Intransigence of malaria in Malawi: Understanding hidden reservoirs, successful vectors, and prevention failures

Project 004: Combined effects of RTS,S vaccination and PBO nets on malaria infection and transmission in Malawi

Principal Investigator

Don P. Mathanga, MBBS, MPH, PhD[1]

Co-Investigators

Miriam K. Laufer MD MPH[2]
Clarissa Valim, MD, MSc, SM, ScD[3]
Themba Mzilahowa, BSc, MSc, PhD[1]
Mark L. Wilson, ScD[4]
Peter A. M. Ntenda. BSc, MSc, PhD[1]
Edward D. Walker, PhD[5]

Participating Academic Institutions

[1]Malaria Alert Centre-Communicable Diseases Action Centre, College of Medicine, University of Malawi, Blantyre, Malawi
[2]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, USA
[3]Dept. Of Global Health, Boston University, School of Public Health, Boston, MA, USA
[4]Blantyre Malaria Project, College of Medicine, University of Malawi, Blantyre, Malawi
[4]Dept. of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA
[5]Dept. of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA

Contact information: Don Mathanga, MBBS, MPH, PhD, Malaria Alert Centre, University of Malawi College of Medicine, Private Bag 360, Blantyre 3, Malawi. Phone: +265-1-870-145 Fax: 265-1-876-217. E-mail: dmathang@mac.medcol.mw

Institutions under whose umbrella the research project will be conducted:

I. Malaria Alert Centre, University of Malawi, College of Medicine

II. Michigan State University

III. University of Maryland School of Medicine

IV. Boston University

V. University of Michigan

CONTENTS

1. LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
CDC	Centers for Disease Control and Prevention
COMREC	College of Medicine Research Ethics Committee
CRF	Case Report Form
CSP	Circumsporozoite protein
DBS	Dried Blood Spot
DMC	Data Management Core
EIR	Entomologic Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Programme on Immunization
F1 mosquitoes	First generation mosquito progeny
GCP	Good Clinical Practice
GPS	Global Positioning System
HC	Health Center (or Centre)
HH	Household
HIV	Human Immunodeficiency Virus
HLC	Human Landing Catch
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ITN	Insecticide-Treated Net
LLIN	Long-Lasting Insecticide-Treated Net
LC50	Single dose that kills 50% of test mosquitos
MoH	Ministry of Health
MOP	Manual of Procedures
mRDTs	Malaria rapid diagnostic tests
NMCP	National Malaria Control Programme

ICEMR	International Center for Excellence in Malaria Research
ITNs	Insecticide-treated bed nets
MDHS	Malawi Demographic and Health Survey
MSU	Michigan State University
NCST	National Commission for Science and Technology
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OPD	Outpatient Department
PBO nets	Piperonyl butoxide-impregnated nets
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PID	Participant Identification Number
RTS,S	Central repeat region of *Plasmodium falciparum* circumsporozoite protein (CSP); T-cell epitopes of the CSP; and the hepatitis B surface antigen (HBsAg).
SAE	Serious Adverse Event
SES	Socio-Economic Status
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SP	Sulfadoxine-Pyrimethamine
SSA	Sub-Saharan Africa
WBC	White Blood Cell
WHO	World Health Organization

2. EXECUTIVE SUMMARY

Introduction: The decline in malaria incidence has stalled globally and incidence is increasing in some high transmission settings of sub-Saharan Africa (SSA), including Malawi. The situation is worsening despite the scale-up of previously effective interventions, raising concerns that the impact of current malaria control and prevention strategies maybe compromised.

Problem: There is an urgent need for innovative approaches to malaria control and Malawi is currently positioned to assess two of the most promising new interventions. The Malawi Ministry of Health (MOH) is launching large scale projects to evaluate a new formulation of insecticide-treated bed nets with a chemical synergist, piperonyl butoxide (PBO), designed to enhance the insecticidal effect of pyrethroids and the new malaria vaccine RTS,S/ ASO1 (RTS,S). In an effort to gain the most information from these, interventions Malawi's National Malaria Control Programme (NMCP) have invited the Malawi International Center for Excellence in Malaria Research (ICEMR) to evaluate the effectiveness of the two interventions (alone and in combination) on malaria prevalence and transmission.

Objective: In this proposed implementation study, we propose to assess the impact of PBO nets and RTS,S vaccine on *Plasmodium* infection prevalence and transmission.

Study type and methodology: We will enroll children in a prospective cohort study in which the follow-up will be at the 2nd, 4th, and 6th month. Using malaria Rapid Diagnostic Tests (mRDTs), all participants will be screened for malaria infection at enrollment. Participants with with positive mRDTs will will be treated with a full, weight appropriate treatment course of dispersible artemether-lumefantrine (LA) by government staff. The mRDTs and treatment of mRDT positive children will be repeated at the final visit. Similarly, all participants will be examined for hemoglobin levels at enrollment and at every two-month follow-up visits using Hemoglobin Color Scale (HCS). Children in households that are scheduled to receive both PBO nets and RTS,S vaccine will be compared to children in households that are not scheduled to receive either of these interventions. The outcomes of interest will be malaria infection prevalence, gametocyte prevalence and entomological evidence of the force of infection.

Results dissemination: Results from this study will be shared with malaria national programs in Malawi and globally, through conference presentations and peer-reviewedpublications, to contribute to the development of policy in rolling out these new interventions.

3. BACKGROUND INFORMATION

3.1 Significance of the problem

Malaria remains a significant global health problem, with an estimated 216 million cases worldwide in 2016 (WHO, 2017). At present, the main approaches to malaria control are insecticide-treated bed nets (ITNs) and prompt diagnosis with effective treatment. Malaria-related morbidity and mortality declined globally over the past decade, however, this progress has stagnated. Indeed, five million more malaria cases occurred globally in 2016 than in 2015, with the greatest increases occurring in countries like Malawi (WHO, 2017). This increased incidence arose despite continued use of previously effective interventions, raising fears about the future usefulness of current malaria control activities, and highlights the urgent need for innovative approaches to malaria control. In Malawi, two new interventions will be introduced over the period of surveillance supported by the Malawi International Center of Excellence in Malaria Research (ICEMR): a new formulation of bed nets that is designed to manage insecticide resistance and a pilot study of RTS,S/ASO1 vaccination in young children. The Malawi ICEMR has a truly unique opportunity to evaluate the implementation and impact on *Plasmodium* transmission of these two interventions.

Implementation and interactions of RTS,S and bed nets: Both the RTS,S vaccine and PBO nets have been studied as part of large clinical trials. RTS,S vaccine has been evaluated in the setting of high ITN use (Agnandji, 2011) and PBO nets have been studied with and without indoor residual spraying. Impacts of these interventions, when implemented on a large-scale programmatic level, must be evaluated for their individual and combined effects, and their cost-effectiveness against other potential interventions. The combined effects of these interventions could be enhanced or diminished by interactions. For example, if young children receiving the RTS,S vaccine are also from households more likely to use ITNs, then vaccine effectiveness could be relatively more. Or it may be that parents of those receiving malaria vaccine believe their children have become protected against malaria illness, and therefore become less vigilant in shielding their children from mosquito bites. Perhaps people's perceptions of PBO nets (benefits over conventional nets or concerns about the added synergist) will alter their use compared to conventional ITNs. The significance of our study lies in our ICEMR's ability to evaluate both of these major new interventions, and systematically collect evidence about the impact of RTS,S vaccination and PBO nets on the incidence of *Plasmodium* infection.

3.2 Novel interventions to prevent malaria

The use of piperonyl butoxide (PBO) nets: Pyrethroids are the only class of insecticide approved for use in ITNs, but resistance among the *Anopheles* vectors in Africa is widespread. In Malawi, pyrethroid resistance is present in the two major malaria vectors, *An. funestus* and *An. arabiensis*, and has recently become more severe (Mzilahowa, 2016). Increased prevalence and intensity of pyrethroid resistance is a key contributor to the observed failure of current ITNs to protect against *Plasmodium* infection in our setting and elsewhere. We recently demonstrated that pyrethroid-based ITNs in southern Malawi provided only modest or no protection against infection and disease (Mathanga, 2015; Buchwald, 2017) yet no alternative insecticide is currently available. However, the addition of a chemical synergist, piperonyl butoxide (PBO), is designed to enhance the insecticidal effect of pyrethroids even in the face of resistance. PBO inhibits the enzyme that detoxifies the pyrethroid, allowing the pyrethroid to act on the mosquito. A recent cluster-randomized trial in Tanzania showed that use of PBO nets compared to conventional pyrethroid nets produced 44% and 33% protective efficacy in the first and second year, respectively. The impact of PBO net use was also detectable in key entomological measures

including *Anopheles* density, sporozoite rate and entomological inoculate rates (Protopopoff, 2018). Following these promising preliminary results, Malawi's National Malaria Control Program (NMCP) will lead a PBO-net mass distribution campaign in six districts, presenting us with the opportunity to study the effectiveness of these nets in the context of real-world program setting. The significance of this opportunity lies in our ability to carefully evaluate the impact on *Plasmodium* transmission and also investigate accompanying changes in pyrethroid resistance to better understand the mechanism of protection.

3.3 Malaria vaccine

A highly effective malaria vaccine remains elusive, but the RTS,S vaccine has progressed through the clinical development stages. RTS,S is a subunit vaccine that includes a portion of the circumsporozoite protein (CSP) co-expressed with Hepatitis B surface antigen combined with ASO1 adjuvant. The Phase 3 trial of three doses administered to 5-17-month-olds confirmed moderate protection, with overall efficacy estimates of 50.4% against clinical malaria and 34.8% against severe malaria after three doses (Agnandji, 2011). Efficacy, which waned over time, was marginally improved by boosting at 18 months. The European Medicines Agency adopted a positive scientific opinion of the vaccine for use outside of the European Union. The WHO has created the Malaria Vaccine Implementation Program (MVIP) and selected Malawi as one of the sites to explore the feasibility, efficacy and safety of RTS,S vaccination in the context of routine use. In addition, when exploring RTS,S feasibility and safety in routine health delivery, little evidence exists regarding how RTS,S vaccine may affect *Plasmodium* infection and transmission. An analysis of several RTS,S trials suggested 70-90% protection against microscopy-defined infection (Penny, 2015), but this does not consider submicroscopic, PCR-detected *Plasmodium* infections containing gametocytes infectious to mosquitoes. The significance of our proposed studies is that RTS,S vaccination may reduce human infection prevalence and transmission to mosquitoes. Thus, RTS,S vaccine may offer indirect protection and decrease infection in other age groups. Alternatively, if vaccination permits low-density *Plasmodium* infections that do not lead to disease and treatment-seeking, vaccinees may actually serve as sources of added household transmission. The Malawi Ministry of Health (MOH) has invited our ICEMR to help analyze impacts of RTS,S vaccination on *Plasmodium* transmission and malaria incidence. Knowledge of these outcomes is critical to future role out of the vaccine.

3.4 Malaria in Malawi

Malaria remains a major public health problem in Malawi. Recent surveys suggest that malaria infection risk exists throughout Malawi, with the highest risk being along the hotter, wetter and more humid low-lying regions, and the lowest in the highland areas (Kazembe, 2007). Despite the scale-up of ITNs in certain locations, there has been no corresponding reduction in malaria risk and morbidity (Roca-Feltrer, 2012; Okiro, 2013; Sisya, 2015). From 2000 to 2010, the number of people living in hyper-endemic conditions actually increased by 37% (MOH-KEMRI, 2013) whilst the number of reported cases of malaria increased from 3.7 million in 2005 to 6.8 million in 2010, an increase of 46% in a population that grew by 14.1% during the same time period. Malaria morbidity burden also remained high in hospital settings (Okiro, 2013). Further, the incidence of malaria ranged from 200 - 600 cases per 1,000 populations per district in 2013, with 9.2 million courses of artemisinin combined treatment (ACT) dispensed in 2013 alone**.** Yet during this time frame, the proportion of under-five children and pregnant women who slept under an ITN the previous night reportedly increased from 25% and 8% to 67% and 62%, respectively, by 2013. Although few data reflecting actual transmission exist, these numbers suggest that the intensity of *P. falciparum* transmission may actually have increased despite a decade of intensified nation-wide investment in malaria control and prevention.

3.5 Preliminary Studies

Evaluation of effectiveness of large-scale bed net projects. Beginning in the early 2000s and continuing into the current decade, we analyzed the effectiveness of ITN (Lindblade, 2015; Mathanga, 2015) programs in Malawi. In a 2012 study, the incidence of infection with *P. falciparum* was 30% lower in ITN users than non-users despite the presence of pyrethroid resistance in the *An. funestus* population (Lindblade, 2015). In parallel, there was no reduction in cases of malaria in children presenting with fever to clinics who used bed nets compared to matched controls not using ITNs, suggesting no effect of bed net use in contrast to a previous systematic review that showed reductions of 50-62% in malaria episodes across different transmission settings (Mathanga, 2015), highlighting the need for more effective interventions across different transmission intensities.

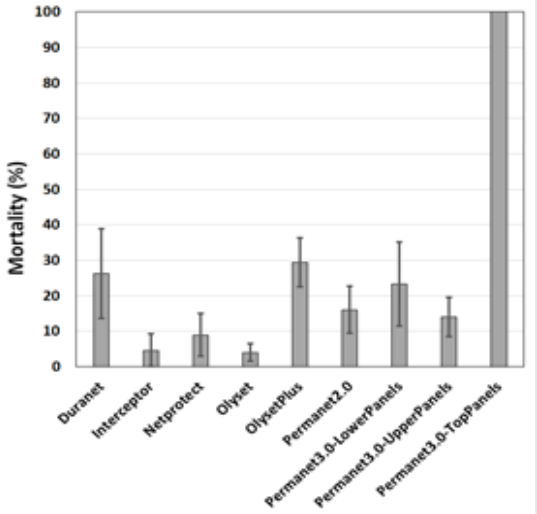

Figure 1. Mortality of *An. funestus* exposed to seven LLIN types was <40% except to PermaNet 3.0.

Insecticide resistance in Malawi. In support of the government campaigns to roll out ITNs, we coupled analyses of insecticide resistance in vector populations to *describe what the IR analyses were coupled to (Mzilahowa, 2008;* Riveron, 2015). Results revealed a spectrum of phenotypic and genotypic resistance attributes and in particular, showed that many populations of *An. funestus* were resistant to presently used pyrethroids used in ITNs in Malawi. We have shown that mortality rates realized by exposing wild-caught *Anopheles* to seven different ITN types, including two dually-treated nets (Olyset plus and PermaNet 3.0) were very low, indicating that the *An. funestus* strains were resistant (**Fig. 1**). Mortality of mosquitoes was complete (100%) only when they were exposed to the top-panel of PermaNet 3.0, which was treated with high doses of deltamethrin and piperonyl butoxide (PBO), a synergist. This study provides an opportunity to measure the impact of PBO nets at the population level in an area of differing insecticide resistance levels and in the context of other interventions.

4. JUSTIFICATION

Despite massive scale-up of malaria control interventions in Malawi, especially ITNs, malaria disease burden still remains high. This study will assess the impact of two new interventions being introduced into the Malawi national malaria control program. The outstanding research question is

a) What is the impact of RSS,S vaccine on *P. falciparum* prevalence and transmission?
The focus of past RTS,S clinical trials has always been the protective efficacy against clinical disease. Given the large-scale cluster-randomized introductions of the vaccines, and the existing ICEMR community-based surveillance infrastructure, we will evaluate the effect of RTS,S vaccination on the prevalence of *P. falciparum* infection in the vaccinated children and their household members and also on various measures of transmission. This critical information has never previously been explored in evaluating malaria vaccine effectiveness. In addition, the use of serological markers will generate measures of transmission for individuals on a finer scale than entomological measures alone and will allow controlling for exposure level in the analysis. Accurate estimations of effectiveness in prior vaccination studies have been hampered by the lack of measures of individual exposure.

b) Does the use of PBO nets decrease exposure to malaria infection and limit transmission? Although PBO nets are likely to have more potent insecticide activity, only one study has been published to date that shows that the implementation of PBO nets decreases human malaria infection (Protopopoff 2018).

c) What are the combined effects of RTS,S vaccine and PBO nets?
While it is possible that the impact of RTS,S vaccine plus the impact of PBO nets is additive, human behavior, parasite dynamics and vector responses may lead to either synergistic or antagonistic effects when they are combined. The factorial study design proposed in this application allows the evaluation of the impact and interaction of these two interventions.

Findings from this study will have implications for policy makers since understanding both the direct and indirect, population-level benefits of malaria vaccination and PBO nets will be critical when countries decide whether to adopt these intensive interventions. Hence the data obtained in this study will be used to enhance program effectiveness, both at national and international level. This study will be divided into two phases. This protocol is particularly focused on detailing the first phase of the study, although it briefly summarizes the proposal of the second phase of the study.

5 OBJECTIVES

5.1 Broad Objective

To assess the combined impact of RTS,S (malaria) vaccination and PBO nets on *Plasmodium* infection and transmission, and how they interact when they are introduced together.

5.2 Specific Objectives

The specific objectives for the study are as follows:

1. To estimate the impacts of PBO nets and RTS,S vaccine on the prevalence and transmission of *Plasmodium* infection. (Phase 1).
2. To assess the feasibility of evaluating the impact of RTS,S vaccine and PBO nets on the prevalence and transmission of *Plasmodium* infection in a larger scale study. (Phase 2).

6 METHODS

6.1 Study Design

This will be a prospective cohort study. This is a preliminary study to determine if we can detect an impact of RTS,S vaccine combined with PBO nets on parasitological, entomological and/or serological evidence of infection and transmission. If this preliminary study yields significant results, we will have the opportunity to apply for funding from our sponsor (US National Institutes of Health) for a much larger study that will assess the impact of each of these interventions individually and how they interact with each other. If we are approved to conduct this multi-arm study, we will submit a new protocol to COMREC and the MSU IRB for review and approval.

Using malaria and vaccine registers, we will establish community-based cohorts of children age-eligible to receive RTS,S vaccinationfrom the two extreme groups: i.e. Nyambi health centre in Machinga and Kalembo health centre in Balaka. The Machinga district, is undergoing implemetation of both RTS,S vaccination and PBO nets (RTS,S and PBO interventions). Balaka households do not receive RTS,S vaccination and only receive conventional nets arms (Table 1). We will enroll children of the age that would be eligible for vaccination and all of the children over nineteen months and under ten years of age in their house. We will conduct interviews and collect specimens at enrolment and at 2, 4 and 6 months, as described below. During these visits, data onindividual, household, social, and behavioral determinants, immunization status, ITN use will be collected.

Table 1: Overview of study design and methods

Specific Objective 1: To estimate the impact of PBO nets and RTS,S vaccine on *Plasmodium* infection prevalence and transmission in Malawi.		
Study design: Cohort study Phase 1: 3,000 children in total: 1000 age-eligible children: 250 children age-eligible for RTS,S vaccination [7 to 18 months] in each of the two sites and in two separate cohorts 2000 siblings: 500 siblings (up to two children >18 months and ≤10 years old from the same households as the vaccine-eligible children) in each of two sites and in two separate cohorts		
Methods	**Data sources**	**Key variables/ outcome**
<u>Phase 1</u>**:** Recruited from Nyambi Machinga health centre – in catchment areas that receive RTS,S vaccination and PBO nets. Recruited from Kalembo health centre in Balaka – in catchment areas that receive conventional nets and no malaria vaccination.	• Interviews of parent/guardians participants • Blood samples • Captured mosquitos • Human reporting about Nets • Finger prick blood specimens	• *Plasmodium falciparum* infection • Gametocyte presence • *Anopheles spp.* abundance • *Anopheles* gravidity • *Anopheles* sporozoite rate • Net usage • Immunization status • Serological markers

6.2 Study location

The study will be carried out in Machinga and Balaka Districts, in Southern Malawi. *Machinga District (14.55 S, 35.40 E),* located ~120 km northeast of Blantyre, is a poor rural district. Malaria transmission is intense and year-round, peaking during the rainy season (November through May). Between July 2013 and June 2014, an estimated 162,500 (1-year risk of 28%) malaria cases were reported and accounted for 24% of the total Outpatient Department (OPD) attendance (the total population of Machinga District is ~572,000 people). In 2015, malaria annual incidence was 367 cases/1,000 individuals. The use of ITNs is widespread in the district; findings from the 2015-2016 MDHS reported that 66.3% of the individuals and 80.5% of under-five children slept under an ITN in the previous night.

Balaka District (15.01° S, 35.01° E), forms a boundary with Machinga and is also a poor, rural district. Malaria is generally high in the district with intense, year-round transmission that also peaks during the rainy season. Between July 2013 and June 2014, malaria cases were accounted for 29% of the total OPD attendance (the total population of Balaka District is ~372,000 people). In 2015, malaria annual incidence was 310 cases/1,000 population. Findings from 2015-2016 MDHS indicated that 67.5% of the population and 87.3% of under-five children slept under an ITN in the previous night.

The timing for this proposal is optimal as we will be able to take advantage of the Malawi government-funded nationwide ITN distribution campaign (2018) and the RTS,S evaluation project (2019). In November 2018, Malawi undertook a country-wide bed net campaign, distributing nets to each household with a target of one net for every two people. In Machinga district, PBO nets were distributed, while neighboring Balaka District conventional pyrethroid-treated bed nets were given. About eight months later, both districts are part of the vaccination of infants and young children with the RTS,S vaccine, where children in selected clusters will receive RTS,S whilst children in some clusters of the same district would act as controls. The Malawi Ministry of Health has asked our ICEMR team, through the College of Medicine Malaria Alert Centre, to support the evaluation of RTS,S vaccination, thus providing a perfect opportunity to assess the combined effects of these interventions.

6.3 Study Population

Children whose age is between 7 and 18 months, which indicates they could receive at least 3 RTS,S doses, will be eligible to participate. Following informed consent from parents/guardians, up to two children who are 18 months but ≤10 years of age, from the same household as the vaccine-eligible child, will also be enrolled. Only households whose families intend to stay in the area for at least six months and whose eligible children will also be expected to use an assigned health center for child vaccinations will be eligible to participate.

Inclusion/Exclusion Criteria

To be eligible for the cohort, participants must meet all the following inclusion criteria:

- Children aged 7 to 18 months of age (age-eligible for at least 3 doses of RTS,S doses) OR being one of not more than two children living in the household of an enrolled age-eligible child and being >18 mos and ≤ 10 years of age.
- Not on cotrimoxazole prophylaxis for HIV infection
- Weight >5 kg
- Permanent residence of Health Centre (HC) catchment area
- Residence within 10 km from the HC
- Written informed consent from parent/guardian for the child to participate in the study

Non-residents of the catchment area and visitors to the study area will be excluded because the study requires follow-up for at least 6 months and access to interventions such as conventional, PBO nets and malaria vaccination.

6.3.1 Study period

We will conduct two consecutive cohort studies with 500 children in the desired age group in each arm, and we will also study up to two children >18 mos and ≤ 10 years of age (anticipated one or two per household) in each household to obtain preliminary data about the impact on transmission in the household (Table 3). Children will be identified and enrolled after obtaining parental consent. They will undergo interviews and one finger-prick blood sample collection every two months for six months (2, 4, and 6 months) after enrollment. Household entomological sampling will occur during the rainy season.

Table 2: Timing of Phase 1 study visits and specimen collection

Timeline	Activity
May-November 2019	Finalizing research plan and approval by IRBs (CoM and MSU)
November 2019	Community consultation with community leaders, Staff recruitment and training, Developing practical arrangements and SOPs and developing the data management system
December 2019	Cohort recruitment
January- 2019-May2020	Cohort follow up (round 1)
May 2020-October 2020	Cohort recruitment and follow up (round 2)
November 2020-March 2021	Data analysis and assessment of Go/No-Go criteria

6.3.2 Sample size

Our sample size estimates are based on previous high and low transmission season surveys conducted during the first ICEMR (2010–2017) in areas similar to the clusters that will be selected for this study. Based on these data, we anticipate that 13%-18% of households will contain a child 7-18 months old. The risk of infection in this age group is estimated to be 21% (30% high and 20% low transmission seasons). In children ≤10 years of age the risk of infection is estimated to be 31% (46% in the high and 32% in the low transmission seasons). The within-cluster correlation is estimated at 0.004 (0.05 in the high and 0.00 in the low transmission seasons) and 0.14 (0.22 in the high and 0.06 in the low transmission seasons) for children aged 7-18 months and ≤10 years of age, respectively. Based on these data, by studying the 25 clusters with 20 households with a child 7-18 months per intervention arm, we will analyze 500 children aged 7-18 months and a similar or larger number of older children. For power calculations, we assumed that these proportions represented the risk of incident cases of infection.

This proposed sample size will allow us to estimate moderate effectiveness of the combined interventions (versus no intervention) with 80% power in 2-sided tests, accounting for within-cluster correlation, and with α-level of 0.05. For instance, based on the cross-sectional estimations of individual risk of malaria infection obtained at enrollment of each cohort, we will be able to

detect the effectiveness of 34% (36% and 33% when stratifying by season) in children 7-18 month-old (Hayes, 2009). In a repeated measure analysis, with within-subject correlation of 1.5 to 2 times larger than within-cluster correlation, we will be able to detect effectiveness of 34-37% in children 7-18 month old (Liu, 2002).

For entomological assessments, 100 households from each of the two arms will be visited for entomological analysis including *Anopheles* abundance, female mosquito parity, and source of blood meal and sporozoite prevalence. Based on our previous experience, we have found an average of one *Anopheles* mosquito per household sampled (although with very heterogeneous distribution), and 75-95% human-biting prevalence.

6.4 Participant recruitment and study procedures

Participant recruitment. Prior to recruiting participants, households in which children have received RTS,S and PBO nets will be identified. (PBO+, RTS,S+). Addtionally, through vaccination records, we will identify households in Balaka where children who received the full scheme of Pentavalent (Diphtheria-pertussis-tetanus; Hepatitis B; Haemophilus influenzae type b) vaccine and have received a standard bed net live. In Nyambi catchment area, by the end of September, an estimated 260 children are expected to be vaccinated with RTSS vaccine. Thus, to achieve an adequate sample size for the significant inferential statistics, children will be enrolled from the Health Centre and tracked back in the community. Children will be identified and RTS,S information will be extracted from the registers by the research team. With the help from the health surveillances Assistants (HSAs) and community health volunteers (CHVs), RTS,S age-eligible children (7-18 months old) will be tracked using information obtained from the registers back to their villages and households where children will be enrolled and followed up for the designated study period. In the Kalembo catchment area, the same process that will be employed in Nyambi will be adopted. However, to make the cohort more comparable, using under two EPI vaccine register, children who completed three doses of Pentavalent (Diphtheria-pertussis-tetanus; Hepatitis B; Haemophilus influenzae type b) vaccine and are in RTS,S vaccine age-eligible will be considered in Kalembo.

In both catchment areas, up to two children in the household aged >18 months and $\leq$ 10 years old residing with the vaccine age-eligible children will also be offered the opportunity to enroll. All invited children will be screened by a study nurse for eligibility using a screening form. During the screening process, the details of the study procedures that will take place in the cohort will be described in detail. For children who meet the eligibility criteria, parents/guardians will then be asked whether they are willing to have their child participate in the study and those that agree will be asked to sign the consent form. A copy of the signed consent form will be given to the head of the parent/guardian. All enrolled will be given a study identification number. Only children from the families intending to stay in the area whose parents provide written informed parental consent will be considered for enrollment. In addition, consent will be sought from the parent/guardian for entomological activities in the household.

Table 3: Overview of study procedures

Visit Type	Home Visit/enrollment	Two-monthly visits: Central location near home
Study procedure		
Potentially eligible participants identified through satellite imagery and predetermined clusters. Households are visited and those with eligible children identified	**X**	
Obtain informed consent to participate in the study	**X**	
Child demographic and HH socioeconomic information	**X**	
Through a finger prick, collect blood sample and DBS for quantitative PCR, serum separation, antibody quantification, preservation of RNA, and storage – done on every two months follow up for entire 6 months.	**X**	**X**
Structured questionnaire interview	**X**	**X**
ITN use and vaccination status	**X**	**X**
Participants reminded of their visit date	**X**	**X**
Household mosquito collection for testing		**One time**
Hemoglobin measurement	**X**	**X**
Malaria rapid diagnostic test	**X**	**Final visit only**

At enrollment, we will conduct household and individual interviews. In this initial household interview, we will collect data on hemoglobin status, SES, household construction, and on ITN use (PBO or conventional net). Through visual inspection of each bed net to determine if it is a PBO or conventional net, we will also identify which other household members aged ≤10 years of age who slept under each bed net and with respective frequency. We will also document vaccination status including RTS,S vaccine administrations. See details of proposed study procedures at the enrollment and every two months follow up for six months in the community. See Table 3 above.

Individual participant's visits. Every two months through the sixth month, participants in the cohort will be invited to the nearest central location for data and specimen collection. A structured questionnaire will be administered to collect information on demographics, net utilization and vaccination status). At each visit, we will review the target child's health passport to document all vaccinations, including the standard EPI vaccines through 9 months of age and the 15-month measles vaccine. We will also document the dates of all RTS,S vaccine administrations.

Human blood samples and testing.

Clinical testing: To establish the study cohort, potential participants will be screened by the study team to assess for their eligibility. At enrollmentthe cohort will be screened for malaria using Malaria Rapid Diagnostic Tests (The SD BIOLINE Malaria Ag P.f/Pan (HRP-II)™ rapid diagnostic test RDT)) and those participants who test positivewill be treated with a full, weight appropriate treatment course of dispersible artemether-lumefantrine (LA) by government Staff. The RDT and treatment of RDT positive children will be repeated at the final visit.

Hemoglobin levels from each participant will also be examined at enrollment and at every two-month follow-up visits. Parents of children with a haemoglobin level under 11 g/dl will be advised to take the child to a health facility for follow-up care and will be given a referral letter with the haemoglobin reading to show to staff at the health facility by the research Team.

Molecular analyses: At enrollment and at every two-month visit, we will collect finger-prick blood samples from all enrolled participatants. These specimens will be used to assess *P. falciparum* infection by quantitiative PCR. Then the specimens will be stored for later determination of gametocyte-specific gene expression and gametocyte density. Serum will be collected to assess for serological evidence of exposure to infection and response to vaccination.

Household mosquito collection and testing. Vector populations will be sampled from 100 study households (from RTS,S age-eligible households) in both intervention arms. The selection of the households for mosquito collection will be done during the recruitment of age-eligible children. In each catchment area CDC miniature light traps will be set in sleeping rooms to capture host-seeking vectors indoors. We will also sample blood-fed and gravid mosquitoes by indoor aspiration and exit trap sampling. Sampling will occur at each house on four consecutive nights during the last four weeks of the rainy season to optimize catch numbers. Light traps will be set up in a single functional bedroom in each of the households. Local personnel in each catchment area will be trained to carry out these procedures.

Species of captured adult mosquitoes will be determined by visual examination under microscopy and PCR-based assays using procedures well established in our laboratories. Blood-fed mosquito abdomens will be tested for the host species that were fed upon using a PCR method based on the vertebrate mitochondrial cytochrome B gene. The separated thorax and head of each female will be tested for salivary gland *P. falciparum* sporozoites using standard ELISA or PCR to estimate infection prevalence. For non-blood fed mosquitoes, we will assess their parity status by dissection and examination of mosquito ovaries.

6.5 Analysis Plan

To describe the study population, evaluate potential confounding, and identify sources of selection bias, baseline information will be summarized and compared across children who do/do not comply with either RTS,S vaccination, or with PBO nets, or with both. These analyses will be primarily conducted at an individual level, but to assess robustness of results, comparisons will also be conducted at cluster levels. Analysis at individual levels will be performed using random-effect models to account for the within subject correlation. Linear, logistic, and over-dispersed Poisson models will be used to analyze continuous, categorical, and count variables, respectively. Baseline information will also be assessed for children ≤10 years old at individual- and household-level through nested random effects accounting for correlation of subjects within households.

For analysis of effectiveness on prevalence and transmission, separate models will be fitted to estimate total and direct effectiveness (Hayes, 2009; Hallaron, 2010) in children aged 7-18 months at six time points. Analysis will be primarily done at the individual level, with intervention

groups compared through nested random effect models (accounting for correlation across time). A subgroup analysis will include children who will have been vaccinated within 3 months from Month (M) 1. Models will be logistic for the occurrence of infection and presence of gametocytes, and overdispersed Poisson or zero-inflated for gametocyte density. In all analysis, the linearity of association of the outcome with predictors will be evaluated through semiparametric regression and, if necessary, predictors will be categorized or transformed. In secondary analyses, all incident events will be assessed in individual-level analysis through negative binomial models (corresponding to discrete proportional hazard multiple event models) with nested random effects. We will also explore comparisons of the number of new infection events per follow-up month in over-dispersed Poisson models. Since adherence to vaccination (post-randomization variable) can introduce selection bias, we will also explore estimating total and direct effectiveness using causal inference models.

To examine antibodies of exposure, two approaches will evaluate the association between levels of exposure antibodies and intervention groups: 1) antibody levels will be summarized using partial least square regression (PLS); 2) levels of individual antibodies will be used. Summary scores and antibody levels will be compared over time between the two groups through joint outcome (shared parameter) models (Verbeke, 2010). Moreover, joint outcome models will be fit for positivity of responses and a zero-inflated model will be fit for the breadth of responses (number of antibodies that are positive in a subject).

For analysis of effectiveness in all household members (in Phase 1, children aged >18 months and $\leq$ 10 years old), indirect effectiveness at the individual level at four-time points will be estimated. Also, in nested random effect models for individual analysis, we will account for correlation across visits, within the household. The household-level analysis will be conducted by summarizing outcomes in the household, and also in nested random effect models, using the same link functions described above.

For entomological outcomes, analysis of effectiveness will be performed comparing intervention groups through zero inflated negative binomial models with random effects, including as offsets hours of capture effort or number of captured mosquitoes, as appropriate. Outcome variables will include abundance of *Anopheles*, sporozoite rates, blood-meal originated from humans, and female parity in households.

6.5.1 Data handling and record keeping

Data for the survey will be collected through questionnaires programmed onto computer tablets. Electronic records (in the form of structured questionnaires) will be made from each data-producing contact between the study participants and the study team. These source data are de-identified (each sheet containing date, participant number, household number and signature of the data collector). Questionnaires will be administered to household owners by enumerators. Every day after the fieldwork the questionnaires will be uploaded loaded to a secure central database. The team leader/ data manager will immediately check for errors. Additionally, periodic reports will check for errors in data capture. The database will be managed at the Data Management Core at the College of Medicine in Blantyre. All documentation regarding the participants will be identified with appropriate participant codes, both on paper and in computer files. Samples will be de-identified and contain only a sample identification code. The names will only appear on informed consent forms and a separate coding list that will be secured stored, separately from the database. Only the Principal Investigators and designated study personnel

will have access to the code list linking study identification numbers with the subject identifiable information.

The investigators shall maintain the records of their copies of the questionnaires and regulatory documents (informed consents, ethical approval) with all records kept in a secure place. Clinical information will not be released without written permission of the subject, except as necessary for monitoring.

6.6 Results presentation and dissemination

The results of this study will be presented through tables, graphs and powerpoint presentations. If maps are used to display information about malaria prevalence, household GPS data will be aggregated at the village or cluster level such that individual households are not identifiable. Locally, the results will be discussed with the villages in the study area, the health centers involved, Machinga and Balaka District Health Office and Assembly. The main findings will also be published in international peer-reviewed journals and the Malawi Medical Journal, and through presentations at local, regional or international conferences/seminars. Results will also be presented to the College of Medicine Research and Ethics Committee (COMREC) and the National Malaria Control Program since they develop and implement policy as well as other stakeholders.

Data will be collected prospectively and this data will be of use to the Malawi National Malaria Control Program, NIH and other malaria control partners in Malawi and beyond. Data from studies will be made available to researchers, policymakers and the wider community at no cost to promote dialogue on the results, encourage further analysis, inform new research questions and disseminate information to the community. This data sharing plan is designed to enable access to as much data as possible while protecting the human subject identity and ensuring proper use.

De-identification of data:
Under this proposal, we will de-identify all the data intended for broader use and variables which could be used to deductively disclose the identity of individual subjects. Examples of data that will be removed include names, date of births or attendance at clinics, medical record numbers, GPS location etc. Only the PI and designated personnel in Malawi will have access to the link between study and sample identification number and the names and medical record numbers of study participants. Only staff and investigators with authorization from the

Data Sharing:
All de-identified data will be made available through NIH supported portals for wide dissemination and access. All de-identified human clinical and epidemiological data will be available through the ClinEpiDB repository whilst genomic data from humans (if any) and mosquitoes will be made available through controlled access to the EuPathDB repository. Any data shared will be subjected to conditions of use governing access to the public release data, including restrictions against attempting to identify study participants, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties, and proper acknowledgment of the data resource.

Data Submission and Release Timeline:
Research data from a completed study will be shared with the scientific community and other interested organizations within a period of one year from completion of the study when the data has been thoroughly cleaned and a draft report has been written. Data will be sent to the repositories no later than the closure date for the grant.

7 ETHICAL CONSIDERATIONS

7.1 General Principles

Strict measures will be taken by the study investigators and study staff members to ensure subject confidentiality. The study protocol, documentation, data and all other information generated will be kept in strict confidence. Each participant will be assigned a unique Participant Identification Number (PID) that will be used for data entry and analysis linked to the different Case Report Forms (CRFs). Records will only be accessible to the data entry team, the individual conducting data entry checks as part of the dual entry system and the authorized study investigators. All consent forms containing identifiable information will be kept in a locked cabinet available only to the Principal Investigator. All data will be stored in and analyzed using password-protected, encrypted secure computers. No information or data of the study participants will be released to any unauthorized third party without prior approval of the study participant and appropriate IRBs. If maps are used to display information about malaria prevalence, household GPS data will be kept in a separate file (to which only the PI and designated personnel involved in data captures will have access).

7.2 Informed Consent

Consent will be sought from all collectors and study participants. Informed consent will be initiated by the study staff prior to anyone agreeing to participate in the study. The study staff members will introduce themselves and explain the purpose of the study to the parent/guardian. In this study, a guardian or caretaker will be defined as any adult of legal age with a blood relation to the child (such as a grandparent, sister or aunt) or a legal guardian who is involved in taking care of the child. The parent/guardian of potentially eligible children will then be asked to read the informed consent in the preferred language either in Chichewa (the local language) or in English. The informed consent will be read and explained verbally in Chichewa to those who are unable to read. This verbal explanation will be attended by an impartial witness who will also sign the consent form. If no impartial witness is available, a member of the study staff other than the person administering consent may serve as a witness and sign the document. Study staff members will then answer any questions that the parent/legal guardian of the child participant may have, following which they will be asked to sign the informed consent form. For those who cannot write, a thumbprint will be placed on the form, with the signature and the date of the witness. A copy of the consent form will be given to the parent/guardian. All consent forms will be IRB approved in Malawi and the USA.

Study participation will be completely voluntary and participants will be informed that they may withdraw consent at any time throughout the course of the study without penalty. The rights and welfare of participants will be protected by emphasizing to the parent/guardian that the quality of the participant's medical care will not be adversely affected if they decline participation. If the parent/guardian decides to withdraw consent, the participant/child will be able to continue to receive health care at the government clinics and hospitals or wherever they choose.

Future Use of Stored Specimens:

The informed consent form for this study has a section that will offer participants a choice about the use of study specimens that remain after the study procedures have been completed. Any use of study samples that is outside the scope of this protocol will be submitted for prior review and approval by the appropriate IRBs when the need arises. Only blood samples collected during finger pricks will be stored for possible future use either as dry blood spots on filter paper or as plasma or serum in cryovial tubes.

7.3 Possible risks and benefits to study participants

7.3.1 Potential Risks

Fingerprick blood sampling: All participating children will be screened for Plasmodium infection during monthly visits, and for disease during unscheduled visits to the HC when they have any illness. Plasmodium infection screening will be done through a routine monthly sample taken for dried blood spots (DBS). Blood samples for all these procedures will be done through a finger prick which may cause participants temporary discomfort. Moreover, blood specimens collected through finger prick will be used to measure serological markers. However, this will also represent a negligible volume of blood collected though finger prick. To prevent any discomfort and risks associated with finger pricks, we will use optimal lancets and will appropriately clean the area before the extraction of blood.

Breach of confidentiality: All data collected during the survey is strictly confidential. There is however a small risk of breach of confidentiality during the study with inadvertent access of personal health information to non-study personnel, Efforts directed to reduce this risk will be a priority by having all study data coded. Each participant will be given a unique Participant Identification number (PID), and all electronic data will be linked to this PID. Directions to the house of the participant will be linked to the PID. It will however, be accessible by the home interviewer only during home interviews and by Data Managers to address queries. All field data collection will be done on PDAs that will be password protected and accessible only to the relevant study personnel staff. Data recorded in the PDA will be transferred to the main data base at the end of each day.

All electronic data will be stored in password-protected computers in a locked room in the data management Core section (DMC). The password protected database files will only be accessible to designated data entry personnel and study investigators. Backup copies of the database files will be maintained in a separate hard disk that will be kept in a locked filing cabinet at DMC. The dataset mapping PID to subject identifiatble information including names and directions to the households will be stored separately from the research database and only the PI and the essential research team will be granted access to this dataset.

Compensation: In accordance with the Malawi National Commission for Science and Technology (NCST) circular dated 22nd August 2019, we will reimburse the parent/guardian (household) with MK 2,000 for the travelling costs each time she/he comes with participants to the central location for blood sample collection and interviews. The amount of money to be reimbursed is an estimated average cost for participants who travel to and from the disignated central location.

7.3.2 Known Potential Benefits

This study will enroll children aged 7 months to 10 years within two government HC catchment areas. In Malawi, this age group is at greatest risk of malaria infection and its complications. The participants will benefit from being intensively followed-up during the six months specifically at (0, 2, 4, and 6 months) study period, and by having mRDT results provided and treatment provided, if indicated at 1 and 6 months. The research team will give the participants health advice and refer them for treatment at the local health center. The study team will support the local health center with laboratory services and drugs so that all participants will be guaranteed access to laboratory diagnostics of malaria and or antimalarial treatment where necessary. The individuals who participate in this study will have access to prompt diagnosis and treatment of malaria when appropriate. We are

going to perform mRDTs to participants at recruitment and 6 months. Similarly, we will also test participants for anemia at recruitment and at all the follow up months. By participating in this study, the children will have access to high-quality and personalized medical attention. In the case of life-threatening emergencies, the study team will use their vehicle to transfer the study participants to the nearest government or private hospital. The minimal risks of this study, associated with finger pricks and the breaches of confidentiality are outweighed by these benefits. In addition, the study will provide support to the health centre in the study with resources mainly in malaria diagnosis and treatment which will benefit the community at large, not just research participants. In addition, the results of this study will help to maximize the implementation and impact of malaria prevention and control measures throughout Malawi. Each enrolled houslehold will be given K2000 for each study visit as compensation for their transport cost during enrolment and monthly visits.

8 POSSIBLE CONSTRAINTS

The main possible problems with participation are hesitation over providing a small volume of blood specimens through a finger prick. Extensive sensitization before and during the survey should significantly mitigate this possibility. In ICEMR, we have successfully conducting the collection of blood specimens through finger prick without notable resistance from communities.

9 REQUIREMENTS

For the evaluations of study participants, each study site will have the regular HC-based team and field teams. The regular HC teams will be comprised of government employed clinical, nursing and laboratory staff, and ICEMR-employed epidemiological field teams of a nurse, enumerator and a village field assistant. The study nurses and enumerators will receive training in human subjects' research, entomological sampling, and data collection using electronic data capture devices. The HC based team will be responsible for passive disease surveillance from participants referred by the field teams during monthly visits and/or from participant's non-scheduled visits to the facilities at any other time during the follow-up. Responsibilities assigned to the study team members will be stipulated in standard operating procedures (SOPs) and will be conducting in-depth interviews. Entomological assessments will be conducted by a different team comprised of an entomology research assistants and field technicians.

A data team lead by Data Managers will be responsible for receiving, archiving and processing all incoming data according to standard operating procedures for local storage and transfer to collaborating institutions. All data will be collected using electronic devices. The nurse, enumerator and an entomology research assistant will be using laptop computers to access the REDCap electronic data capture system for data collection. The REDCap system will be accessed using the internet, however, if internet connectivity is lost, an offline version of REDCap installed on the laptops will be used. The field worker will use the tablet computer to collect data.

Other requirements include local transportation (a vehicle, motorcycles, and bicycles will be procured), office administration (computers, office supplies, space rentals), laboratory and pharmaceutical consumables, including kits for Luminex assays and calibration reagents for the Luminex machine in the laboratory of Malawi, and protective wear. Details are highlighted in the budget below.

10 TRAINING PROVIDED

The study offers training to 1 Malawian post-doctoral research fellow from the College of Medicine, University of Malawi who will lead the project implementation and will supervise core local staff. Other PhD students/post-docs include:

- Jimmy Vareta (molecular epidemiology of malaria transmission)
- Christopher Stanley (biostatistician with expertise in longitudinal analysis)
- Rex Mbewe (impact of PBO nets on entomological indicators)

11 REFERENCES

Agnandji ST, Lell B, Soulanoudjingar SS, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med*. 2011;365(20):1863-1875. doi:10.1056/NEJMoa1102287.

Amatya A, Bhaumik DK. Sample size determination for multilevel hierarchical designs using generalized linear mixed models. *Biometrics*. September 2017. doi:10.1111/biom.12764.

Buchwald AG, Coalson JE, Cohee LM, et al. Insecticide-treated net effectiveness at preventing Plasmodium falciparum infection varies by age and season. *Malar J*. 2017;16(1):32. doi:10.1186/s12936-017-1686-2.

Buchwald AG, Sixpence A, Chimenya M, Damson M, Sorkin JD, Wilson ML, Seydel K, Hochman S, Mathanga DP, Taylor TE LM. Clinical implications of asymptomatic Plasmodium falciparum infections in Malawi. *Clin Infect Dis*. 2018;10(1093).

Chouaïbou M, Bingham G, Knox TB, Pates H, Bonfoh B. Acta Tropica Synergist bioassays : A simple method for initial metabolic resistance investigation of field Anopheles gambiae s . l . populations. *Acta Trop*. 2014;130:108-111. doi:10.1016/j.actatropica.2013.10.020.

Gray A, Clarke P, Wolstenhome J, Wordsworth S. *Applied Methods of Cost-Effectiveness Analysis in Healthcare*. Oxford University Press, Oxford; 2011.

Halloran M, Longini I, Struchiner C. *Design and Analysis of Vaccine Studies*. Springer. New York; 2010.

Hauck K, Smith P GM. The economics of priority setting for heath care: a literature review. HNP discussion paper. 2004.

Hayes R, Moulton L. *Cluster Randomized Trials*. Chapman & Hall/CRC Press. Boca Raton; 2009.

Johnson PCD, Barry SJE, Heather MF MP. Power analysis for generalized linear mixed models in ecology and evolution. *Methods Ecol Evol*. 2015;6:133-142.

Kazembe LN, Kleinschmidt I, Holtz TH, Sharp BL. Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. International journal of health geographics. 2006;5:41.

KC. *Constructing Grounded Theory : A Practical Guide through Qualitative Analysis*. SAGE Publications, Inc; 2006.

Lindblade KA, Mwandama D, Mzilahowa T, Steinhardt L, Gimnig J, Shah M, et al. A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. Malaria journal. 2015;14:31.

Liu A, Shih WJ, Gehan E. Sample size and power determination for clustered repeated measurements. *Stat Med*. 2002;21(12):1787-1801. doi:10.1002/sim.1154.

Mathanga DP, Mwandama DA, Bauleni A, et al. The effectiveness of long-lasting, insecticide-treated nets in a setting of pyrethroid resistance: a case-control study among febrile children 6 to 59 months of age in Machinga District, Malawi. *Malar J*. 2015;14:457. doi:10.1186/s12936-015-0961-3.

Ministry of Health (MoH). National Malaria Control Programme - NMCP/Malawi and ICF. 2018. Lilongwe, Malawi, and Rockville, Maryland UN and I. *Malawi Malaria Indicator Survey 2017*.

Mzilahowa T, Ball AJ, Bass C, Morgan JC, Nyoni B, Steen K, et al. Reduced susceptibility to DDT in field populations of Anopheles quadriannulatus and Anopheles arabiensis in Malawi: evidence for larval selection. Medical and veterinary entomology. 2008;22(3):258-63.

Mzilahowa T, Chiumia M, Mbewe RB, et al. Increasing insecticide resistance in Anopheles funestus and Anopheles arabiensis in Malawi, 2011-2015. *Malar J*. 2016;15(1):563. doi:10.1186/s12936-016-1610-1.

Ochomo EO, Bayoh NM, Walker ED, et al. The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance. *Malar J*. 2013;12(1):1. doi:10.1186/1475-2875-12-368.

Okiro EA, Kazembe LN, Kabaria CW, Ligomeka J, Noor AM, Ali D, et al. Childhood malaria admission rates to four hospitals in Malawi between 2000 and 2010. PloS one. 2013;8(4):e62214.

Penny MA, Pemberton-Ross P, Smith TA. The time-course of protection of the RTS,S vaccine against malaria infections and clinical disease. *Malar J*. 2015;14:437. doi:10.1186/s12936-015-0969-8.

Protopopoff N, Mosha JF, Lukole E, et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two fact. *Lancet*. 2018;391(10130):1577-1588. doi:10.1016/S0140-6736(18)30427-6.

Ranson H, Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends Parasitol*. 2016;32(3):187-196. doi:10.1016/j.pt.2015.11.010.

Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS, et al. Rise of multiple insecticide resistance in Anopheles funestus in Malawi: a major concern for malaria vector control. Malaria journal. 2015;14:344.

Roca-Feltrer A, Kwizombe CJ, Sanjoaquin MA, Sesay SS, Faragher B, Harrison J, et al. Lack of decline in childhood malaria, Malawi, 2001-2010. Emerging infectious diseases. 2012;18(2):272-8.

Sisya TJ, Kamn'gona RM, Vareta JA, Fulakeza JM, Mukaka MF, Seydel KB, et al. Subtle changes in Plasmodium falciparum infection complexity following enhanced intervention in Malawi. Acta tropica. 2015;142:108-14.

Sonnenberg FA, Beck JR. Markov models in medical decision making: a practical guide. *Med Decis Making*. 1993;13(4):322-338. doi:10.1177/0272989X9301300409.

Verbeke G, Fieuws S, Molenberghs G, Davidian M. The analysis of multivariate longitudinal data: a review. *Stat Methods Med Res*. 2014;23(1):42-59. doi:10.1177/0962280212445834.

Vontas J, Grigoraki L, Morgan J, et al. Rapid selection of a pyrethroid metabolic enzyme CYP9K1 by operational malaria control activities. *Proc Natl Acad Sci U S A*. 2018;115(18):4619-4624. doi:10.1073/pnas.1719663115.

World Malaria Report 2017. Geneva: World Health Organization, 2017. License: CC BY-NC-SA. *World Malaria Report 2017.*

12 BUDGET

	Item Description	Posts	Salary (USD)	Salary (MK)	Effort	Total Salaries (USD)	Total Salaries (MK)	Totals (USD)	Totals (MK)
A	**Study Staff Salaries**							**212,510.00**	**155,132,300.00**
	Post Title / Personnel	**No. Posts**	**Salary/post /month**	**Salary/ post /month**	**Effort in mos/Yr**	**Total Salaries/Yr**	**Total Salaries/Yr**		
	Field-based staff								
1	Grant administrator - finance and admin	1	4,000	2,920,000.00	0.50	2,000.00	**1,460,000.00**		
2	Grant administrator - HR extended to all 5 years	1	4,000	2,920,000.00	1.00	4,000.00	**2,920,000.00**		
3	Procurement Coordinator extended to all 5 years	1	1,200	876,000.00	1.00	1,200.00	**876,000.00**		
4	Project Coordinator	1	2,500	1,825,000.00	6.00	15,000.00	**10,950,000.00**		
5	Data Manager	1	3,000	2,190,000.00	4.80	14,400.00	**10,512,000.00**		
6	Nurses (2 in year1 and 2) Epi	2	1,000	730,000.00	12.00	12,000.00	**8,760,000.00**		
7	Enumerators (2 in yr1 and 2 yr 2) Epi	2	690	503,700.00	12.00	8,280.00	**6,044,400.00**		
8	4 Ento field workers (6 months in yr 2)	4	690	503,700.00	24	16,560.00	**12,088,800.00**		
9	1 lab technician (12 months) EPI	1	0	-	12	0.00	**-**		
10	1 lab technician (12 months in yr 2) Ento	1	1,000	730,000.00	0	0.00	**-**		
11	1 lab tech, Molecular Core (SA1)	1	1,500	1,095,000.00	6	9,000.00	**6,570,000.00**		
12	1 Driver	1	570	416,100.00	6	3,420.00	**2,496,600.00**		
13	Undergrad Intern	1	500	365,000.00	36	18,000.00	**13,140,000.00**		
	Sub-total for Field-based Staff			-		***103,860.00***	**75,817,800.00**		
	Post Doctoral Associates			-			**-**		

1	EPI Postgraduate (TBR)	1	3,000	2,190,000.00	12	36,000	**26,280,000.00**		
2	Ento Postgraduate (TBR)	1	0	-	0	0	**-**		
3	EPI Research Assistant (TBR)	1	1,200	876,000.00	6	7,200	**5,256,000.00**		
4	Ento Research Assistant (TBR)	1	1,200	876,000.00	6	7,200	**5,256,000.00**		
5	Ento Research Assistant	1	1,200	876,000.00		0	**-**		
	Subtotal Post-docs salary			-		50,400.00	**36,792,000.00**		
	Graduate Students stipend/ pay			-			**-**		
1	EPI Intern (TBR)	1	1,000	730,000.00	12	12,000.00	**8,760,000.00**		
	Subtotal Grad students stipend/ pay			-		**12,000.00**	**8,760,000.00**		
	Sub-total support staff			-			**-**		
	Professional staff			-			**-**		
1	Overall PI	1	12,000	8,760,000.00	2.50	30,000.00	**21,900,000.00**		
2	Entomological Co-PI	1	6,500	4,745,000.00	2.50	16,250.00	**11,862,500.00**		
	Sub-total professional staff					**46,250.00**	**33,762,500.00**		
	Total salary support					**212,510.00**	**155,132,300.00**		
B	**Travel Costs**							**5,510.00**	**4,022,300.00**
C	**Office Rental Costs**							**3,744.00**	**2,733,120.00**
D	**CLINICAL SUPPLIES (for sample collection and analysis for 12 months)**							**67,300.00**	**49,129,000.00**
E	**Equipment**							**24,828.00**	**18,124,440.00**
	Total Direct Cost							**313,892.00**	**229,141,160.00**
	F&A, College of Medicine (8%)*							**23,125.12**	**16,881,337.60**
	Total							**337,017.12**	**246,022,497.60**