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PHOSPHORYLATION OF *PLASMODIUM* EUKARYOTIC INITIATION FACTOR 2α IN RESPONSE TO ARTEMISININ THERAPY

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Artemisinin and its derivatives are the most potent anti-malaria drugs. Nevertheless, artemisinin monotherapy is associated with accumulation of dormant ring stages and with recrudescence of *Plasmodium* infection, which is considered a treatment failure. The molecular mechanism(s) leading to parasite dormancy are under investigation. Here we report that dormancy is associated with phosphorylation of the parasite's eukaryotic initiation factor-2α (eIF2α). In an attempt to reveal which one of three *Plasmodium* kinases, eIK1, eIK2, or PK4 is involved we generated knockouts of the enzymes. Following artesunate treatment, the eIK1(-) and eIK2(-) parasites phosphorylated their eIF2α and entered dormancy like wild type. We could not obtain knockouts of PK4 because it is essential for blood stage development where the gene targeting takes place. Nevertheless minutes after drug treatment PK4 dimerized, autophosphorylated and phosphorylate eIF2α indicating that it is the enzyme that controls dormancy. Artesunate-induced dormancy is extended by salubrinal, a selective inhibitor of the eIF2α-P phosphatases, which provides evidence for the mechanism that *Plasmodium* phophorylates eIF2α causing dormancy in response to artemisinin treatment.

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EFFECTIVE SCALING-UP OF SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO

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Burkina Faso scaled-up SMC implementation in 2015, covering 17 districts with a population of about 900,000 children under 5 years of age. Delivery, primarily door-to-door, was for four months starting late July. Each month the first dose of the 3-dose regimen was administered by a health worker and the remaining doses left with the caregiver. To evaluate the effectiveness of SMC delivery at scale, a survey was conducted at the end of the transmission season in 11 districts where SMC was delivered through the ACCESS-SMC project. 50 villages were selected with probability proportional to size, and households selected using compact segment sampling. SMC record cards were inspected and caregivers were asked about adherence, side effects, reasons for missed treatments, the time and any costs involved to obtain SMC for their child, the caregiver's level of education and socioeconomic status. Utilisation of insecticide-treated bednets by household members was also recorded. Children up to 7 years old were included in order to determine if children above the recommended age limit were being treated. Data were collected using Android tablet devices. 1000 children were surveyed. 741 of these were eligible to have received 4 cycles of SMC (aged between 3months and 5years at the first cycle). Of these 94% had received an SMC card and at least one SMC treatment. 83% received at least 3 cycles. 97% of caregivers reported that they had administered both unsupervised doses of amodiaquine the month before the survey. The mean percentage of children who attended for SMC who did not receive the treatment from the health worker, obtained from health worker tally sheets, was about 1% to 2%, mostly because the child was unwell. Of 95 children aged 6 to 7 years at the survey, 80% reported having received SMC. 89% of children slept under a bednet the night before the survey. In conclusion, a high level of coverage was achieved during 2015, in the first phase of implementing SMC on a large scale in Burkina Faso. Sustaining this achievement will be challenging.

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PREVALENCE OF MUTATIONS ASSOCIATED WITH SULPHADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN *PLASMODIUM FALCIPARUM* SAMPLES FROM THE GENERAL POPULATION AND PREGNANT WOMEN IN NANORO, BURKINA FASO

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Pregnant women are at increased risk of *Plasmodium falciparum* infection, which can result in maternal anemia, low birth weight babies and other sequelae. Most Sub-Saharan African countries have therefore implemented intermittent preventive treatment for pregnant women (IPTp) using sulphadoxine-pyrimethamine (SP). However, concerns are rising about its continuous efficacy because of increasing resistance against SP. Point mutations in the *Dhps* and *Dhfr* genes of *P. falciparum* are associated with resistance, especially combinations like the triple *Dhfr* mutant (51I, 59R, 108N), the double *Dhps* mutant (437G, 540E) and moreover the quintuple mutant. The aim of our study was to estimate the current levels of SP resistance in Nanoro, Burkina Faso and to test whether the mutation rate increases during pregnancy. Filter paper samples from pregnant women at first antenatal care visit (ANC1) and at delivery were collected from March 2014 till September 2015 as part of an intervention trial (COSMIC). Furthermore, samples from the general population (GP) were collected from March till May 2015. DNA was extracted and *P. falciparum* positive samples were detected by qPCR. Next, nested PCR was used to amplify the *Dhps* and *Dhfr* genes and products were sent for sequencing. We found a high prevalence of *Dhfr*-51, -59 and -108 overall, with a trend of higher levels in GP and delivery samples compared with ANC1 samples (70.7%, 74% and 61.5% triple *Dhfr* mutants respectively). Statistical analyses will follow, but this trend could possibly indicate selection of resistant parasites during pregnancy. However, the concurrent higher mutation rate in GP samples needs to be explored. *Dhps*-437 also showed high mutation rates (89.4%, 84% and 83.4% respectively) without a clear trend. The *Dhps*-540 mutation was found in one GP sample and in two delivery samples, of which one was a quintuple mutant. To our knowledge this is the first time the *Dhps*-540 mutation is found in Burkina Faso. This finding and the high prevalence of the other mutations raises concerns about efficacy of IPTp-SP in the future. Other drug combinations to tackle malaria in pregnancy should therefore be explored.

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ON THE ADEQUACY OF A 28 DAY FOLLOW-UP PERIOD FOR ARTEMETHER LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Clinical trials are the gold-standard for deriving information on antimalarial efficacy from which policy decisions can be made. These estimates are vulnerable to methodological approaches such as the duration of study and loss to follow-up. The aim of this work is to explore the optimal duration of follow-up for capturing PCR confirmed recrudescences following treatment with artemether-lumefantrine (AL), and to assess the sensitivity of the recommended minimum follow-up duration of 28 days in patients from Africa and Asia. The cumulative baseline hazard, estimated from Cox regression models with shared frailty on study-sites and fractional polynomials to capture nonlinear associations, was used to estimate the probability density of recrudescences. The area under the

density curve (AUC) was calculated to determine the optimal follow-up period; accuracy of which was evaluated using simulation techniques. Data were available from 54 efficacy trials on AL (n=7,735; 2002-2014) in children less than 5 years in Africa and 10 trials in Asia in patients of all ages (n=1859, 2000-2010) with a minimum follow-up of 28 days. There were 221 (2.9%) recrudescent infections in Africa and 41 (2.2%) in Asia within 63 days. In studies with follow-up duration of 42 days or longer, 43% (47/109) of these recrudescences in Africa and 24% (10/41) in Asia were missed with a day 28 follow-up. The missed proportions were even higher when estimated using the AUC approach, which makes use of all available data. A shift to the left of the probability density function (i.e. recrudescences occur earlier) was observed for Asia compared to Africa. Effects of baseline parasitaemia and treatment dose on the shape and location of the density function were also studied. This pooled analysis confirms that the current recommended follow-up duration of 28 days remains inadequate for accurately determining AL efficacy and fails to identify an estimated 62% of the recrudescences in Africa and 49% in Asia. The feasibility and cost-effectiveness of a longer follow-up duration warrants further investigation while also considering misclassification errors associated with genotyping.

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PLASMODIUM FALCIPARUM PARASITE CLEARANCE IN THE PERUVIAN AMAZON AS PART OF A DOD HARMONIZED CLINICAL TRIAL

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There is a global threat of *Plasmodium falciparum* resistance to artemisinin-based combination therapies observed in South-East Asia. Three DoD laboratories in Kenya, Peru and Thailand conducted a harmonized clinical trial to determine parasite clearance time after receiving artesunate. Data from Peru is presented.Participants between 5-65 years old with uncomplicated, microscopy confirmed, *P. falciparum* mono-infection, with asexual parasite density between 1,000 - 100,000 parasites/µL were enrolled. Participants were hospitalized for three days and received 4 mg/kg artesunate on Days 0, 1 and 2; 15 mg/kg mefloquine on Day 3, and 10 mg/kg mefloquine on Day 4. The parasite clearance rates were determined by microscopy every 4 h during first 12 h and then every 6 h until 72 h after first being treated with artesunate. Clinical and parasitological responses were assessed for 42 days.Between June 2014 and November 2015, 482 people with *P. falciparum* mono-infection were identified at eight health centers in Iquitos, in the Peruvian Amazon Basin, but most did not comply with the study requirements. Seventy-four subjects were consented and screened, and among them, 55 subjects with uncomplicated *P. falciparum* malaria were enrolled. Two participants were excluded due to serious adverse events and mixed infection. Participants cleared parasitemia by hour 42 (PCR uncorrected).The mean age of participants was 31.2 (95% CI 26.8 - 35.6), and 22 (41.5%) were female. The geometric mean parasite count at admission was 5514 parasites/µL (95% CI 4095-7426). The clearance rate constant (by hour) median was 0.32 (IQR: 0.29-0.39). The slope half-life median is 2.18 hours (IQR: 1.78-2.39). Finally, 50% and 99% parasite clearance median times (PC50 and PC99) were 5.84 (IQR: 3.07-7.28) and 17.02 (IQR: 15.22-19.35) respectively. All participants completed Day 42 follow-up and met the adequate clinical and parasitological response endpoint.No suggestion of resistance to artesunate was found among the participants evaluated in the Peruvian Amazon. However, surveillance using molecular markers such as K13 should be used as a complementary regional strategy.

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DOES METHYLENE BLUE ENHANCE THE *EX VIVO* ANTIMALARIAL BLOOD SCHIZONTOCIDAL ACTIVITY OF ARTESUNATE-AMODIAQUINE?

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Reports of falciparum malaria patients in Cambodia and Vietnam failing treatment with dihydroartemisinin-piperaquine highlights the urgent need to contain and reduce the spread of artesunate based combination therapy (ACT) resistant strains. As part of this effort a triple drug strategy approach should be investigated to extend the useful life of ACTs. The objective of the present study was to determine whether methylene blue (MB) can enhance the pharmacodynamic (PD) *ex vivo* antimalarial activity of artesunate-amodiaquine (ASAQ). If ASAQ+MB can be demonstrated to be more potent than ASAQ alone, then the triple combination may provide a better option to treat ACT resistant malaria infections. In an open labelled, randomized cross-over design, a single oral dose of either ASAQ (2 tablets, with each tablet containing 100 mg AS and 270 mg AQ) or ASAQ (2 tablets)+MB (5 tablets, with each tablet containing 65 mg MB) was administered to 16 healthy Vietnamese volunteers. After an 8 week washout period the same participants received the alternative drug combination. Serial blood samples were collected up to 28 days after the last dose of either ASAQ or ASAQ+MB. The *ex vivo* antimalarial activity of ASAQ and ASAQ+MB was assessed by subjecting the participant's plasma samples collected after drug administration against an artemisinin-sensitive and an artemisinin-resistant *Plasmodium falciparum* line *in vitro*. Based on the participant's plasma inhibitory concentration profiles the preliminary *ex vivo* data revealed MB to enhance the blood schizontocidal activity of ASAQ by at least 2-fold against both *P. falciparum* lines. Additionally, using LC/MS/MS we determined the pharmacokinetic (PK) properties of the partner drugs including their principal active metabolites. The PK-PD relationship of ASAQ and ASAQ+MB will be compared and their implications for the treatment of multidrug-resistant falciparum malaria will be discussed.

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PARASITE CLEARANCE AND DECLINES IN ARTEMETHER EXPOSURE OVER THE COURSE OF ARTEMETHER-LUMEFANTRINE TREATMENT FOR *PLASMODIUM FALCIPARUM* MALARIA IN UGANDAN CHILDREN

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We sought to assess the association between parasite clearance parameters and artemether (AR) and dihydroartemisinin (DHA) pharmacokinetic (PK) exposure in HIV-infected and HIV-uninfected children in Uganda treated with artemether-lumefantrine (AL) for malaria. Children ≤8 years underwent intensive PK sampling post-1st dose of AL followed by every 12-hour blood smears. AR and DHA exposure was compared to post-last AL dose PK in concurrently enrolled children using non-compartmental analysis. Parasite clearance parameters were calculated using the WWARN Parasite Clearance Estimator. Post-1st and last dose PK parameters were estimated in 103 children (77 HIV-uninfected and 26 HIV-infected) and 142 children (51 HIV-uninfected and 91 HIV-infected), respectively. In HIV-uninfected children, post-last dose AR area-under-