Statistical Analysis Plan

Repeat Ivermectin Mass Drug Administrations for MALaria Control II (RIMDAMAL II): a double-blind, cluster-randomized control trial for integrated control of malaria.

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Statistical Analysis Plan
RIMDAMAL II Trial

1. OVERVIEW OF THE STATISTICAL DESIGN FOR RIMDAMAL II

1.1 Design overview: RIMDAMAL II is a double-blind cluster randomized controlled trial of repeated ivermectin (IVM) mass drug administration (MDA) in addition to standard malaria control (SMC) for integrated control of malaria. The primary hypothesis is that repeated IVM MDAs coinciding with SMC rounds and distribution of long-lasting insecticidal nets (the intervention) will significantly reduce the cumulative incidence of malaria in children ≤ 10 years of age compared to SMC and long-lasting insecticidal nets (LLIN) alone. The primary objective is to determine the efficacy of adding seasonal ivermectin mass drug administrations (IVM MDA) to the standard-policy malaria control measures in the Sahel (SMC in children, LLIN coverage, IPTp in pregnant women), for reducing the incidence of uncomplicated malaria episodes in enrolled village children (≤ 10 years of age) assessed by active case surveillance. IVM or placebo MDA will be given monthly over 4 months of the rainy season to the eligible village population, each as 3-day course of 300 µg/kg/day for two consecutive years.

1.2 Study population: The RIMDAMAL II study population is comprised of fourteen spatially-delineated villages in southwest Burkina Faso, equally divided between placebo and IVM. Villagers are indigenous Burkinabé from various ethnic groups, most of whom farm and rear livestock. The village population sizes range between approximately 150-450 people with approximately 100 children between 0-10 years old in each village.

1.3 Sample size and power: The primary endpoint for the trial is the incidence rate of malaria episodes in children ≤10 years of age as assessed by active case surveillance by study nurses with weekly visits to each child, starting at the first round of MDA and continuing for 4 months in year 1 (1 month after MDA #4), and continuing for another 4 months in year 2 (starting with MDA #5 and ceasing 1 month after MDA #8). The sample size and power for RIMDAMAL II is based on data from our previous trial (RIMDAMAL) and another published trial, IVERMAL. Calculations assume seasonal incidence rates (per year) of approximately 1.088 and 0.619 in the control and intervention arms, respectively, thus corresponding to an incidence rate ratio of 0.619/1.088 = 0.569 (i.e., an incidence rate reduction of 0.431). The initial sample size evaluation shows that RIMDAMAL II has 80% power with 6 villages per arm and 48 children per cluster assuming that the coefficient of variation between clusters is approximately 0.258. During trial initiation, sample size was increased from 12 to 14 villages (7 per arm) with approximately 100 children per village/cluster. The larger sample size provides over 90% power in the detailed simulation studies of the statistical models that were considered for the primary analysis of trial results (Jackson, et al. *Trials* 2021).

2. ANALYSIS PLAN

2.1 Primary analysis

The primary outcome will be analyzed as intention-to-treat (ITT). The number of episodes per week among the active case detection (ACD) cohort (children age ≤ 10 years) will be used as the primary measurable outcome to evaluate the impact of the intervention when compared to the control arm. The active case detection cohort includes all enrolled children regardless of

eligibility or receipt of MDA or SMC. A mixed effects regression analysis with Poisson distribution will be used to assess the impact. The primary analysis will include a random effect for cluster (village), and 3 fixed effects (treatment effect, year, and sex; all binary covariates, no interactions evaluated). Subgroup analyses will include an evaluation of fixed effects such as LLIN use, age, and treatment with SMC or IVM. Estimates for the intervention effect from the primary analysis as well as subgroup analyses will be reported along with 95% confidence intervals. The statistical program SAS v9.4 (SAS Institute Inc., Cary, NC) will be used for primary statistical analyses. Secondary data generated from Aim 1 will include adverse events (AE), severe adverse events (SAE), and their relationship to the intervention/placebo. The risk of SAE and AE will be compared between intervention and control arms by calculating risks ratios and attributable risks with 95% confidence intervals.

2.1.1 Definition of analysis populations

Primary incidence analysis – We will use ITT analysis method for our prospective randomized trial and the population for the primary incidence outcome will be all enrolled children aged 10 years and younger.

Secondary safety analysis – Safety is being assessed with active and passive surveillance of all enrolled study participants, regardless of age. AEs will be further categorized by their relationship to the intervention and as SAEs based on their severity.

2.1.2 Definition of primary efficacy endpoint

A malaria episode is defined as the presence of a temperature of ≥37·5°C and/or history of fever in the last 24 hours, along with a positive rapid diagnostic test (RDT) for *Plasmodium* species (any species).

A participant's time-at-risk will be considered as censored for 14 days following a diagnosis and treatment of malaria due to the impacts of treatment on risk of recurrent malaria. Person time will be defined in 7-day consecutive intervals, with one or more visits occurring during this time considered as an eligible person-week.

Eligibility for MDA is determined at the beginning of each intervention season and is retained for the remainder of that season.

- Newborns are eligible for inclusion and will be considered exposed beginning at the time of their 1st ACD visit.
- Children who turn 11 years of age during an intervention season will remain eligible for the ACD cohort for the remainder of that season.
- If a participant ages out of ACD cohort in the first season, they will not be eligible for the ACD cohort in the second season.
- Eligibility for SMC is determined by the MoH community workers, not by the RIMDAMAL II study team. Participants may age out of SMC (>59 months) in the 1st season and become eligible for MDA (≥90 cm and >59 months) in the second season. They will not become eligible for MDA during the season where this occurred.

2.1.3 Analysis methods for primary endpoint

Primary efficacy analysis:

- Intention-to-treat analyses will include the assigned treatment arms and results from all clusters. Incidence rates will be determined using a Poisson regression that models

counts of malaria episodes and includes an offset that accounts for log person-weeks. This yields a malaria rate per week per child per rainy season for intervention and control villages (averaged across year 1 and 2).

- The estimate for the primary analysis is the exponentiated beta coefficient for the treatment covariate across both seasons. This rate ratio corresponds to the difference in rates between treatment and control villages.
- Statistical Model:
  - The model below shows the outcome (malaria episodes), a coefficient for treatment group (intervention vs control), a coefficient for year (rainy season 1 vs rainy season 2), a coefficient for sex, an offset term to account for person-weeks, and a random effect to account for clustering at the village level.

Let Y_{hijk} be the number of malaria episodes for subjects k within village j within treatment i within year h

$$log(Y_{ijk}) = \alpha + \beta_1 X_i + \beta_2 X_{ijk} + \beta_3 X_{hijk} + log(t_{hk}) + u_{ij}$$

where $X_i = 0$ (control) or $X_i = 1$ (Ivermectin), $X_{ijk} = 0$ (female) or $X_{ijk} = 1$ (male) and $X_{hijk} = 1$ (Year 1) or $X_{hijk} = 2$ (Year 2) and:

$$\begin{aligned} \alpha &= \text{Intercept} \\ \beta_1 &= \text{Log of the rate ratio treatment vs control} \\ \beta_2 &= \text{Log of the rate ratio males vs females} \\ \beta_3 &= \text{Log of the rate ratio Year 1 vs Year 2} \\ log(t_{hk}) &= \text{Denotes log person-weeks} \\ u_{ij} &= \text{Denotes the random village effect} \end{aligned}$$

- Detailed simulation studies were used to determine the appropriate analysis procedure for this trial (Jackson, et al. *Trials* 2021)
- Software:
  - SAS v9.4 (SAS Institute Inc., Cary, NC) will be used for the primary statistical analyses.
  - A Kenward Roger degrees of freedom correction will be applied to account for the limited number of clusters
  - Code Example:

```
proc glimmix data=WORK.DataExample;
class Village_code Treatment YEAR Sex;
Model Episodes = Treatment  Year Sex/ s dist=Poisson ddfm=kr offset =
lnPersonWeeks;
Random  INT / subject= Village_code;
Run;
```

2.2 Secondary endpoints

2.2.1 Adverse events analysis: Safety is a secondary endpoint and will be assessed by relative risk (RR).

a. **Groups**

i. **All participants** will be followed actively and passively for adverse events and the Risk Ratios (RR) of AEs in those in the intervention versus control clusters will be compared.

ii. **Active Case Detection Cohort**

1. **SMC-treated children only** – children age 3-59 months who receive SMC. RR of AEs in children who receive SMC and live in IVM-treated clusters versus those who receive SMC and live in placebo-treated clusters
2. **IVM/placebo-treated only** – children ≥90cm and eligible for MDA/placebo. RR of AEs in those children receiving MDA versus those receiving placebo.

iii. **Non-ACD participants** that were enrolled in the study were actively followed in the study on the days of study intervention through 24-hours post study intervention, and then passively for the rest of the MDA round.

b. **Type** – AEs will be further classified by seriousness, severity, relationship to the intervention, outcome, and organ system.

i. Classification (AE, SAE, etc)

Adverse Event: (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product.

Serious AE (SAE): An AE is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

1. Death,
2. A life-threatening AE: An AE is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization,
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
5. A congenital anomaly/birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

ii. **Severity** (grade)
- Grade 1 (mild)
- Grade 2 (moderate)
- Grade 3 (severe)
- Grade 4 (potentially life-threatening)
- Grade 5 (death)

iii. **Outcome**
- recovered/resolved without sequelae (grade for the event returned to <2)
- recovering/resolving (grade for event is decreasing and not greater than 2)
- not recovered/not resolving (adverse event still present but stable)
- recovered/resolved with sequelae (grade for the event returned to <2, however some pathological condition resulting from a disease, injury, or other trauma)
- Death
- unknown (example for using this code is when a participant is lost to follow up and outcome cannot be determined)

iv. **By body system and preferred term** - using MedDRA coding (27 groups)

2.2.2 Parasitological secondary endpoints and analysis.

The following are key secondary parasitological endpoints, and the method of analysis will depend on the type of data collected:

- Parasite prevalence. Parasite prevalence of *Plasmodium falciparum* will be assessed either by:
  - molecular assay (PCR-based off of filter paper from capillary finger-prick samples). Filter paper samples are obtained at the beginning and end of each intervention season, and monthly, prior to each MDA in the ACD cohort. In addition, filter paper was obtained in the entire cohort at the beginning and end of the 2020 intervention season.
  - blood smear (only for ACD children at the time of diagnosed malaria infection by RDT)
- Multiplicity of infection (MOI, also sometimes referred to as complexity of infection (COI). This is defined as the number of unique clones per individual at the time of diagnosis of malaria infection (count data). It is based either on microsatellite typing or amplicon sequencing
- Molecular force of infection (mFOI; also sometimes further subclassified as molFOB (molecular force of blood stage infection). Defined as the number of new *Plasmodium* clones acquired over time (count data). Will be determined using monthly filter paper samples in ACD children only.

An approach consistent with the primary analysis will be adopted here with both unadjusted and adjusted regression models. For parasite prevalence, a binomial regression model will be used, and for MOI and mFOI, Poisson and Negative Binomial regression model will be used, with the final model choice being indicated by the distribution of the dependent variable.

2.2.3 Entomological secondary endpoints and analysis

The tests used will depend on the type of data collected (e.g., survivorship of blood fed mosquitoes in each arm per collection interval, proportion of mosquitoes parous/arm per collection interval, etc…) The following are key secondary entomological outcomes.

- Human biting rate (HBR). This is defined as the number of mosquitoes captured per person per time period. For aspirated, indoor resting, blood fed mosquitoes, the HBR is calculated by dividing the house or household catch by the number of humans living in that house or household. For host-seeking mosquitoes caught in light traps placed next to individual persons sleeping in tents (outdoors) or under bednets (indoors), the HBR is simply the catch number per 1 person.
    - This will be compared between intervention and control arms and further analyzed by mosquito species (*An. gambiae* s.l [or *An. gambiae* s.s., *An. coluzzi*, *An. arabiensis*]; *An. funestus*)
        - Indoor blood fed HBR
        - Indoor host-seeking HBR
        - Outdoor host-seeking HBR
        - Total HBR
- Sporozoite rate. A representative proportion of mosquitoes captured per time period and per mosquito species will be tested for prevalence of infection with *Plasmodium* sporozoites by genetic testing of their heads+thoracies.
    - This will be compared between intervention and control arms and analyzed in total.
    - This will also be analyzed by identified *Plasmodium* species: *P. falciparum*, *P. ovale*, *P. malariae*, or *P. vivax*.
- Changes in participants' antibody responses to *Anopheles* saliva protein(s). Antibodies will be eluted from filter paper samples containing a representative proportion of participants' capillary finger-prick blood that were collected at the beginning and end of each intervention season. Paired sets antibodies from individual participants (beginning and end of season collections) will then be tested for binding to *Anopheles* saliva protein(s), the binding change per person calculated, and then these data compared between participants from the intervention and control arms.
- The survival rate of blood fed mosquitoes. These blood fed mosquitoes were collected per time period, brought back to the field insectary and held for 3 days to measure their survivorship over that time. Survivorship will be compared between intervention and control arms and analyzed by time period captured, by mosquito species, and by location in clusters where they were captured.

These data (except for survival rate, see below) will also be used to make generalized linear and non-linear mixed effects models in R and SAS to understand the functional relationships of measured entomological and parasitological variables and the relative

contributions of various independent variables on variation in the dependent variable. Final model choices will be indicated by the distribution of the dependent variable as well as assessments for the need to model an additional parameter to account for variability in the data (e.g., Poisson vs Negative Binomial). The survival rate of blood fed mosquitoes will be analyzed using a log-rank test and visualized using a Kaplan Meier curve.

2.2.4 Missing data

Missed weekly ACD visits will be accounted for by using person-weeks at-risk. If an ACD child does not have a documented visit during a 7-day period, this will be considered a missed person-week at-risk. Missed visits may be due to the child not being present (school, travel, etc..), refusal, or incomplete data entry in the REDCap instrument. Participants can enter the cohort and exit the cohort as described in 2.1.2, and this will be accounted for using person-time at-risk as well.

2.3 Tertiary and exploratory analyses

2.3.1 Tertiary clinical/parasitological endpoints

2.3.1.1 Drug resistance mutations – The prevalence of parasite mutations associated with reduced susceptibility to antimalarials used both for treatment and prevention will be compared between intervention and control arms. These include mutations in transporters *Pfcrt*, *Pfmdr1*, and antifolate pathways (*DHFR* and *DHPS*).

2.3.1.2 Prevalence of anemia- IVM may improve anemia by reducing malaria and/or soil-transmitted helminths in the intervention populace. Changes in the prevalence of anemia will be compared between 1) ACD children in the intervention and control villages; 2) individuals in cross-sectional HHs in the intervention and control villages.

2.3.2 Tertiary entomological endpoints

2.3.2.1 Measures of insecticide resistance- Changes in mosquito phenotypic susceptibility to standard insecticides will be analyzed between times this was tested at the study site and related to the prevalence of mosquito SNPs (or quantity of gene transcripts) that correlate with known, genetic insecticide resistance markers.

2.3.2.2 Age structure of the mosquito population over time- This will be defined as the estimated median age of the adult female *An. gambiae* s.l. population per unit time or the proportions estimated in certain age classes per unit time. Estimates will be done with traditional and novel age grading techniques.

2.3.3 Subgroup analysis

2.3.3.1 SMC-treated children only – incidence will be compared between those children who received SMC only (age 3-59 months)

2.3.3.2 IVM/placebo-treated children only – incidence will be compared between those children who received MDA/placebo only (≥90 cm and ≤10 y/o)

Other notes regarding covariates/effects to consider:

- Analyze the IRs by cluster and arm over short time periods (by week) for each season - using regression approaches

        - Malaria transmission intensity naturally varies over each season due to changing weather and mosquito vector species and densities and changing human immunity.
        - Each of the 4 ivermectin MDAs per season acts like a “pulse” of insecticidal blood that starts high and then the mosquito-killing efficacy wanes over time as the ivermectin is eliminated from treated persons’ blood. We estimate the mosquito killing activity lasts for 2-3 weeks but is highest in the week after the MDA is given. On top of this, there would be a time delay, as the killing would reduce biting, and then a week to 2 later, the numbers of parasitemic individuals would decrease
- Spatial effects between and within clusters
    - The incidence difference based on child/ HH location in cluster – inner vs. outer edge of the cluster
    - Differences based on the density of non-study populations nearby (within several km of the cluster boundaries)
- Random effect within HHs in clusters (due to HH size, proportion of males to females in a HH, age proportion in a HH proportion, HH members treated and/or treated over time)

3. PLANNED TABLES, FIGURES, LISTINGS

Manuscript Tables:

1. Participant characteristics
2. Primary efficacy analyses
3. Adverse events
4. Subgroup analysis of malaria risk

Manuscript Figures:

1. CONSORT diagram
2. Map of study area
3. Rainfall and weekly malaria incidence

Supplementary appendix tables:

- S2.1: Percentage reduction in clinical incidence during the trial period
- S3.1: Risk in epidemiological strata
- S3.2: Exploratory analysis of the frequency of malaria episodes per child in each group

Figures: Supplementary appendix

- S1.1: Map of study area
- S2.1: Estimated reduction in clinical incidence
- S3.1: Flow diagram for all RIMDAMAL participants with respect to MDA participation and AE analysis.
- S3.2: Weekly malaria incidence per person-year in the RIMDAMAL study cohort children plotted over the study period.

Supplementary appendix Listing:

- S4.1: Descriptions of reported adverse events (AE) in RIMDAMAL villages (excluding uncomplicated malaria recorded from the child cohort.
- S4.2: Summary of deaths during intervention season

Additional figures/tables:

- Seasonality: Risk by month with rainfall
- Interaction of Interceptor G2 bed net distribution by cluster/HH/individual (only in 2020)
- Interaction of proportion of people treated per cluster and over time.
- MOI
- mFOI
- anemia
- entomology outcome figures

4. LITERATURE INFORMING THIS SAP

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5. APPENDIX: FUTURE WORK TO BE CONSIDERED

1. Pharmacokinetic/dynamic parameters
    a. Impact of age on IVM PK
    b. Evaluation of IVM metabolites
    c. Relationship of IVM PK with blood-feeding results done at CSU
    d. Safety of IVM (clinical, hematology, chemistry)
    e. Comparison on capillary and venous measurements of IVM
2. Parasite data
    a. Parasitemia [Count data] – done by quantitative PCR – compare (log10) distributions in populations between arms
        i. SMC-treated only
        ii. IVM/placebo-treated only
        iii. all cohort
    b. Multiplicity of Infection (MOI) per episode or sampling timepoint and molecular Force of Infection (mFOI) per child [Count data] – done by either genotyping microsatellites or amplicon deep sequencing
        i. Regression analysis with Poisson distribution, in an unadjusted analysis followed by an adjusted analysis taking into account the random effects of the cluster (village) and household and fixed effects such as sex, LLIN use, age, and treatment with SMC or IVM.
3. Entomology data
    a. Survivorship bioassays of blood fed mosquitoes [Survival assays]
        i. Consider time variable
    b. Catch density [count data]
        i. Consider time variable
    c. Age structure
        i. Age distribution of mosquitoes in Intervention vs. Control clusters
        ii. Proportion of mosquitoes caught over an age cut-off (>10 days)
            1. Consider time variable
    d. Proportion of mosquito blood meals taken from humans vs other animals
        i. Consider time variable
    e. Proportion of mosquitoes with sporozoites
        i. Consider time variable
    f. Entomological inoculation rates (human biting proportion x sporozoite rate)
        i. Consider time variable
4. Other studies
    a. Impact of IVM on the prevalence of drug and insecticide resistance
    b. Impact of IVM on all-cause anemia in children
    c. Household survey – association of household factors and vector density