## Title

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# Child Health and Infection with Low Density (CHILD) malaria: a protocol for a randomised controlled trial to assess the long-term health and socioeconomic impacts of testing and treating low-density malaria infection among children in Tanzania

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## ABSTRACT

**Introduction** As malaria declines, low-density malaria infections (LMIs) represent an increasing proportion of infections and may have negative impacts on child health and cognition, necessitating development of targeted and effective solutions. This trial assesses the health, cognitive and socioeconomic impact of two strategies for detecting and treating LMI in a low transmission setting.

**Methods and analysis** The study is a 3-arm open-label individually randomised controlled trial enrolling 600 children aged 6 months to 10 years in Bagamoyo district, Tanzania. Children are randomised to one of three arms: active case detection with molecular (ACDm) testing by high volume quantitative PCR (qPCR), passive case detection also with molecular testing (PCDm) and a control of standard PCD using rapid diagnostics tests (RDTs). Over the 2-year trial, ACDm participants receive malaria testing using RDT and qPCR three times annually, and malaria testing by RDT only when presenting with fever. PCDm and PCD participants receive malaria testing by RDT and qPCR or RDT only, respectively, when presenting with fever. RDT or qPCR positive participants with uncomplicated malaria are treated with artemether lumefantrine. The primary outcome is cumulative incidence of all-cause sick visits. Secondary outcomes include fever episodes, clinical failure after fever episodes, adverse events, malaria, non-malarial infection, antibiotic use, anaemia, growth faltering, cognition and attention, school outcomes, immune responses, and socioeconomic effects. Outcomes are assessed through monthly clinical assessments and testing, and baseline and endline neurodevelopmental testing. The trial is expected to provide key evidence and inform policy on health, cognitive and socioeconomic impact of interventions targeting LMI in children.

**Ethics and dissemination** Study is approved by Tanzania NatHREC and institutional review boards at University of California San Francisco and Ifakara Health Institute. Findings will be reported on ClinicalTrials.gov, in peer-reviewed journals and through stakeholder meetings.

**Trial registration numbers** NCT05567016; PACTR202311470737025.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The trial may be the first randomised controlled trial to assess the impact, safety and cost-effectiveness of detecting and treating low-density malaria infection (LMI) on child health and well-being.
⇒ Trial will allow assessment regarding potential effectiveness of using highly sensitive molecular diagnostics to reduce malaria-associated disease burden in low malaria transmission settings.
⇒ Trial will enable an assessment regarding the potential impact of clearing LMI on malaria immunity.
⇒ As an open-label trial, there is potential for biases resulting from investigator or patient expectation.

## INTRODUCTION

The scale-up of malaria control interventions such as bed nets and effective drugs for case management and chemoprevention has resulted in dramatic declines in malaria cases and deaths worldwide.[1 2] However, as transmission declines, low-density malaria infections (LMI) or infections that are below the detection limit of standard microscopy and rapid diagnostic tests (RDTs), represent an increasing proportion of all infections.[3–5] Left undetected and untreated, LMI can persist as

a chronic infection for months and even years.[6] Although the importance of LMI for malaria transmission is well recognised,[7 8] LMI has received less attention due to the prevailing perception that they are asymptomatic, induce protective immunity against future infection, and because they are difficult to detect due to the limited sensitivity of microscopy and standard RDTs.

The potential morbidity associated with LMI has received less attention due to cohort studies suggesting that LMIs are asymptomatic and immune-protective.[9–12] However, these studies were conducted in high transmission settings, where converse associations have also been observed.[13] In low transmission settings, growing evidence suggests that LMIs are associated with febrile and non-febrile illness,[14 15] chronic anaemia,[16] growth faltering,[17] immune dysregulation and increased vulnerability to other infections,[17–19] and negative impacts on cognition and school attendance.[14 18 20–23] Given these associations, the causal impacts of LMI on child health, well-being and productivity are likely to be significant.

Chemoprevention strategies such as seasonal and perennial malaria chemoprevention provide treatment and prevention of LMI, but these approaches have limited application in settings where antimalarial resistance is a challenge. Also, in low transmission settings, the benefit of these interventions may become overshadowed by drug toxicity risks and lower perceived malaria risk, leading to decreased acceptability and adherence.[24] While a 'test-and-treat' approach may minimise overtreatment and maximise acceptance and adherence, evidence to date suggests limited effectiveness to decrease malaria,[25 26] mainly due to the limited sensitivity of standard diagnostics. In community surveys, use of molecular methods identifies 2–3 old more infections than microscopy or RDT.[3 5 25]

In passive case detection (PCD) of symptomatic individuals presenting to health facilities, standard RDTs miss a large proportion of PCR-detectable infection: in low transmission settings, standard RDTs miss 24%–48% of infections (primarily LMI) in febrile patients.[27–30] Given the low prevalence of infection in these communities, the unexpectedly high proportion of low-density infections cannot solely be explained by background parasitaemia.[31] Due to declining immunity, LMI may be more likely to cause acute febrile malaria when transmission declines.[32] Indeed, the parasite density pyrogenic threshold for *Plasmodium falciparum* malaria has been found to be as low as 10 parasites/µL in malaria-naïve individuals.[33] LMI, as a subacute or chronic infection, may also contribute to morbidity from other infections, for example, through its effects on malnutrition and decreased host defences.

Molecular methods make it increasingly possible to detect LMI in afebrile and febrile individuals not detectable by currently available rapid tests. Recent advancements in quantitative PCR enable detection of infections with parasite densities as low as <0.1 parasites/mL,[34] providing roughly 10-fold and 1000-fold higher sensitivity compared with traditional nested PCR methods and RDT, respectively. While molecular testing is still resource-intensive, convenient and affordable highly sensitive diagnostics and high-throughput platforms are likely to become available, particularly when leveraging systems used for other conditions such as HIV or SARS-CoV-2.

Novel approaches for detecting and treating LMI are needed. Here, we present the protocol for a randomised controlled trial to evaluate two different approaches for detecting and treating P. *falciparum* LMI and assess their impact, safety and cost-effectiveness for improving child health and well-being. The study will take place in the low transmission setting of Bagamoyo district, Tanzania. The interventions include PCD using molecular testing (PCDm) and active case detection using molecular testing (ACDm). Each of these interventions will be separately compared with the standard of care based on PCD using RDT. All malaria cases detected during the study will be treated with artemether-lumefantrine (AL), the standard of care for uncomplicated malaria in Tanzania.

### Aims and objectives

The overall objective of this trial is to evaluate the impact of detecting and treating *P. falciparum* LMI on child health and well-being. The primary aim is to assess the impact of interventions on the incidence of all-cause child sick visits during the 24-month follow-up period. We hypothesise that detection and treatment of LMI will be associated with ≥20% reduction in all-cause sick visits in intervention arms compared with the control. The secondary aims include assessing the impact of interventions on anaemia, growth, safety, malaria, fever episodes, clinical symptoms, clinical failure after fever episodes, antibiotic use, immune responses, neurodevelopment, school outcomes and socioeconomic effects.

## METHODS AND ANALYSIS

The Standard Protocol Items: Recommendations for Intervention Trials guidelines[35] were referenced when developing this protocol.

### Patient and public involvement

Patients and/or the public were not involved in the design of the study but will be involved in the trial conduct, and reporting and dissemination of results through community meetings.

### Study design

The study is a three-arm open-label individually randomised controlled superiority trial aimed at evaluating the impact of detecting and treating LMI (defined as RDT (−)/quantitative PCR (qPCR) (+) infection). The standard of care based on PCD using RDT will be compared with ACDm (arm 2) and PCDm (arm 3). Each intervention will be separately compared with the standard of care as the health effects, logistical and cost requirements of treating *P. falciparum* LMI through active or PCD may differ.

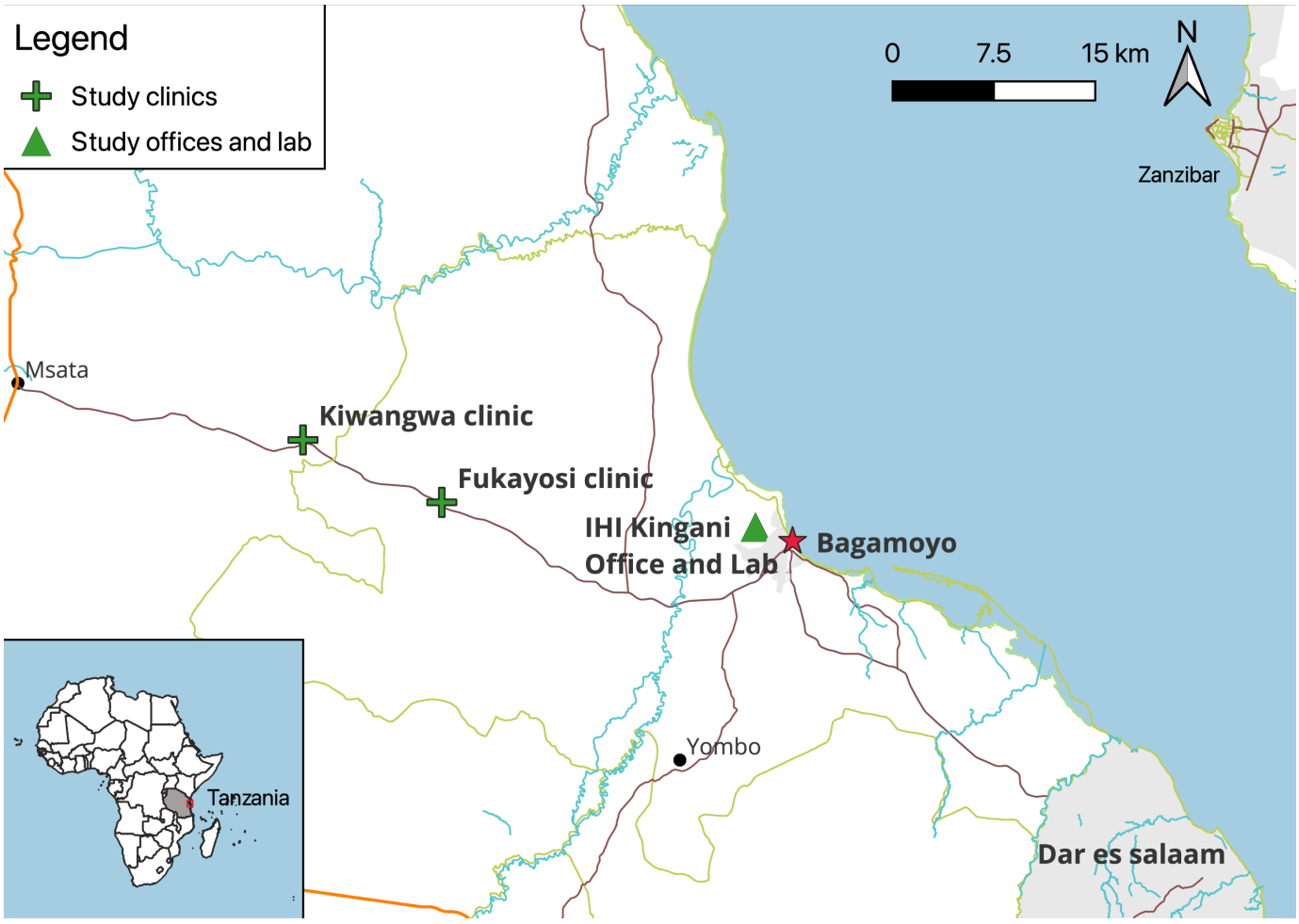


**Figure 1** Study site in Bagamoyo, Pwani region, Tanzania, showing location of study clinics and lab.

### Study setting and trial preparations

The study will be conducted between 18 July 2023 and 28 November 2025. The study site is Kiwangwa and Fukayosi, two neighbouring wards in Bagamoyo district, Tanzania with a total population of approximately 43 000.[36] (figure 1). *P. falciparum* is the predominant malaria species, and transmission is perennial with two peaks (June–July and October–November). Transmission intensity was previously moderate to high, but after successful scale-up of interventions such as insecticide-treated bed nets, RDTs and effective treatment with artemisinin combination therapies, transmission is now considered low by WHO criteria (annual incidence is <100–250/1000 and prevalence 1%–10% by RDT or microscopy for all ages).[37] A high proportion of LMI in febrile and afebrile children has previously been documented in Bagamoyo. Infection prevalence by RDT and qPCR was 14% and 57.5% in asymptomatic children, and 67.5% and 89% in symptomatic children, respectively.[27 38]

The study area is served by Kiwangwa and Fukayosi clinics, which will be locations for study activities including the baseline evaluation, follow-up visits and standard medical care during the study. Malaria qPCR testing will be conducted at the Kingani Clinical Trials Unit lab, located within 40 km of the study clinics to facilitate 0–3 days turnaround time for results.

To create the sampling frame, a roster of households within a 5 km radius of each study clinic will be generated, along with line listings of children 6 months to 10 years. Prior to recruitment, community sensitisations meetings will be held with local leaders, community stakeholders and parents/guardians of potential study participants to discuss the study and its goals in a culturally appropriate fashion.

### Study procedures

An overview of recruitment, enrolment and subsequent procedures is shown in figure 2.

### Recruitment and enrolment

Approximately 660 children aged 6 months to 10 years (assuming 10% ineligibility) will be randomly selected from the household listings and invited for informed consent and screening to assess study eligibility. To limit contamination and clustering effects from the intervention, only one child per household will be eligible. The initial encounter will occur at the household where

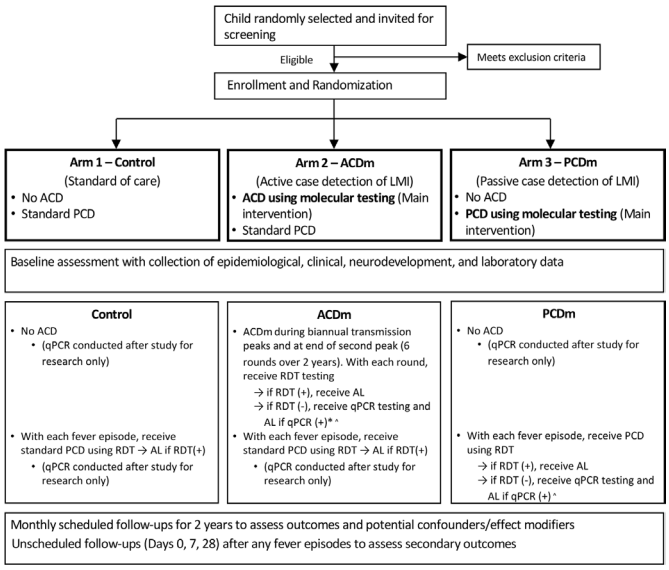


**Figure 2** Flow of study procedures. ACDm, active case detection with molecular; AL, artemether-lumefantrine; LMI, low-density malaria infection; PCDm, passive case detection also with molecular; qPCR, quantitative PCR; RDT, rapid diagnostics test. *ACDm will be conducted during scheduled follow-up visits. ^qPCR result available within 0–3 days.

informed consent and initial screening for eligibility (table 1) will occur.

Enrolment will commence with the administration of a household questionnaire for eligible and consented participants. The questionnaire will collect demographic characteristics, vector control coverage and water, sanitation and hygiene information. Enrolment will be completed at the first visit to the study health facility, at which point random allocation to study arms and the baseline assessment will be completed.

Enrolled participants will not be permitted to participate in other trials during the 2-year study period. They may also be withdrawn due to severe adverse events (AEs), consent withdrawal, loss to follow-up, non-compliance with study procedures, ineligibility that was overlooked during screening or death.

## Consent process

Written informed consent will be obtained by trained study staff from parents or guardians of potential participants prior to start of study procedures. Consent forms will include a statement about future testing of samples collected. Parents' or guardians' comprehension will be verbally assessed before signing of the consent form. The written consent will be obtained in Swahili, the national language. Per local guidelines, written minor assent is not standard for participants <13 years and will, therefore, not be obtained as none of the study participants will be 13 years or older during the study period.

## Randomisation and masking

Eligible and consented participants will be assigned to 1 of the 3 study arms in a ratio of 1:1:1, or 200 per arm by an individual not directly involved in the conduct of the study using computer-generated randomisation stratified by age category (6 months to ≤5 years; 6–10 years), location (Kiwangwa or Fukayosi) and gender. Randomisation will be conducted sequentially as participants are enrolled using the minimisation method[39] to maximise balance in baseline characteristics between study arms.

As the trial is open-label, study participants and clinicians will be unblinded. However, staff performing laboratory analyses and final formal data analyses will be blinded to study intervention. To mitigate bias, the assessment of primary outcomes will be standardised through rigorous training and annual retraining, and use of the same case record forms across arms. All parents/guardians will be encouraged to seek care or call the study nurse if their child is not feeling well to mitigate bias in health-seeking by participants.

## Baseline evaluation

The baseline assessment will comprise epidemiological, clinical and neurodevelopmental assessment, as well as blood, urine and stool collection, to assess for baseline health and to measure potential confounding factors. Neurodevelopment will be assessed through validated

**Table 1** Inclusion and exclusion criteria for study enrolment

| Inclusion criteria | Exclusion criteria |
|---|---|
| ► 6 months to 10 years.<br>► Primary residence in the study area during the study period*<br>► Agree to come to study clinic for any illness.<br>► Agree to avoid medications outside of study, even herbal medication. | ► Another child from household already randomly selected for recruitment.<br>► Not able or does not provide informed consent.<br>► Need for emergency intervention.†<br>► Known history of chronic illness requiring regular specialty care including diabetes mellitus, cancer or stage 3 or 4 HIV/AIDS.<br>► Contraindications to artemether-lumefantrine including history of allergic reaction, weight under 5 kg.<br>► Participation in another active/ongoing intervention trial. |

*Currently stays overnight in a home located in Kiwangwa or Fukayosi majority of the time and intends to continue doing so during the 2-year study period.
†Need for emergency intervention only applies at the time of recruitment and at the time enrolment.

tests of sociobehavioural development, cognition and attention in young and school-age children.

### Study interventions

Control arm (PCD): Participants will receive the standard of care when febrile, which includes PCD of malaria using RDT and treatment with AL if RDT-positive.

ACD using molecular testing (ACDm): As in the control arm, study participants will receive standard PCD when febrile. Additionally, participants will receive six total rounds of ACDm (3× annually), conducted during the two high transmission season peaks (June–July, then October–Novmber) and at the end of the high transmission season (Feb). At each round of ACDm, blood will be collected for RDT testing and RDT-positives will receive AL while RDT-negatives will have qPCR testing conducted, and those testing positive receiving AL.

PCD using molecular testing (PCDm): As in the control arm, study participants will receive standard PCD when febrile. If participants are RDT-negative, a second malaria testing by qPCR will be done. Uncomplicated malaria cases detected by RDT or qPCR will be treated with AL.

### Scheduled follow-up of study participants

Participants will be followed up for 2 years to capture subacute and chronic effects of the interventions. Study outcomes will be measured passively and actively at monthly encounters (table 2) at the health facility (even numbered visits) and in household visits (odd numbered visits) through surveys, review of health records and laboratory assessments. If a participant misses their scheduled visit, they or their emergency contacts will be called by the study staff to be reminded to attend or reschedule within a 7-day window.

### Follow-up for sick visits

After initial assessment (day 0), all participants with fever (at visit or within previous 48 hours) will receive follow-up at day 7 and day 28 to measure secondary outcomes of safety, clinical failure and immunological responses. RDT-positive (or qPCR-positive for the PCDm arm) participants who meet criteria for uncomplicated *P. falciparum* malaria will receive standard doses of AL (twice a day×3 days) via directly observed therapy (DOT) in the clinic and community.

### Malaria testing and treatment during study

RDT and qPCR results will be used to inform treatment during visits for PCD, PCDm and ACDm interventions. Due to the persistence of *P. falciparum* histidine rich protein 2 (HRP-2) antigen, microscopy will used to inform treatment if the participant was diagnosed with malaria using RDT in the prior 2 weeks. Cases of uncomplicated malaria detected in the study participants will be treated with AL, with complicated cases treated with artesunate, per local guidelines. AL will be administered by DOT, with dosing following weight-based recommendations. All other testing for malaria (table 2) will be conducted at the end of study and for research purposes only.

### Illness during the study

At all times, participants will receive free healthcare for acute illnesses (eg, malaria, anaemia, diarrhoea, and acute upper and lower respiratory infections) at the Kiwangwa or Fukayosi clinics and will have access to an on-call nurse for medical consultation. If needed, study staff will refer and facilitate transport to the district hospital. Routine specialty care for chronic illnesses will not be covered by the project unless the illness is deemed to have been caused by one of the diagnostics strategies.

### Safety and tolerability

Risks associated with AL are expected to be low, due to its excellent safety profile, and its use as a first line of treatment for uncomplicated malaria in Tanzania. AEs will be monitored actively during all routine follow-up visits, and passively, as participants are encouraged to seek care at the study health facility or contact study nurses in case of any issues. AE severity will be graded using the Division of Aids (DAIDS) toxicity table for grading severity of paediatric AEs, and local reference intervals for laboratory results. Management of AEs will follow local IRB standard guidelines, including withholding drugs, follow-up tests and evaluations, and reporting. Participants with severe adverse reaction associated with any of the diagnostic strategies may be withdrawn from the study. Grade 3 AEs or higher will be reported per regulatory guidelines and included in safety data reports shared with the data safety and monitoring board (DSMB). Participants with abnormal clinical findings and laboratory values will be followed at least weekly until toxicity resolves to less than grade 2 or no further improvement is anticipated.

AL dosage will be weight based and will be administered by DOT, providing an additional opportunity to monitor participants for AEs. Participants will be observed for at least 30 min post-AL administration, with redosing done in case of vomiting. Alternative intravenous malaria treatment will be provided in case of repeated vomiting. AE monitoring related to AL will continue for 28 days following drug administration.

### Neurodevelopmental assessment

Neurodevelopment will be assessed through validated tests of sociobehavioural development, cognition and attention in young and school-aged children. Prior to trial launch, neurodevelopment assessment tools will be piloted and validated for the local context. For cognition, the Global Scales for Early Development[40] will be used for children aged <3.5 years, the International Development and Early Learning Assessment[41] for children 3.5 to <6 years of age, and the East African Neurodevelopment Assessment Tool,[42] a locally adapted modification of the Kaufman Brief Intelligence Test second Edition, for children 6–12 years. For sustained attention, the Pencil Tap Test[43] will be used for children aged 5 to <9 year, and the Code Transmission Test, a local adaptation of the Test of Everyday Attention for Children,[21 43–46] will be used for children aged 9–12 years.

**Table 2** Schedule of follow-up assessments

| | Month (schedu led routine visits) | | | | | | | | | | | | | | | | | | | | | | | | | Sick visit | Day (follow-up for fever or malaria) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 0 | 7 | 28 |
| Primary outcome | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| All-cause sick visits | | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| Secondary outcomes | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Haemoglobin (Hb)* | • | | • | | | | • | | • | | | | • | | • | | | | • | | • | | | | • | † | | |
| Clinical signs and symptoms‡ | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| All-cause fever episodes | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| Adverse events | | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | • | • |
| Growth measurements | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | • | | |
| Malaria parasitaemia/ clinical malaria§ | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | • |
| Non-malarial pathogen detection | | | | | | | | | | | | | | | | | | | | | | | | | | ¶ | | ¶ |
| Antibiotics prescribed | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| Inflammation/ immunological testing | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CRP** | | | | | | | | | | | | | | | | | | | | | | | | | | ¶ | | |
| Antimalarial antibodies | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | | • | ¶ | | ¶ |
| Complete blood count (CBC) | • | | | | | | • | | | | | | • | | | | | | • | | | | | | • | | | • |
| Cellular immune responses | • | | | | | | • | | | | | | • | | | | | | • | | | | | | • | | | • |
| Cytokine panel | • | | | | | | • | | | | | | • | | | | | | • | | | | | | • | | | • |
| Vaccine and pathogen antibodies | • | | | | | | • | | | | | | • | | | | | | • | | | | | | • | | | • |

Continued

**Table 2** Continued

| | Month (schedu led routine visits) | | | | | | | | | | | | | | | | | | | | | | | | | Sick visit | Day (follow-up for fever or malaria) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neurocognitive assessment | • | | | | | | | | | | | | | | | | | | | | | | | | • | | | |
| Socioeconomic assessment | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| School absenteeism | | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| School advancement | | | | | | | • | | | | | | • | | | | | | | • | | | | | • | | | |
| Parental missed work, healthcare costs | | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| Potential confounders or effect modifiers | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Stool testing for intestinal parasites†† | • | | | | | | | | | | | | • | | | | | | | | | | | | • | | | |
| Urine filtration for schistosomiasis†† | • | | | | | | | | | | | | • | | | | | | | | | | | | • | | | |
| HIV | | | | | | | | | | | | | | | | | | | | | | | | | † | | | |
| RBC polymorphisms and iron studies | | | | | | | | | | | | | | | | | | | | | | | | | † | | | |
| Clinical and epidemiological factors | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | | |

*Hb data will be collected by Hemocue if not already collected by CBC.
†As clinically indicated.
‡Assessed as: categories of symptoms, reported fever or illness prompting care-seeking. With fever episodes, assessed for clinical failure (persistent or worsening illness).
§qPCR result to guide treatment for PCDm and ACDm only; in standard PCD in control and ACDm arms, qPCR will be done after the study period.
¶In a sample, CRP, antimalarial antibody immune responses and non-malarial pathogen detection using molecular methods such as metagenomic next-generation sequencing (mNGS) may be conducted day 0 and with convalescence visit. mNGS to be conducted as combined oropharyngeal or mid-turbinate swabs and blood samples.
**Other markers of inflammation such as procalcitonin and erythrocyte sedimentation rate may be assessed.
††Stool and urine samples collected within a week of these visits, though timing may vary as we will aim to collect these before annual deworming campaigns. Stool testing to be conducted by examination for ova and parasites and/or PCR.
ACDm, active case detection with molecular; CRP, C reactive protein; PCDm, passive case detection also with molecular; qPCR, quantitative PCR; RBC, Red Blood Cells.

### Costing data collection

Detailed costs from the healthcare provider and patient will be collected. Programme costs of ACDm, PCDm and standard of care PCD will be collected through careful documentation of project expenses including capital expenditures, consumables, personnel, training, transport and infrastructure. Data will be collected from participants on care-seeking and associated costs (medications, transport costs, time lost from school or work).

### Data management

Data will be collected using forms programmed on Castor Electronic Data Capture (EDC), and on password-secure tablet computers. Staff will be trained prior to start of the trial, annually and/or when necessary, on data collection procedures and use of electronic tablets. Data will be validated, queried, reviewed, verified and corrected in real time. To protect participant's privacy, computer records of participants will be password protected, while paper records kept in a locked file at Kingani clinical trial facility, with only authorised personnel having access. Study records will be retained for 20 years after the end of the clinical trial and will be readily available to ethics and regulatory committees for inspection and auditing.

### Laboratory methods

Parasitaemia will be detected using RDT, microscopy and qPCR. Testing with standard RDTs (Abbott Bioline Malaria Ag Pf/Pan) will be performed per manufacturer instructions. Thick blood smears will be assessed for asexual parasites by examination of 100 high power fields by 2 independent microscopists, with a third microscopist breaking the tie in case of discordant results. Species identification will be conducted using thin smear microscopy. DNA extraction from whole blood followed by *P. falciparum*-specific qPCR method and genotyping will be conducted using previously described methods.[6 34] PCR for non-falciparum species as well as RT-qPCR for gametocytes will also be conducted.[38]

Point-of-care tests used in sick care management will include testing for haemoglobin (Hb), glucose, pulse oximetry, urinalysis and complete blood counts with differential. Rapid HIV-1/2 testing will be done as clinically indicated. Stool and urine samples will be used to assess intestinal parasites by Kato Katz and urine filtration microscopy, respectively. Molecular testing of soil transmitted helminths and schistosomiasis will be done using previously published methods. For children with persistent anaemia of unclear aetiology, an assessment for aetiology will be done, including peripheral blood smear, iron studies and Hb electrophoresis. To understand the role of LMI clearance on malaria immunity, humoral and cell-mediated immunity will be assessed using assays including ELISA to test sera for antibody levels to a select panel of malaria antigens, and flow cytometry on isolated Peripheral blood mononuclear cells (PBMC) to assess the role of Vδ2+T cells on LMI. Non-malaria specific responses will be examined by looking at cytokine and serum immunoglobulin responses to common bacterial and viral pathogens using phage immunoprecipitation sequencing. Aetiologies of fever will be assessed using metagenomic next-generation sequencing of nasopharyngeal/oropharyngeal swabs and plasma.

### Study outcomes and measures

The primary outcome is the cumulative incidence of all-cause sick visits over the 2-year study period. A sick visit is defined as any sick visit to the health facility excluding a related illness diagnosed within 2 weeks of another or planned admissions for medical care, elective surgery and trauma. Children will be considered 'at risk' during all days during the follow-up period excluding 14 days after sick visit, and in case of premature study withdrawal, up to the midpoint from last documented visit. Secondary outcomes include fever episodes, clinical failure after fever episodes, malaria infection, non-malarial infection, antibiotic use, anaemia, safety, child growth faltering, neurodevelopment, socioeconomic effects, school outcomes, immunological responses to malaria and inflammation. Study outcomes and potential confounders or effect modifiers will be assessed at scheduled and unscheduled visits and cross-validated through review of the health facility registers. Study outcomes and their metrics are summarised in table 3.

### Sample size and power calculation

The estimated mean baseline incidence of all-cause sick visits in our study population is 4.3/person-year of follow-up (SD 2.9).[47–52] With a sample size of 200 per study arm and assuming a 15% loss to follow-up, the study is powered to detect ≥20% decrease in sick visits relative to the control group (85% power, 95% significance). For the primary outcome, the sample size also provides adequate power (80%–90%) across a range of standard deviations (online supplemental appendix A). To ensure 600 participants are enrolled and assuming a 10% refusal/ ineligibility, 660 households with at least one child 6 months to 10 years of age will be approached for recruitment.

The study is also powered for stratified analyses of primary outcome by age category. In children ≤5 years and sample size 300 or 100 per arm, the study is powered to detect ≥25% decrease in sick visits (90% power; 95% significance, 15% loss to follow-up) from a baseline estimate of 5.0 sicks visits/person-year (SD 2.9). In children aged 6–10 years at enrolment and sample size 100 per arm, the study is powered to detect ≥35% decrease in all-cause sick visits (90% power, 95% significance, 15% loss to follow-up) from a baseline estimate of 3.3 sick visits/person-year (SD 2.8). For stratified analyses of primary outcome by age, adequate power is also maintained across a range of standard deviations (online supplemental appendix A).

The estimated baseline prevalence of the secondary outcome of anaemia (Hb<110 g/L) in the study population is 45%.[27 50 53 54] With a sample size of 200 per arm the study is powered to detect ≥35% decrease in the

Table 3 Summary of primary and secondary outcomes

| Study outcomes | Definition |
|---|---|
| Primary outcome | |
| Incidence in all-cause sick visits | Number of sick visits to health facility (excluding planned admissions for medical care, elective surgery and trauma) per person-time. |
| Secondary outcomes | |
| Prevalence of anaemia | Proportion of routine Hb measurements that are low (<110 g/L) or moderate-to-severe low (<80 g/L). A separate assessment for delayed haemolytic effect of ACT will be made at subsequent follow-up.[57] |
| Incidence and prevalence of child growth faltering | Underweight (weight for age), stunting (height for age), wasting (weight for height), body mass index and mid upper arm circumference, moderate malnutrition: z-score of –3 to <–2, severe malnutrition z-score <–3. |
| Incidence of adverse events (AEs) | Any grades 3–4 AE or serious AE; individual AEs; or AEs relate to study drugs, per person time.[58] |
| Tolerability of study drugs | Proportion of children vomiting following administration of study drugs and measures of non-adherence. |
| Incidence of clinical symptoms | Number of days with overall symptoms reported as moderate (3 on a 5-point scale) per person time.[14 59–62] |
| Incidence in all-cause fever episodes | Number of fever episodes (reported fever in the past week and/or axillary temperature of 37.5°C) per person time. |
| Clinical failure after fever episodes | Persistent or worsening symptoms assessed 7 and 28 days after initial evaluation. If PCDm participants have fever and LMI is found, they receive treatment days 0–3. |
| Incidence of clinical malaria | New episodes of positive malaria test (with fever or other clinical symptoms) per person time. |
| Prevalence of parasitaemia | Proportion of routine samples with parasites detected by microscopy or qPCR. Parasitaemia will be classified as any, new, recrudescent or persistent by genotyping. |
| Incidence in antibiotics prescribed | Number of antibiotic regimens prescribed per person time. |
| Prevalence of systemic inflammation | Proportion of routine visits with elevated biomarkers of inflammation, for example, CRP. |
| Cognitive ability | Standardised scores from validated tests will be used at baseline and 24 months: 0 to <3.5y: Global Scales of Early Development[40] 3.5 to <6y: International Development and Early Learning Assessment[41] 6 to 12 years: East Africa Neurodevelopment assessment tool [42]. |
| Sustained attention | Standardised scores from validated tests will be used at baseline and 24 months: 5 to <9 years: Pencil tap test[43] 9 to 12 years: Code Transmission Test (a local adaptation of Test of Everyday Attention for Children).[21 43–46] |
| Learning and school performance | Mean days of school absenteeism, proportion of children with school advancement to the next grade each year. |
| Socioeconomic costs | Cost to participant: Estimated long-term income loss due to reduced early childhood development. Costs to family: days of parental work absenteeism, costs of sick visits Costs to health system: Costs of testing and treatment for sick care. |
| Cost effectiveness | Cost per outcome averted (eg, per sick visit averted, per disability-adjusted life-years and per economic dollar saved). |
| Exploratory secondary outcomes | |
| Antimalarial humoral responses | Proportion with antimalarial antibodies against *Plasmodium falciparum* antigens, will also assess quantitatively using optical density (O.D.) or antibody concentration. |

Continued

Table 3 Continued

| Study outcomes | Definition |
|---|---|
| Antimalarial cell-mediated responses | Proportion with Vδ2+T cell-mediated cytokine production following stimulation with parasite lysate. |
| Biomarkers of inflammation | Proportion with changes in cytokines detected in Luminex 10-plex panel. |
| General immune responses to vaccines and other common pathogens | Proportion with antibody responses to a chip with an array of antigens to vaccines and common pathogens will also assess quantitatively using O.D. or antibody concentration. |

CRP, C reactive protein; Hb, haemoglobin; LMI, low-density malaria infection; PCDm, passive case detection also with molecular; qPCR, quantitative PCR.

prevalence of anaemia (85% power, 95% significance (two sided), 15% loss to follow-up) from the estimated baseline prevalence. For children aged ≤5 years and sample size of 300 or 100 per arm, the study is powered to detect ≥40% decrease in prevalence of anaemia (80% power, 95% significance(two sided), 15% loss to follow-up) from a baseline estimate of 60%. For children aged 6–10 years with an estimated baseline anaemia prevalence of 30%, the sample size of 300 does not provide adequate power to a detect difference in anaemia prevalence.

### Statistical analyses

Baseline characteristics will be compared across arms using t-tests for continuous variables, Wilcoxon rank sum test for non-parametric data and $\chi^2$ for categorical variables. Primary analyses will be unadjusted unless baseline characteristics are not balanced between study arms; if so, analyses will be adjusted for baseline characteristics.

An intention-to-treat approach will be used when analysing all the study outcomes and will include all study participants randomised and with outcomes of interest measured, and all follow-up time until the study participants complete the study or early study termination regardless of whether the intervention was stopped due to an AE. Children in the trial who are prematurely withdrawn from the study or are not able to provide data for specific outcomes will be considered unevaluable and will not be included in the primary trial analysis.

The primary and secondary incidence outcomes in ACDm and PCDm arms will be compared with the control arm using negative binomial regression models (with the indicator of intervention as covariate and the logarithm of person-time as an offset), with results presented as incidence rate ratio. Separate statistical models will be fit to estimate effects of ACDm and PCDm. For clinical malaria, we will conduct a time-to-event analysis using Cox proportional regression analysis, with result presented as Kaplan-Meier survival curves and as the HR. Linear regression will be conducted for outcomes with continuous variables. For prevalence outcomes, difference between study arms will be compared using log-binomial models and intervention effect reported as a prevalence ratio (PR). For repeated measured outcomes (eg, parasite prevalence, anaemia), we will construct generalised mixed-effect models where child random effects are included to account for correlation among observations from the same subjects. Models including an interaction term between follow-up time and intervention assignment will be fit to assess differences in trends over time between arms. We will explore effect modification on the additive and multiplicative scales by age category, sex, baseline malaria parasite density, baseline anaemia, reinfection status, antimalarial use outside study and comorbidities. For PCD versus PCDm comparison, we will also look at clinical diagnosis at presentation and duration of the illness prior to presentation. For neurocognitive development, we will compute the point in time cognitive function construct at baseline and endline and assess for potential differential development by study intervention.

A complete-case analysis will also be performed. If participant attrition exceeds 20% or is differential between study arms, we will perform an inverse probability of censoring weighted analysis that uses weights to reconstruct the original study population.[55]

An economic analysis will be conducted from provider, patient and community perspectives. The socioeconomic costs of LMI under each study arms will be quantified. Quantification of direct costs will be conducted using ingredients-based analysis, and indirect costs discussed and where possible, quantified, including surrounding uncertainties for each variable. Using a life-course approach,[56] schooling measures will be converted to lifetime earnings, which will be added to individual health benefits and health system savings to assess overall societal impact of these programmes. For each variable examined, a sensitivity analysis will be conducted examining plausible higher and lower bounds, and to determine its contribution to the total aggregate cost and benefit across each scenario.

## ETHICS AND DISSEMINATION

### Ethical considerations

The study protocol has been approved by the Tanzania National Health Research Ethics Review Committee (Ref: NIMR/HQ/R.8a/Vol.IX/4204) and the IRB boards at University of California, San Francisco (Ref: 22-36146) and Ifakara Health Institute (Ref: IHI/IRB/No.08-2023).

Written approval will be sought from the above ethical bodies for all protocol amendments.

Prior to recruitment, community sensitisation meetings with local stakeholders and parents/guardians of potential participants will be held, where study goals and procedures will be explained in a culturally appropriate manner. Verbal comprehension will be assessed before obtaining informed consent from guardians/parents of study participants. Participants will be assigned a unique identifier and personally identifiable information kept separate from medical information. Tablets used to collect data will be encrypted and participant records locked, with controlled access to reduce confidentiality breaches. AEs will be actively and passively monitored and reported to the regulatory bodies and the DSMB board within stipulated timelines. Participants will receive compensation for travel and time associated with study activities to reduce financial burden due to participating in the study. Furthermore, all participants will receive free on-demand care during the duration of the study. There is the potential that children randomised to certain treatment arms will experience improved health, cognition, and learning, and socioeconomic benefits. The trial will expand knowledge on health and socioeconomic impacts of treating LMI and may trigger policy discussions and new recommendations at global, national and regional levels if study shows clear benefits to treating LMI.

### Oversight, monitoring and audit

The study team will be adequately trained prior to trial launch and annually thereafter, with regular evaluation and supervision by project manager. Data will be audited weekly to ensure high-quality data are collected and to identify areas for retraining. AEs in the study will be actively and passively identified. Study staff will receive extensive training in the identification and management of AEs. Additionally, children and their parents/guardians will be educated about potential AEs associated with malaria treatment and encouraged to seek care. Grading of AEs and assessing for causality will be done by the lead clinician. Any serious AE (SAE) irrespective of relationship to study drug will be reported informally within 24 hours and formally within 7 days to the IRBs and local regulatory bodies.

Trial monitoring will be provided by a steering committee, senior management team, local and external monitors, IRBs, and an independent DSMB. The DSMB includes four content experts in clinical trials, malaria, paediatric malaria, biostatistics and safety monitoring of paediatric trials. The DSMB will meet prior to trial launch to review the study protocol and data safety monitoring plan and will meet annually to review study progress and safety, and to vote on study continuation versus termination. An interim safety analysis may be triggered should any significant AE (SAEs and grade 3 or 4 AE beginning within 2 days after study drug and persisting at grade 3 or 4 for over 48 hours) exceeds 5% after at least 100 subjects are enrolled or if more than 3 total SAEs are registered prior to the enrolment of 100 subjects. The study may be terminated for early evidence of intervention safety problems after a thorough review of any submitted interim report and the accompanying meeting with study investigators. The trial will not be stopped for futility or achievement of efficacy as an interim analysis for efficacy or futility is not planned.

Technical oversight of the study will be provided by the steering committee which will meet quarterly via teleconference and annually in person and comprises of senior management team (PI and consortium co-PIs), coinvestigators and collaborators. The senior management team which includes core study personnel will meet weekly in person and in teleconferences to track study progress and implementation.

## DISSEMINATION

The results of the trial will be shared at international scientific meetings, in reports and policy meetings with local, regional and global stakeholders, and published in peer-reviewed journals. On publication of the main trial results, data will be made available for research purposes to other individuals in the scientific community on request to the PI and consortium PIs. The study results will be shared on ClinicalTrials.gov.

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