasite becomes resistant to every drug that we develop, identification and development of novel drug candidates is essential. Many studies have screened compounds designed to target the clinically important blood stages. However, to eradicate malaria, new drugs that block transmission of the parasite between the mammalian host and the mosquito vector are essential. Malaria infection is initiated when mosquitoes inoculate 10 to 100 sporozoites as they probe for blood. Sporozoites actively move in the skin in order to find and enter the blood circulation and be carried to the liver. As sporozoite inoculum is small and motility is critical to their ability to exit the inoculation site and establish infection, we have initiated a study to screen the Pathogen Box compounds for their activity on sporozoite motility. To this end, we have established a moderate throughput assay using sporozoites of the human malaria parasite, Plasmodium falciparum. Sporozoites isolated from infected Anopheles mosquitoes, were incubated with 400 drug-like compounds from the Pathogen box provided by Medicines for Malaria Venture. Compounds exhibiting
inhibitory effects on sporozoite motility were further assessed against asexual stage growth and transmission-blocking activity. Six compounds had a significant inhibitory effect on in vitro P. falciparum sporozoite gliding at 1 µM concentration. Of these 6 compounds, 5 compounds showed significant inhibition on asexual parasite growth and 3 compounds demonstrated significant inhibitory activity on both asexual stage and transmission stage in mosquitoes. In summary, we have identified 3 compounds that have significant inhibitory effects on multiple stages of P. falciparum, including both transmission stages. Further studies are required to reveal the mechanism of action of these compounds. Our findings provide new antimalarial drug candidates for combination therapy as well as prophylaxis.

Using Antonovsky’s Salutogenic Model of Health as new lenses for malaria research
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Malaria as a major public health problem remains uncontested despite global efforts. Malaria elimination and control has been a feature point in the 2005 millennium development goals as well as the 2016 sustainable development goals but that notwithstanding the problem remains. So far, the fight against malaria and other poverty-related diseases (PRDs) have been disease focused and has hardly highlighted efforts people put in place to combat infectious diseases. The future of malaria research therefore needs to see malaria eradication through different lenses. We propose a salutogenic approach to the fight against malaria. Salutogenesis asks the question: what creates health? Instead of focusing on risk factors and prevention strategies, the salutogenic model focuses on how people identify and use generalised and specific resistance resources around them to not only understand the stressor called malaria but also to overcome. In this paper, we would present a two-case mixed method study involving students of two universities in Cameroon and Cameroon Development Corporation camp dwellers to highlight how the salutogenic model of health (SMH) reveals coping strategies with malaria. The salutogenic perspective lays emphasis on efforts people undertake to stay in the ease end of the ease-dis (ease) continuum. Our results confirmed malaria as the most commonly perceived PRD by over 90% of respondents in both settings. The presence of malaria was attributed to poor hygienic conditions of the environment. The study revealed that people with a higher sense of coherence (SOC) were most likely to identify and use resources in their environment using varied mechanisms in order to cope with malaria. The results of this study call for the consideration and inclusion of the SMH in future malaria research for better and more sustainable outcomes and the promotion of health in affected populations.

A Hierarchical Bayesian Model for Background Correction of Protein Microarrays
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Protein microarrays allow users to characterize large numbers of proteins in parallel and have the potential to provide information about cumulative exposure to Plasmodium antigens with a cross-sectional study design. However, to extract this information from a protein microarray, attention must be paid to removing noise while retaining the biologically relevant signal. While methods have been developed to correct for background on DNA microarrays, the unique properties of proteins including variable size and charges, structural complexity and binding affinity warrants background correction methods optimized for protein microarrays. We propose a hierarchical Bayesian model that includes measured foreground and background signals at each probe. Markov chain Monte Carlo produces the posterior distribution for the true signal, and the posterior mean and other summaries are available, or the full distribution can input to the next phase of analysis. We show that this model leaves high intensity signals, for which background noise has low impact, close to the directly computed estimate. Low intensity signals receive a substantial and beneficial adjustment, while keeping the mass of the posterior distribution on positive values. Moreover, our proposed modeling approach impacts the rank of the fluorescent intensity of several antigens in Plasmodium falciparum-specific antibody arrays run with serum samples from high and low malaria transmission settings in Zambia and Zimbabwe.

Identification of Expressed Var genes in Whole Blood Clinical Samples with a Custom Capture Array Versus RNA Enrichment Methods

The Johns Hopkins Malaria Research Institute 5th Annual Future of Malaria Research Symposium, November 18th, 2019
The var gene family encodes *Plasmodium falciparum* erythrocyte membrane protein-1 ( PfEMP1) antigens. These highly diverse antigens are displayed on the surface of infected erythrocytes and play a critical role in immune evasion and sequestration. Studies of var expression using non-leukocyte-depleted blood are challenging due to the predominance of host genetic material and lack of conserved var segments. To address these barriers, we compared two enrichment methods for parasite RNA extracted from whole blood clinical samples—globin and rRNA depletion followed by polyA selection vs. a custom capture array based on Roche’s SeqCap EZ Enrichment System. The capture array was designed with probes covering the 3D7 reference genome and an additional >4,000 full-length var gene sequences. We tested each method on the same samples from Malian children with severe or uncomplicated malaria infections, and sequenced using Illumina. Var-like transcripts were identified from the de novo assembly of non-human reads and annotated. For each sample, we compared transcript length and number of unique transcripts generated from each enrichment method. To determine if each method yielded identical var sequences, we compared sequences generated by each method. We then quantified var expression to determine if expression correlated between methods. Depletions of the most abundant human RNAs followed by polyA selection produced transcripts with greater median length in samples with the highest parasitemias compared to the capture method. The capture array produced the longest maximum length and largest numbers of transcripts for each sample, particularly for samples with low parasitemia (<2,000 parasites/µL). The capture method produced more unique fragments, including up to 20 distinct acidic terminal sequence domains per sample. Further evaluation will include expression analyses of samples with known var repertoires to determine the method best suited to analyze var expression in studies with both low and high parasitemia samples.

**A robotic system for extracting salivary glands from anopheles mosquitoes for malaria vaccine production**

Henry Phalen, Prasad Vagdargi, Hongtao Wu, Michael Pozin, Andrew Shaughnessy, Sumana Chakravarty, Gregory S. Chirikjian, Julian Iordachita, Russell H. Taylor

This talk reports progress toward the development of an automated system for extracting salivary glands from *anopheles* mosquitoes, as part an ongoing collaboration between Johns Hopkins and Sanaria, Inc. (Rockville Maryland) to facilitate the production of a live organism vaccine for malaria using *Plasmodium falciparum* sporozoites (PFSPZ). In previous work, we have developed a “semi-automated” system, in which mosquitoes are sorted manually into cartridges so that their heads extend between cutter blades. Then the blades are actuated to decapitate all the mosquitoes in a cartridge, and a comb-like device is used to extrude all the salivary glands, which are then collected via a suction device. Based on this experience, we have begun development of a fully automated system. This talk focuses on a key step in our automated process, the picking and placing of mosquitoes from a staging apparatus into a dissection assembly using a 4 degree-of-freedom robot under the guidance of a computer vision system. Mosquitoes are autonomously grasped from a mesh platform and pulled to a pair of notched dissection blades to remove the head of the mosquito, allowing access to the salivary glands. Placement into these blades is adapted based on output from computer vision to accommodate for the unique anatomy and orientation of each grasped mosquito. In this pilot test of the system on 50 mosquitoes, we demonstrate a 100% grasping accuracy and a 90% accuracy in placing the mosquito with its neck within the blade notches such that the head can be removed. This is a promising result for this difficult and non-standard pick-and-place task. An analysis of the failure cases provides insights for improvements to be implemented as this robotic pick-and-place system is integrated into a larger automated mosquito dissection system under development.

**Mapping the Aedes aegypti antennal lobes**

Shruti Shankar, Conor J. McMeniman

*Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland*
The antennal lobes (ALs) are the primary olfactory centre of the brain of the yellow fever mosquito, *Aedes aegypti*. In mosquitoes, the ALs mediate processing of a diversity of volatile odorants that drive innate behaviors such as host seeking, egg laying and foraging. Anatomically, the ALs are composed of functional units of neuropil known as glomeruli. An individual glomerulus is the point at which the axonal projections of olfactory sensory neurons expressing a single type of chemoreceptor, or complement of chemoreceptors converge and synapse with the dendritic arbors of projection neurons and local interneurons. We present 3D models based on manual image segmentation and reconstruction of the left and right ALs from 10 male and 10 female mosquito brains. We observe that the AL is entirely composed of 77-80 olfactory glomeruli, and based on depth and spatial position, along the anterior-posterior, ventral-dorsal and medial-lateral axes, we classify 13 glomerular spatial groups in the male and female ALs. Although we did not identify any sexually dimorphic glomeruli in the *Ae. aegypti* ALs, we found overall that the volume of the female antennal lobe, was approximately 1.5 times larger that of the male mosquitoes. Furthermore, using the binary QF2-QUAS binary expression system, we trace the neuronal projections of three classes of chemosensory neurons, to find non-overlapping subsets of 62 *Orco*, 14 *Ir8a* and 1 *Gr* positive glomeruli in each AL. These studies describe a revised model of the *Aedes aegypti* ALs. The high-resolution anatomical map presented here will serve as a useful reference for future functional imaging studies that aim to identify glomeruli activated by human odors to decode the molecular and cellular basis of mosquito attraction to humans.

**Identifying proteins that are differentially expressed in *Asaia bogorensis* under bloodmeal-like conditions**

Marisa Guido, David J. Lampe

*Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania*

Malaria is one of the deadliest vector-borne diseases and claims thousands of lives yearly. Both the causative agent of malaria, *Plasmodium sp.*, and the *Anopheles* mosquito that transmits it are gaining resistance to many current methods of control. By genetically engineering symbiotic bacteria within the mosquito midgut, a process termed paratransgenesis, antiplasmodial effectors can be secreted to reduce *Plasmodium* transmission. *Asaia bogorensis* is a Gram-negative, rod shaped bacteria that naturally colonizes the midgut, ovaries, and salivary glands of *Anopheles* mosquitoes, making it an ideal candidate for modification. However, there are concerns that introducing foreign DNA sequences will cause the transgenic *A. bogorensis* to be outcompeted by wild type. Inserting antiplasmodial effectors downstream of secreted proteins that are upregulated in the presence of blood would introduce the least amount of foreign DNA, and may be the least disruptive system for transgenic *A. bogorensis*. FASTA sequences for proteins both upstream and downstream of promoters known to be bloodmeal inducible (BMI) were collected, and the PSORT program was used to predict the localization of the candidate proteins. Five candidates were identified around the Hemin promoter; one was a predicted extracellular protein downstream, three were predicted outer membrane proteins upstream, and one was a predicted extracellular protein upstream. Primers were then designed for the candidate proteins to observe expression with or without blood. *A. bogorensis* was grown on both minimal media and chocolate agar containing lysed red blood cells. The RNA was then harvested and used in RT-qPCR to determine if there was differential expression of these proteins in bloodmeal-like conditions. Proteins that appeared to be upregulated in bloodmeal conditions were then selected for further modification.

**Assessing Rapid Diagnostic Test and Blood Smear Microscopy outcomes with clinical diagnoses for malaria in Kano, Nigeria**

Michael Vera, Nirmal Ravi

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Malaria is a leading global health issue that disproportionately affects sub-Saharan Africa more than any other region in the world. In 2017, Nigeria accounted for the greatest malaria burden at approximately one quarter of the estimated 219 million global cases. Rapid Diagnostic Test (RDT) and Blood Smear Microscopy (BSM) are the principal diagnostic methods used by clinicians to supplement clinical evaluation. Due to their affordability, robustness, and high sensitivity and specificity, clinicians rely on these parasitological tests for case confirmation. The purpose of this study was to investigate RDT and BSM test outcomes reported from a private health facility in Northcentral Nigeria to inform the clinic’s management of test positive rates and laboratory adherence to testing policy. A secondary analysis to determine the associations between final diagnostic outcomes and patient visit characteristics was performed. An examination of 386 patient visit records (60.3% visits male, 28.5 years (SD=11.9) average patient visit age, 24.8 individual patients) from July 2018 to March 2019 revealed RDT and BSM test positive rates of 8.8% (n=38) and 24.3% (n=93), respectively. Malaria prevalence in Kano was previously reported at 60.6% (n=551) in 2016. Test positive rates reported for RDT and BSM in nearby Kaduna were 11.9% and 10.5% (n=295), respectively. A disease outcome status
algorithm reliant on RDT, BSM, Loop-Mediated Isothermal Amplification, prescribed anti-malarial medication, and clinic policy was developed and found a malaria patient visit case-positive period prevalence of 13.5% (n=385). Univariate analysis of the association between malaria case-positive outcome and patient visit characteristics found that subjective reporting of fever (OR=8.3, 95% CI=2.5-27.1), pain (OR=2.1, 95% CI=1.2-3.8), and loss of appetite (OR=2.8, 95% CI=1.2-6.5) were statistically significant. Presumed selection bias due to the higher patient socioeconomic status required for clinic services and discrepancies between this investigation's findings and published findings suggests further bias analysis.

PP.47

The effect of an artificial blood meal, SkitoSnack, on vector competence of Aedes aegypti and Anopheles stephensi for Plasmodium gallinaceum and P. falciparum.

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The Aedes aegypti mosquito is a vector for the yellow fever, dengue, chikungunya, and Zika viruses, and the Anopheles stephensi mosquito is a vector for the malaria parasites, Plasmodium falciparum and P. vivax. With urban expansion of Ae. aegypti and An. stephensi, innovative vector control strategies are needed to reduce the spread of mosquito-borne disease. For research and some control approaches, mass rearing of mosquitoes in insectary facilities requires large amounts of vertebrate blood, which is expensive and may introduce pathogens. Several artificial blood meals have therefore been created and tested on Aedes aegypti mosquitoes and, more recently, Anopheles mosquitoes, but little is known about potential influences of these meals on vector competence. We have tested one of these meals, SkitoSnack, on the competence of Ae. aegypti for the avian malaria parasite, Plasmodium gallinaceum, and of An. stephensi for the human malaria parasite, P. falciparum. Mosquito eggs from NIH-established Ae. aegypti and An. stephensi colonies were hatched and maintained under standard insectary conditions. Adult female mosquitoes, 3-7 days old, were fed a bovine blood or SkitoSnack meal via an artificial membrane feeding system and were bred on these meals through several consecutive generations. To determine vector competence, Ae. aegypti females were fed on an anesthetized P. gallinaceum-infected chicken, while An. stephensi females were fed through an artificial feeder containing P. falciparum-infected blood. Mosquito midguts were dissected 7 days post infection and oocysts were stained and counted under a light microscope. We found the P. gallinaceum parasite loads of Ae. aegypti raised on bovine blood vs. SkitoSnack for 1, 3, and 5 generations were similar. Results from SkitoSnack-raised An. stephensi are pending. SkitoSnack as a blood substitute will support pathogen-free mosquito lines and may help to reduce costs of large-scale mosquito production in insectary facilities.

PP.48

A recombinant system for the creation of stable antiplasmodial paratransgenic Asaia sp.

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Malaria, a disease caused by the parasite Plasmodium sp., is responsible for hundreds of thousands of deaths each year. Reductions in infection and lethality of malaria have stalled in recent years, so new control strategies must be sought. One such strategy includes the transgenic modification of symbiotic bacteria within the mosquito midgut to produce antiplasmodial effectors in a process known as paratransgenesis. Asaia sp., a Gram-negative rod-shaped bacterium which colonizes the midgut, ovaries and salivary glands of Anopheles mosquitoes, has been identified as an ideal candidate for antiplasmodial paratransgenesis. Previous work developed paratransgenic strains of Asaia which were shown to be effective at reducing oocyst prevalence within infected mosquitoes. However, these strains are plasmid-based and rely on drug-selection for stability, which is unsuitable for field-release. One way to develop stable strains of paratransgenic Asaia is to insert the antiplasmodial effectors into the chromosome. Site-specific recombination via sacB counterselection was used to insert the general antimicrobial peptide scorpine downstream of the native conditional bloodmeal induced promoter HlyC within the chromosome of Asaia SF 2.1. This strain was grown in bloodmeal-like conditions to induce production of scorpine. Western blots were performed on both cell lysate and supernatant fractions and compared to uninduced transgenic controls. Further testing will confirm the efficacy of this strain against Plasmodium in a rodent model.

PP.49

Comparative analyses of parasites with a comprehensive database of genome-scale metabolic models

Maureen A. Carey, Gregory L. Medlock, Michael Stolarczyk, William A. Petri, Jennifer L. Guler, Jason A. Papin,
Protozoan parasites cause diverse diseases with large global impacts. Research on the pathogenesis and biology of these organisms is limited by economic and experimental constraints. Accordingly, studies of one parasite are frequently extrapolated to infer knowledge about another parasite, across and within genera. Model in vitro or in vivo systems are frequently used to enhance experimental manipulability, but these systems generally use species related to, yet distinct from, the clinically relevant causal pathogen. Characterization of functional differences among parasite species is confined to post hoc or single target studies, limiting the utility of this extrapolation approach. To address this challenge and to accelerate parasitology research broadly, we present a functional comparative analysis of 192 genomes, representing every high-quality, publicly-available protozoan parasite genome including 44 genomes for 20 Plasmodium species, as well as Toxoplasma, Cryptosporidium, Entamoeba, Trypanosoma, Leishmania, Giardia, and other species. We generated an automated metabolic network reconstruction pipeline optimized for eukaryotic organisms to serve as biochemical knowledgebases for each parasite, enabling qualitative and quantitative comparisons of metabolic behavior across parasites. We identified putative differences in enzyme essentiality and pathway utilization to facilitate the comparison of experimental findings. We predict that Toxoplasma gondii is a better model system for Cryptosporidium species than for Plasmodium species. Moreover, the enzyme essentiality of P. falciparum 3D7 is more similar to P. vivax Sal 1 than to P. berghei ANKA. We also discovered that phylogeny is not the sole predictor of metabolic similarity. More broadly, this knowledgebase represents the largest collection of genome-scale metabolic models for both pathogens and eukaryotes; with this resource, we can predict species or strain-specific functions, contextualize experimental results, and optimize selection of experimental systems for fastidious species.

**Cholesterol-dependent enrichment of understudied erythrocytic stages of human Plasmodium parasites**

Audrey C. Brown¹, Christopher C. Moore³, Jennifer L. Guler¹²

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Plasmodium parasites undergo rounds of asexual replication inside human erythrocytes, progressing from ring stage, to trophozoites and schizonts, before egress and reinvasion. Given the discovery of ring-specific artemisinin tolerance and quiescence in Plasmodium falciparum, there is great urgency to better understand ring stage biology. However, the lack of an effective enrichment method has left rings and related parasite stages understudied compared to their late stage counterparts, which can be easily isolated due to their paramagnetic properties. Here, a method for separating all Plasmodium infected erythrocytes from uninfected erythrocytes is presented. This approach takes advantage of streptolysin-O (SLO) to preferentially lyse uninfected erythrocytes as previously shown by Jackson, et al. Following lytic treatment, Percoll gradient centrifugation removes lysed cells, leaving an intact cell population enriched in infected erythrocytes. This SLO-Percoll (SLOPE) method is effective on stages from the entire erythrocytic cycle, including previously inaccessible forms such as circulating rings from malaria-infected patients and artemisinin-induced quiescent parasites. Furthermore, the utility of SLOPE is extended to multiple media formulations used for the propagation of two human Plasmodium species. The alteration of external cholesterol levels modulates SLOPE effectiveness, demonstrating the role of erythrocyte membrane cholesterol in lytic discrimination. Importantly, enrichment does not impact parasite viability, which establishes the non-toxic nature of SLOPE. Targeted metabolomics of SLOPE-enriched ring stage samples confirms the impact on treated samples; parasite-derived metabolites are increased and contaminating host material is reduced compared to non-enriched samples. The SLOPE method is an accessible, scalable, rapid (30-40min), and nontoxic enrichment method that is broadly effective on many erythrocytic stages. This method is ideal for use upstream of a variety of sensitive analyses, which will increase experimental quality in virtually all areas of asexual Plasmodium parasite research.

**PP-51**

*In vitro evolution of high-level resistance to P. falciparum cytochrome B inhibitors*

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The major energy production pathway in Plasmodium falciparum is cytosolic glycolysis, however, the mitochondrial membrane potential is vital in all stages (erythrocytic, liver, mosquito). Thus, electron transport chain (ETC) enzymes...
remain attractive drug development targets. The sole licensed ETC inhibitor, atovaquone (ATQ), binds the cytochrome B (CytB) quinol oxidation (Q₉) pocket; resistance is conferred by mutations of methionine 133 (in vitro) or tyrosine 268 (in vivo). Resistance to CytB inhibitors ATQ or RYL-552 in 106/1 mapped Q₉ site mutations tyrosine 268 to serine (Y268S, ATQ) or valine 259 to leucine (V259L, RYL-552). There was no cross-resistance to ATQ or RYL-552, although V259L was cross-resistant to the CytB inhibitor CK-2-68. All three small molecules bind the Q₉ pocket in a similar location. We asked if selection on Y268S or V259L with a second compound would result in further mutations and differential drug response phenotypes. Secondary selections were Y268S (RYL-552), V259L (ATQ), and both Y268S and V259L (CK-2-68). No additional CytB mutations were identified after Y268S secondary drug pressure, however the lines were highly ATQ resistant and markedly susceptibility to plumbagin (PL), a succinate dehydrogenase inhibitor. V259L (ATQ) resistant lines gained the additional CytB substitutions L144S or V284A. Both L144S/V259L and V259L/V284A demonstrated mid-level ATQ resistance, high-level resistance to antimycin A (AMA, CytB inhibitor of the quinone reduction pocket), and decreased PL sensitivity. V259L (CK-2-68) evolved a Y126C substitution, conferring high-level CK-2-68 and AMA resistance. Docking studies with CytB models demonstrated Y126C may confer high-level CK-2-68 resistance on the V259L background. Given a dwindling pipeline of effective chemotherapeutics, the emergence of multi-drug resistant parasites is an active threat to eradication efforts. These data suggest that CytB inhibitors targeting the same enzymatic domain are effective chemotherapeutics in vitro, lacking substantive cross-resistance. Combinations of these inhibitors may prove useful against future P. falciparum infections.

PP-52

Tracking mosquitoes over time: testing the role of aestivation in dry season persistence

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Despite its recognized importance, tracking mosquitoes over extended time has been beyond medical entomology’s toolkit. Stable isotope enrichment of mosquito breeding sites enables marking of mosquitoes without human handling. Larvae developing in [³H]-enriched water are structurally marked for life, providing a unique opportunity to test the hypothesis that Anopheles coluzzii, but not A. gambiae s.s and A. arabiensis, locally persists in the Sahel through the dry season via aestivation. A large-scale experiment to test this hypothesis began in September 2017 in two Sahelian villages in Mali. We aimed to estimate the contribution of aestivation to persistence of mosquitoes through the 7-month long dry season by marking at least 10% of the A. gambiae s.l. adults by the end of the wet season and assess the proportion of marked adults through the dry season, and immediately after the first rain in June 2018. If aestivation is the only way A. coluzzii persists, the frequency of marked mosquitoes should be similar throughout. Finding no marked mosquitoes would be evidence against aestivation. Twenty-four natural larval sites were enriched from late September. The marked adult proportion by the end of the wet season was above 60%. Five months later a similar frequency of marked adults was detected. Notably, nearly 8 months after enrichment, 6% showed clear marking. All the marked mosquitoes detected after the onset of the DS were A. coluzzii, in accord with aestivation in this species alone. Because [³H]-marking in mosquitoes has steadily weakened over time, we suspect some of the seemingly un-enriched mosquitoes had either lost their marking or dispersed to neighboring villages. Accordingly, we find that the distribution of [³H] in mosquitoes after the first rain exhibits an exceptionally heavy right tail, indicating that in addition to naturally un-enriched mosquitoes there are many enriched mosquitoes that lost much of their enrichment but are clumped in that zone. Our results provide, for the first time, hard evidence of population-wide aestivation in A. coluzzii.

PP-53

Manufacture of aseptic, purified, cryopreserved Plasmodium vivax sporozoites

Sumana Chakravarty, Natasha KC, Stephen L. Hoffman

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Plasmodium vivax (Pv), the second most important human malaria parasite, causes more than 80 million cases annually including severe, fatal disease. Prevention and control are challenged by emerging drug resistance and relapses from dormant liver stage parasites called hypnozoites. The only therapy against relapse, primaquine, causes life threatening acute hemolytic anemia in patients with G6PD deficiency, the most prevalent human genetic disorder, affecting 8% of people in malaria-endemic nations. This barrier to treatment results in repeated Pv attacks, aggravating the problem of control. Efforts to develop better drugs or produce a much-needed vaccine are further hampered by the inability to propagate blood stages of Pv parasites in vitro, unlike Pf. Therefore, generating infected mosquitoes for controlled human malaria infection (CHMI) as a means to assess anti-Pv
drugs and vaccines, is entirely reliant on feeding of mosquitoes on fresh, Pv-infected blood from patients with Pv malaria. Together, these bottlenecks make the task of developing and testing robust interventions against Pv malaria more challenging compared to Pf. We have now overcome this major limitation by using Pv gametocyte-infected Saimiri boliviensis (Sb) non-human primates (NHPs) to produce PvSPZ. In fact we are the only laboratory with an inventory of viable PvSPZ made from NHP-infected blood, having produced as much as 80 million PvSPZ vialved in 1 day from 2,000 mosquitoes. These cryopreserved PvSPZ are 1) infectious to hepatocyte cell lines in vitro in traditional monolayer formats over 3-6 days and in micro-patterned co-cultured primary human hepatocytes over 12-21 days, and 2) infectious to NHPs in vivo. In our efforts to increase regulatory compliance to manufacture a product for human use, we next attempted to produce aseptic, purified, cryopreserved, infectious PvSPZ (PvSPZ Challenge) by using a specific germ-free (SPF) colony of the permissive Sb as the source for Pv-infected blood. Under an NIH phase I SBIR funding we have now successfully vialled aseptic, purified Plasmodium vivax (Pv) sporozoites (SPZ) that were generated in aseptic mosquitoes using infected blood from SPF Sb. The vialled product met ascepticity criteria in all in-process steps as well as release assays, similar to analogous PfSPZ-based products. This newly established novel pipeline is intended to generate cGMP-compliant, controlled batches of PvSPZ, an innovation by Sanaria that will offer a consistent, quality-controlled stock of cryopreserved PvSPZ to promote well-controlled, reproducible in vitro and in vivo studies in Pv including CHMI. This enabling technology will support the development and testing of anti-Pv drugs and vaccines in CHMIs world-wide, just as PFSPZ Challenge has done for Pf CHMIs. It will also form the basis of a powerful vaccine approach to preventing Pv malaria when administered with anti-malarial chemophrophylaxis, the PvSPZ chemophrophylaxis vaccine (PvSPZ-CVac).

Whole-genome analysis of Plasmodium falciparum to understand clinical immunity to malaria

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After repeated P. falciparum infections, individuals in high-transmission areas acquire clinical immunity to malaria. However, the mechanisms important in determining clinical immunity are not entirely known. Here we take a whole-genome approach to identify genes that may be involved in acquisition of clinical immunity to malaria by sequencing and analyzing parasite genomes collected from infected individuals followed over time as part of a longitudinal study in Malawi. We compared parasite genomes from individuals with varying levels of clinical immunity, defined using an individual’s proportion of symptomatic infections. We also examined pairs of parasites collected at different time points from the same individuals, hypothesizing that these parasites will be more different from each other than expected by chance, at loci important for clinical immunity. Using FST as a measure of genetic differentiation, we identified several SNPs, including SNPs in vaccine candidate antigens such as AMA1, LSAPa and RESA, that are significantly genetically differentiated between individuals with varying levels of clinical immunity. Analysis of infections from the same individuals showed that there is lower identity-by-descent between parasites from the same individual compared to parasites from different individuals, highlighting the role of allele-specific immunity. We also found that several vaccine candidate antigens, such as SERA5, MSP6, AMA1, etc., differ more than expected by chance in parasites from the same individual compared to parasites from different individuals. SNPs in genes such as ama1 and claq2, as well as several genes encoding proteins of unknown function, were identified by both analyses, lending further support for their involvement in the development of immunity. We will analyze in silico predicted T/B-cell epitope regions in loci these genes to understand which regions of these proteins might be immunologically important. Identifying and further analyzing these genomic regions will provide insights into mechanisms involved in acquired immunity and antigenic escape.

Evaluation of PFCRT mutations associated with piperaquine resistance in Cambodia

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Resistance to artemisinins and key partner drugs such as piperaquine (PPQ) have become well-established in regions of Cambodia and neighboring countries. In 2016, Cambodia replaced dihydroartemisinin-piperaquine with artesunate-mefloquine as the first-line therapy. We and others have previously shown that mutations within the Plasmodium falciparum chloroquine resistance transporter (PfCRT) are associated with reduced susceptibility to PPQ. Subsequent gene-editing studies have shown that these mutations can independently confer PPQ resistance in parasites with single copy plasmepsin II. To better understand the emergence and distribution of PfCRT mutations associated with PPQ resistance, we are examining parasites from 478 clinical infections collected from eight provinces in Cambodia from 2009-2017. We are using PacBio amplicon sequencing or whole genome sequencing data to identify PfCRT mutations and quantitative PCR (or read coverage for samples with whole genome sequences available) to estimate plasmepsin II (pfpm2) copy number. In this dataset, amplified pfpm2 is observed in <1% of samples in 2009, does not reach high frequency until 2013 (62%) and 2014 (79%), then decreases in frequency in 2016 (48%) and 2017 (55%). Our preliminary data from a site in northern Cambodia identify multiple PfCRT mutations, including mutations H97Y, F145I, A128V, I218F, and G353Y. F145I is first observed in 2013 (24%), peaks in 2014 (34%), then decreases in prevalence in 2016 (24%) and 2017 (13%). However, H97Y, I218F, and G353Y each increase in prevalence to 24% in 2017 from 10%, 13%, and 20% in 2016, respectively. Whole-genome sequencing will be performed on parasites with PfCRT mutations of interest to determine parasite origins. This work provides insight into the emergence, dynamics, and origins of PfCRT mutations contributing to PPQ resistance.

In silico read capture and assembly enables allele reconstruction of highly variable loci

Theresa K. Hodges, Ankit Dwivedi, James Matsumura, Kara A. Moser, Andrea A. Berry, Shannon Takala-Harrison, Jonathan Crabtree, Joana Carneiro Da Silva

Theresa K. Hodges, Ankit Dwivedi, James Matsumura, Kara A. Moser, Andrea A. Berry, Shannon Takala-Harrison, Jonathan Crabtree, Joana Carneiro Da Silva

Cecilia S. Engdahl, Eric Caragata, Raul Saraiva, Hannah MacLeod, Luisa M. Otero, George Dimopoulos

Vector control plays a key role in reducing the number of mosquito-borne diseases. Today's vector control strategies heavily rely on synthetic insecticides that struggle with resistant mosquito populations. An alternative and promising approach involves the implementation of natural product-based insecticides (biopesticides) into vector control programs. Mechanism of resistance related to synthetic insecticides is commonly grouped into three categories; metabolic resistance, target site resistance and behavioral

Will mosquitoes develop resistance to the Chromobacterium sp Csp_p biopesticide?

Cecilia S. Engdahl, Eric Caragata, Raul Saraiva, Hannah MacLeod, Luisa M. Otero, George Dimopoulos

Vector control plays a key role in reducing the number of mosquito-borne diseases. Today's vector control strategies heavily rely on synthetic insecticides that struggle with resistant mosquito populations. An alternative and promising approach involves the implementation of natural product-based insecticides (biopesticides) into vector control programs. Mechanism of resistance related to synthetic insecticides is commonly grouped into three categories; metabolic resistance, target site resistance and behavioral
resistance. These all pose a serious threat to the public health efforts of vector control and are therefore well studied, while very little is known about the pathways affected by selection with bioinsecticides. A recently isolated Chromobacterium specimen from Panama (Csp_p) has become interesting for its potential use as a bioinsecticide. This gram negative soil bacterium display mosquitoicidal activity against both larvae and adults of multiple vector species; Anopheles, Aedes and Culex. In this study we aim to investigate if, (when and how) our biopesticide candidate Csp_p will cause mosquitoes to develop resistance, and how costly this would be. We will select for survival to a dose initially corresponding to LD20 in two separate lines of Aedes aegypti over several generations. The change of life history traits such as development time will be recorded and once the survival rate is equal to that for non-treated mosquitoes, genome-wide changes in transcription levels in the selected lines relative to unselected controls will be explored.

PP-59
Re-designing malaria control and surveillance: an engineering case study in sub-Saharan Africa

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High-quality mosquito surveillance is crucial to the control of malaria, a fatal disease that disproportionately affects sub-Saharan Africa. Despite a significant scale-up of interventions over the last decade, malaria cases continue to rise. This study aimed to reinvigorate malaria elimination efforts by identifying innovation opportunities in sub-Saharan Africa. To achieve this aim, an interdisciplinary team of engineers spent August 2019 observing and participating in control and surveillance activities in Zambia and Uganda. The team identified three critical opportunities for innovation: first, there is a need for more reliable quality control of implemented interventions. Currently, interventions are assessed using Human Landing Catches (HLCs). Volunteers expose themselves to malaria-carrying mosquitoes, collecting data on biting rates, peak biting times, and vector populations. This method is highly susceptible to human error, as HLCs may fall asleep or fail to accurately follow collection instructions. A standardized method of collecting vector data would produce more representative information, thereby improving quality control. Secondly, there was a need for inexpensive outdoor control methods. Currently, Long-Lasting Insecticide Treated Nets (LLINs) and Indoor Residual Spraying (IRS) are the primary control mechanisms used. These methods fail to provide coverage during peak mosquito biting hours, times at which rural citizens are often outside frequenting the numerous outbuildings that make up their homes. Finally, insecticide resistance monitoring is insufficient. Current practices are labor-intensive, measure only one variable, and often lead to geographically unrepresentative data. A scalable method of monitoring regional insecticide resistance would allow for the timely, bespoke deployment of control interventions in a given region. It would also shed light on the impact resistance has on deployed interventions. Identifying challenges guides the development of new technologies to improve current practices. Future work will utilize these findings to develop a technical solution in hopes of advancing the road to elimination.

PP-60
Semi-field measurements of mosquito olfactory preference

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Female mosquitoes detect volatile chemicals emitted by their hosts to locate them. Differences in the chemical composition of human body odor may influence mosquito olfactory preference for certain humans within disease-endemic communities, with important implications for malaria epidemiology. However, the molecular and cellular basis of mosquito olfactory preference for particular humans remains unknown. Laboratory experiments have been useful to study mosquito host preference, however, they often fail to capture the full composition of odorants emitted by whole humans. Semi-field experiments allow us to overcome this limitation and to study mosquito behavior in a more natural context. We studied mosquito olfactory preferences in a large 20m x 20m semi-field cage in Macha, Zambia using an odor guided thermotaxis assay (OGTA) and video tracking. The OGTA consisted of a landing arena heated to 35°C and illuminated with infrared LEDs, and a video camera on top. 8 tents around the cage were connected to it with air conditioning ducting that ended next to each arena. Air from the tents was pumped into the cage. Each tent was baited with either CO2 or humans, and control tents were empty. 100 An. gambiae females were starved and released into the flight cage and their preference was assessed by recording landings between 22:00-04:00 using video tracking for analysis. The OGTA allowed us to successfully assess olfactory preferences of An. gambiae in semi-field conditions. Mosquitoes preferred landing on CO2 baited arenas vs clean air. Additionally, mosquitoes were more attracted to arenas baited with humans vs CO2. This pilot data indicates that this multi-choice assay may be a promising system to screen for...
highly attractive humans in the future. An improved understanding of the odor profile of highly attractive humans may facilitate development of potent chemical lures that improve mosquito trapping for malaria surveillance and control.

**Gut microbiome predicts lumefantrine pharmacokinetics in healthy mice**

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The antimalarial drug lumefantrine exhibits highly variable pharmacokinetics—absorption, distribution, and clearance—between individuals with up to 16-fold differences in drug exposure. Differences are due, in part, to poor absorption which is mitigated by administration with fatty food. We hypothesized that the intestinal microbiome may also play an important role in lumefantrine disposition. Four cohorts of isogenic mice from different vendors (n=24) previously shown to harbor distinct enterotypes were administered a humanized dose of lumefantrine (150 μg/g) by gavage. Gut microbiome was characterized by 16S rRNA amplicon sequencing of fecal pellets and enterotypes were classified according to β-diversity using weighted UniFrac distance. Plasma was collected at 0, 0.5, 1.5, 10, and 24 h post dose for drug analyte quantitation by liquid chromatography tandem mass spectrometry. Drug exposure measured as maximal concentration (C_max) and area under the drug concentration-time curve (AUC_0-24) was estimated by the linear-log trapezoidal method in non-compartmental analyses. Oral clearance (Cl/F) and apparent volume of distribution (Vd/F) were estimated using covariate analysis of enterotype with stepwise selection in population-based compartmental models. Four discrete enterotypes were identified. Enterotypes 1 and 2 had significantly greater taxonomic abundance than 3 and 4, and β-diversity plots showed clustering of the same pairs. Mice with enterotypes 1 and 2 had higher lumefantrine exposure than enterotypes 3 and 4 (C_max = 542 and 768 vs. 392 and 380 ng/ml, P≤0.04, for pairwise comparisons; AUC_0-24 = 511,000 and 657,000 vs. 344,000 and 333,000 ng*h/ml, P≤0.02). Results of compartmental analyses suggest that enterotype-related differences in Cl/F and not Vd/F correlated with drug exposure. The gut microbiome has previously been shown to contribute to inter-individual variation in metabolism of orally administered drugs. Here, we demonstrate that gut microbiome differences may partially account for variation in lumefantrine exposure.
employing for the selection of parasite lines transformed with plasmid constructs expressing human DHFR. Early studies sourced WR99210 from Walter Reed Army Institute of Research directly, and subsequent studies obtained the compound from Jacobus Pharmaceutical (JP) or, more recently, Sigma Aldrich (SA). Effective compound concentrations (EC50s) can vary depending on source. Indeed, following relatively ineffective selections of transformants with WR99210 from SA, we established that P. falciparum DD2 parasites survived constant drug pressure of 2nm WR99210 from SA, but not from JP. Further testing indicated greatly different EC50s of 0.62nM vs. 547nM for the JP- and SA-sourced WR99210, respectively. Potential causes of these discrepancies have been investigated. Assured and effective sources of WR99210 will be vital to the continued utility of plasmid constructs.

Innovative tool development is essential for continued advancement in malaria control and depends on a deeper understanding of the molecular mechanisms that govern transmission of malaria parasites by Anopheles mosquitoes. Targeted disruption of genes in mosquito vectors is a powerful method to uncover the underlying biology of vector-pathogen interactions, and genome manipulation technologies can themselves form the bases of mosquito and pathogen control strategies. However, the embryo injection methods used to genetically manipulate mosquitoes, and in particular Anopheles species, are difficult and inefficient, particularly for non-specialist laboratories. We have adapted a strategy called ReMOT Control (Receptor-mediated Ovary Translocation of Cargo) to deliver the Cas9 ribonucleoprotein complex to adult mosquito ovaries and generate targeted and heritable mutations in the malaria vector Anopheles stephensi. We found that gene editing by ReMOT Control in Anopheles mosquitoes was comparable to the technique in Ae. aegypti and as efficient in editing as standard embryo injections. The adaptation of this technology to Anopheles mosquitoes opens up the power of reverse genetics to malaria vector labs that do not have the equipment or technical expertise to perform embryo injections and establishes the flexibility of ReMOT Control for gene-editing in non-Aedes species.
The impact of glyphosate on the melanin-based immune system of Anopheles gambiae

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Despite global campaign efforts to eradicate malaria, over 200 million people fall sick from the mosquito-borne disease annually. A major strategy to combat malaria includes manipulating the mosquito’s immune system in order to reduce susceptibility to diseases that could then be transmitted to humans. Due to the growth of agriculture in malaria-endemic regions, and the accompanied increased use of herbicides, we examined the effect of glyphosate, the active ingredient in the widely used herbicide Roundup, on mosquitoes. We found that glyphosate is toxic when chronically given to the malaria vector, An. gambiae females at concentrations consistent with agricultural glyphosate application on crops. Interestingly, low doses of glyphosate appear to prolong mosquito survival compared to non-drugged adults. We found that glyphosate inhibits the ability of An. gambiae to produce melanin. Melanin pigment encapsulation of pathogens is a crucial component of the insect immune response against Plasmodium infection. Without it, the insects could have a higher disease burden. We see that glyphosate-drugged mosquitoes have an increased parasite burden when they are infected with Plasmodium falciparum, the etiological agent of most lethal cases of malaria. These “immune-compromised” mosquitoes with a dampened melanization response may therefore pose an increased threat to human health. We further validated these experiments in Galleria mellonella wax moth larvae, another insect model of infection. In G. mellonella larvae, we see a dose dependent inhibition of melanization with glyphosate, and an increased susceptibility to Cryptococcus neoformans fungal infection when first treated with a dose of glyphosate. Our data suggests that glyphosate may increase Anopheles ability to serve as a vector by posing a double threat: elongating lifespan at some environmentally relevant concentrations, and making these longer-lived individuals more susceptible to pathogens that could be transmitted to people.

Comparative genome analysis identifies conserved gene amplification features between Plasmodium species

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Gene amplifications, a type of DNA copy number variation (CNV), facilitate overexpression of drug targets and contribute to adaptation. Long monomeric A/T tracks are found at the breakpoints of many P. falciparum resistance-conferring CNVs. We have applied a novel sequencing analysis pipeline to obtain single nucleotide breakpoint resolution of unique amplifications in 35 P. falciparum clones with 19 previously identified unique amplifications, 7 P. vivax clinical samples with 5 confirmed amplifications, and one human-adapted P. knowlesi clone with a previously confirmed amplification. Very long A/T tracks (32bp on average) were found to be the breakpoint for virtually all P. falciparum samples and stable hairpins were found near all known future breakpoint locations. We therefore hypothesized that CNV formation “trigger sites” are formed by A/T tracks near other proximal sequence features, such as DNA hairpins. Five shared breakpoints were in proximity to extremely stable hairpins (top 0.2% genome-wide) which adds further support. This “trigger site” model is thus far corroborated by the P. vivax and knowlesi samples. Breakpoints for the previously identified amplifications in P. vivax and knowlesi were all A/T tracks and were 18bp on average for each. Preliminary comparisons between species indicate that trigger sites are enriched in all three species. As expected, based on overall genome A/T content there are fewer long AT tracks (>9bp) in vivax and knowlesi genomes but very long tracks (>20bp) are 95% of those found in the falciparum genome. Current investigations include expanding our data set with more P. vivax and knowlesi amplifications, refining comparisons between Plasmodium species genome-wide, and investigating trigger sites in syntenic blocks of sequence.

Impact of targeted IRS on vector counts and malaria cases in a high transmission setting in Nchelenge District, Zambia

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Luapula Province in northern Zambia is an area of high malaria transmission with a parasite prevalence of 32.5% in children under 5 years. In this province, targeted indoor residual spraying (IRS) and LLINs are used as vector control to prevent malaria. The objective is to determine if targeted IRS can lower the risk of mosquitoes in a household, which in turn can reduce the risk of malaria infection in the broader population. Nchelenge District in Luapula Province is a study site for the Southern and Central Africa International Centers of Excellence for Malaria Research. Targeted IRS campaigns are conducted annually. Data are from cross-sectional household surveys and mosquito counts as well as passive surveillance at health centers from 2012 through 2019. Climate data were extracted from the Africa Flood and Drought Monitor. The association of mosquito counts per household and IRS was modeled using quasi-Poisson regression, stratified by mosquito species. The association of mosquito counts per household and malaria infections were modeled using a log-linear regression model. All models included a flexible time function to account for seasonality. Households self-reporting IRS had a 52% reduction in relative risk of *A. funestus* in their household (RR: 0.48, 95% CI: 0.34-0.67). However, there was no association with self-reported IRS and the risk of having *A. gambiae* captured in their household. There was also no association between the number of mosquitoes of either species in the household and *Plasmodium falciparum* infection. Households targeted for IRS had a lower risk of mosquitoes in their home for only one of the two dominant malaria transmitting species. Given differences in these two species, it is possible that once a year spraying is insufficient to last through both population expansions. Vector counts within a household were not associated with the number of parasite infections in a household.

High-throughput parasite genomics is increasingly useful in assessing prevalence of clinically important mutations, parasite transmission patterns, and the impact of interventions. As malaria elimination efforts shift to high transmission regions, a fuller understanding of local and regional parasite diversity and population structure is needed so that genomic surveys can help inform intervention efforts. However, many genotyping methods, such as whole genome sequencing, can be cost-prohibitive for large surveys needed for such discrimination. Here, we use molecular inversion probe (MIP) panels to investigate *P. falciparum* population structure using nearly 1,000 Tanzania clinical infections collected from 13 districts across the country. We found that parasites in Tanzania are comprised of two main populations, separating parasites from the northern and southern regions of the country. There was also a relationship between genetic and geographic distance, with isolates from districts close to one another more likely to be genetically related (by identity-by-descent) compared to parasites sampled from more distant districts. Drug resistance mutations in the *pfmdhs* and *pffrt* genes also showed differential frequencies by geographic location; several other SNPs not known to directly contribute to antimalarial resistance also appeared to segregate by population structure. In addition, demographic data from the 2017 Malaria Indicator Survey corresponded with genetic findings from the MIP data, including a positive correlation with average complexity of infection estimates with malaria prevalence estimates by district. These analyses will provide information on the genetic diversity and population structure of Tanzanian
parasites to the National Malaria Control Program, on which assessments of public health interventions can be made.


Mallory Cox

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In 2018, under the auspices of the Yale University Archaeology Laboratory Malaria Project, I began exploring the use of magnetic methodologies to identify hemozoin in ancient skeletal remains. Here I present preliminary data to assert the usefulness of Archaeomagnetism to ancient malaria research. I scraped all samples from the marrow cavities of long bones, where hemozoin is known to sequester. First, 100 mg samples were fixed on a borosilicate glass plate, mounted to an amber probe, and inserted into the Alternating Gradient Force Magnetometer (AGM). In samples from Panama (2500 BC-1600 AD) and Puerto Rico (0AD-1400 AD), I observed super and paramagnetic behavior. Interestingly, both magnetic state domains are reported in the literature as characteristic of hemozoin, with some groups observing paramagnetic behavior while others report superparamagnetism. Then, I utilized an MPMS2 Cryogenic Susceptometer (Institute for Rock Magnetism-IRM) to analyze samples at low temperatures (2K) and high temperatures (400K) to induce crystallographic transitions that are diagnostic of the magnetic mineral composition. In addition to magnetite, we identified magnetotactic bacteria to explain the presence of iron-bearing crystals <50nm in some samples. Notably, as 50% of the magnetic signature in some samples was unidentifiable by leading geologists and archaeomagnetic scientists at the IRM. To our knowledge, hemozoin from skeletal remains has not been analyzed using magnetometry. With the help of colleagues, YUALMP is building magnetic profiles for malarial hemozoin and other iron-bearing minerals that occur naturally in soils, to enrich the methodologies available for studying malaria in the archaeological record.

Comparing luciferase expression in transfected stages of Plasmodium falciparum and P. knowlesi

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Transfection of malaria parasites provides an important means to investigate the roles of specific genes in parasite biology and pathogenesis. Plasmodium falciparum and P. knowlesi are two species responsible for extensive malaria morbidity and mortality; unlike other widespread human parasites including P. vivax, P. falciparum and P. knowlesi can be readily cultivated and transfected under laboratory in vitro conditions. Here we describe the efficiencies of direct-electroporation on both P. falciparum and P. knowlesi using a firefly luciferase reporter construct at different intra-erythrocytic stages. Preliminary data show variations in luciferase expression among different P. falciparum stages: signals in these stages are higher from the reporter construct introduced by direct-electroporation of rings than by direct-electroporation of schizonts. In contrast, previous literature has reported that direct-electroporation of mature P. knowlesi schizont stages ("segmenters") with luciferase plasmids yielded greater expression than did direct-electroporation of late trophozoites or young developing schizont stages. These differences may reflect a relative robustness of P. knowlesi relative to P. falciparum segmenters and merozoites, so that P. knowlesi segmenters with developed merozoites can withstand direct-electroporation better than corresponding stages of P. falciparum. Experiments to directly compare luciferase expression from electroporated late-schizont/segmenter stages of P. falciparum and P. knowlesi are underway.

Characterization of Plasmodium berghei parasites lacking O-Fucosyltransferase 2 Activity

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Malaria is a mosquito-borne disease caused by protozoan parasites of the genus Plasmodium, which continue to impact public health. The parasite has a complex life cycle involving both its mosquito and mammalian hosts, several of which are motile and must move through host tissues to establish infection. In the mosquito host ookinetes move through the midgut to establish oocysts in which sporozoites develop. Sporozoites are also motile and use their motility to enter mosquito salivary glands, and in the mammalian host, exit the skin inoculation site and invade hepatocytes. In the sporozoite stage, both TRAP (thrombospondin-related anonymous protein) and CSP (circumsporozoite protein), are required for motility. Both proteins are known to have type I thrombospondin repeat (TSR) domains, that are glycosylated...
by an O-fucosyltransferase 2 enzyme (POFUT2). Studies performed to elucidate the role of POFUT2 in the parasite have led to controversial results and few questions unanswered. In this study, we have deleted POFUT2 in the rodent malaria parasite *Plasmodium berghei* and shown by mass spectrometry that both TRAP and CSP are not fucosylated in their TSR domains in this mutant. We then set out to determine whether these parasites are defective in their ability to establish infection in both mosquito and mammalian hosts. The POFUT2 KO parasite showed no deficiency in gametocyte production and mosquitoes feeding on mice with equivalent gametocyte counts had similar numbers of oocysts. POFUT2 KO sporozoites harvested from these mosquitoes had similar infectivity to control sporozoites when administered intravenously. Furthermore, POFUT2 KO sporozoites inoculated into mice by mosquito bite also showed no difference in infectivity compared to controls. These results demonstrate that fucosylation of TRAP and CSP do not play a significant role in parasite motility in the skin, blood vessel entry, and liver infectivity. We also looked at the role of fucosylation in CSP synthesis and found no significant difference between the POFUT2 KO and WT parasites. Thus, fucosylation does not appear to be essential for gametocyte formation, oocysts formation, sporozoite motility, liver infectivity, and CSP expression in sporozoites.

**Blocking Plasmodium host cell invasion using small molecule inhibitors targeting an essential protein-protein interaction**

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Malaria, a mosquito-borne infectious disease caused by Plasmodium parasites, infects over 200 million individuals worldwide, causing nearly 400,000 deaths every year. Current antimalarial drugs such as Artemisinin target intracellular parasite development. Despite substantial fall in the morbidity and mortality of malaria, the disease is still a major global concern as increasing resistance to these drugs is slowing down effective clearance of the parasites. This calls for new approaches and alternative targets to sustain the anti-malarial pipeline. Invasion is a rapid process that begins with an initial weak interaction of the merozoite with the red-blood cell (RBC), followed by its apical re-orientation. The formation of a tight junction between the apical end of the merozoite and the RBC commits its entry into the RBC. Targeting conserved regions of the protein-protein interactions that are essential to this committed step will serve as novel means to overcoming the threat of drug resistance. Recent high-throughput studies have shown that targeting one such interaction between AMA1 and RON2 with small molecule inhibitors have been successful in blocking merozoite invasion. Following these studies, a pilot screen of over 50,000 small molecules from the NCATS (NIH) and TCAMS (GSK) was performed using several biochemical and cell-based assays. We are conducting follow-up studies of several hits from this screen to test the on-target efficacy in using multiple P. falciparum lab strains as well as field isolates. We have developed a flow cytometry-based short-term invasion assay using mature schizonts. We are selecting for compounds that specifically block host cell invasion by disrupting AMA1-RON2 interaction. The aim of this project is to identify potential therapeutics that can have synergistic effects on reducing disease severity in combination with existing frontline antimalarial drugs.

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