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A Phase 1/2a single centre, randomised, placebo-controlled, double-blind, dose escalation, age de-escalation, study to evaluate the safety, tolerability, pharmacokinetics, and Plasmodium falciparum transmission-reducing activity of monoclonal antibody TB31F in malaria-exposed Malian adults and children

Statistical manual

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SIGNATURE SHEET

The signature below constitutes approval of this statistical manual.

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List Of Abbreviations And Relevant Definitions

ADA	Anti-drug antibody
AE	Adverse event
AUC 0-∞	Area under the concentration versus time curve extrapolated to infinity
Cmax	Maximum observed serum concentration
CL	Drug clearance
CV	Coefficient of variation
DMFA	Direct Membrane Feeding Assay
DSF	Direct Skin Feeding
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-Linked Immuno Sorbent Assay
GMT	Geometric mean titers
mAB	Monoclonal Antibody
PD	Pharmacodynamics
PK	Pharmacokinetics
SAE	Serious adverse event
SAP	Statistical analysis plan
SMFA	Standard Membrane Feeding Assay
SOP	Standard operating procedure
$T_{1/2}$	Terminal half-life
TBA	Transmission blocking activity
TRA	Transmission reducing activity
V_d	Volume of Distribution

Purpose

The purpose of the statistical analysis plan (SAP) is to outline the analyses that will be applied to the data generated in the described phase I/IIa trial, for the primary research outputs of this trial (i.e. the scientific paper/s reporting the primary and secondary outcomes). The SAP serves as a supplement to the protocol and contains further details about the study procedures to analyze the safety data, pharmacokinetics (PK) data and direct skin feeding (DSF), direct membrane feeding (DMFA) and standard membrane feeding assay (SMFA) data of participants of the trial.

The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and if reported will be carried out as far as possible in line with the principles of this analysis strategy and the source of the suggestion will be acknowledged.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial.

Introduction

TB31F-Mali (full title 'A Phase 1/2a single centre, randomised, placebo-controlled, double-blind, dose escalation, age de-escalation, study to evaluate the safety, tolerability, pharmacokinetics, and *Plasmodium falciparum* transmission-reducing activity of monoclonal antibody TB31F in malaria-exposed Malian adults and children') is a Phase 1/2a single-centre, randomised, double-blind trial that aims to assess the safety and tolerability of monoclonal antibody (mAb) TB31F in Malian adults and school-age children, assess the pharmacokinetics of mAb TB31F in Malian adults and school-age children and assess the *P. falciparum* transmission reducing activity (TRA) of TB31F in naturally *P. falciparum* infected Malian individuals.

Objectives

Cohort 1: Safety

Primary objectives

- To assess the reactogenicity, tolerability, and safety of increasing doses of mAb TB31F in adults and school-age children
- To assess the population pharmacokinetics (pK) of a single subcutaneous injection of mAb TB31F in Malian adults and school-age children

Exploratory objectives

- To assess the impact of potential baseline and biochemical covariates on the population pharmacokinetics (pK) of a single sub-cutaneous injection of mAb TB31F in Malian school-age children
- To assess human genomic variation in the study population
- To assess the presence of plasma biomarkers in the study population

Cohort 2: Efficacy

Primary efficacy objective

- To assess the transmission-blocking activity of mAb TB31F in serum as assessed in the Direct Membrane Feeding Assay (DMFA) at each dose level in naturally *P. falciparum* infected individuals

Primary safety/pharmacokinetics objectives

- To assess the reactogenicity, tolerability, and safety of increasing doses of mAb TB31F in Malian individuals
- To assess the population pharmacokinetics (PK) of a single sub-cutaneous injection of mAb TB31F in Malian individuals

Secondary efficacy objectives

- To assess the transmission-blocking activity of mAb TB31F assessed in the Direct Skin Feed (DSF) and DMFA at each dose level in naturally *P. falciparum* infected individuals
- To assess mosquito infection rate as measured by the proportion of dissected mosquitoes with any number of oocysts at each dose level in naturally *P. falciparum* infected individuals
- To assess the intensity of mosquito infection as measured by the average number of oocysts in dissected mosquitoes at each dose level in naturally *P. falciparum* infected individuals
- To assess participant infectivity after mAb TB31F administration at each dose level in naturally *P. falciparum* infected individuals

- To assess the transmission-reducing activity of participant serum after TB31F administration as assessed in the Standard Membrane Feeding Assay (SMFA) at each dose level in naturally *P. falciparum* infected adults and children

Exploratory objectives

- To develop an integral pharmacokinetic/pharmacodynamic (PK/PD) model describing the relationship between TB31F dose, time, individual characteristics, serum TB31F concentrations and SMFA and DSF/DMFA
- To assess the transmission-blocking activity of participant serum after TB31F administration as assessed in the Standard Membrane Feeding Assay (SMFA) at each dose level in Malian individuals
- To assess the quantitative relationship between SMFA, DSF, and DMFA results
- To assess the IC_{80} serum concentration after TB31F administration resulting in 80% transmission-blocking activity (TBA) in naturally *P. falciparum* infected individuals
- To assess the IC_{80} serum concentration after TB31F administration resulting in 80% transmission-reducing activity (TRA) in naturally *P. falciparum* infected individuals
- To assess the impact of naturally acquired TRA, present prior to TB31F administration, on transmission endpoints and TB31F efficacy estimates
- To assess human and parasite genomic variation and association with parasite measures
- To assess the impact of plasma biomarkers on malaria transmission efficiency

Method

Study design

The study is designed as a Phase 1/2a single-centre, double-blind, dose escalation, age de-escalation trial in malaria-exposed Malian adults and children. Two sequential stages of clinical research are conducted, each recruiting a distinct cohort of participants to confirm: 1. TB31F safety and 2. TB31F efficacy. Pharmacokinetic measurements will be performed in both safety and efficacy cohorts.
In total, a minimum of 165 participants will be recruited and enrolled; 45 adults and 30 children for the safety study, and ≥ 90 adults and children for the efficacy study. In the tables below, the cohorts are described with the number of participants, the duration of follow-up and the dose of TB31F administered.

Table 1. Study design for Cohort 1 (Adults): Safety and pharmacokinetics (18-50 years of age)

Group	Dose	N	Volume TB31F/Saline	Sample timepoints*	Safety follow-up after each dose administration
1	1A: Control (normal saline)	5	0.2 mL	Day 0 (Baseline), 1, 5, 7, 14, 21, 28, 42, 56, 84	Solicited local and systemic AEs (days 1-7); Unsolicited AEs (study duration); SAEs (study duration)
	1B: 10 mg mAb TB31F	10			
2	2A: Control (normal saline)	5	2 mL		
	2B: 100 mg mAb TB31F	10			
3	3A: Control (normal saline)	5	4 mL		
	3B: 200 mg mAb TB31F	10			

** Group 1 participants will be followed-up until day 28*

Table 2. Study design for Cohort 1 (Children): Safety and pharmacokinetics in school age children (≥10-15 years)

Group	Dose	N	Volume TB31F/Saline	Sample timepoints	Safety follow-up after each dose administration
4	4A: Control (normal saline)	5	0.2 mL	Day 0 (Baseline), 1, 5, 7, 14, 21, 28, 42, 56, 84	Solicited local and systemic AEs (days 1-7); Unsolicited AEs (study duration); SAEs (study duration)
	4B: 10 mg mAb TB31F	10			
5	5A: Control (normal saline)	5	2.0 mL		
	5B: 100 mg mAb TB31F	10			

Table 3. Study design for Cohort 2: Efficacy in a mixed age cohort (≥10-50 years of age)

Group	Dose	N	Volume TB31F/Saline	Sample timepoints	Safety follow-up after each dose administration
6.1	6AB: Control (normal saline)	15	0.6* mL	Day 0 (Baseline), 1, 5, 14, 21, 28, 56, 84	Solicited local and systemic AEs (days 1-7); Unsolicited AEs (study duration); SAEs (study duration)
	6B: 30 mg mAb TB31F	30	0.6* mL		
6.2	6AC: Control (normal saline)	15	2.0 mL		
	6C: 100 mg mAb TB31F	30	2.0 mL		

AE = Adverse events, SAE = Serious adverse events. Participants will be randomised in a 1:2:1:2 ratio to the 6AB, 6B, 6AC, or 6C group.

** This dose will be informed by findings from the safety cohort and is expected to be ~30 mg; the volume will be adjusted accordingly.*

Randomization

Eligible participants will be assigned the next sequentially numbered study ID number based on a pre-printed Study ID List created by an independent statistician. Eligible participants will be randomised within each dose-group to either the control sub-group or the TB31F sub-group using a 1:2 ratio for Cohort 1 and Cohort 2. The randomisation codes will be provided to the study pharmacist. The study pharmacist in Mali will prepare the intervention for the study participants. For cohort 2, randomization will be performed in blocks of varying sizes.

Sample size

The sample size is standard for the safety cohorts without formal sample size estimation. Sample size is informed by the anticipated efficacy from an earlier Phase I study [1] and the transmission blocking effects observed in similar Malian cohorts when treated with transmission blocking drugs [2-5]; this is done for the efficacy cohort only. This sample size for the efficacy cohort (n=30 per group) will give us 97% empirical power to detect >80% reduction in infectivity (defined as the percentage of mosquitoes with at least one oocyst) with a one-tailed test with a 0.025 level of significance when dissecting at least 40 mosquitos per participant at each timepoint. This power calculation is based on an online tool based on mixed effects logistic regression,[6] an expected reduction in infectivity of 90%,[1] an expected baseline proportion of infectivity of 15% for the average participant (accounting for non-infectious participants),[3-5] and a conservative expected intra-cluster correlation of 0.5.[6] Of note, this includes non-infectious individuals. In the four years that we performed NECTAR studies with a highly similar

study population, 66.0-79.5% of individuals infected at least one mosquito at enrolment with 14.2-17.0% of mosquitoes becoming infected. The power calculation given above includes all individuals (infectious and non-infectious). If we retain 30 individuals per arm and exclude those individuals who are not infectious at baseline (≤10), the expected percentage of infected mosquitoes will increase from 15% (in the situation where some individuals infect no mosquitoes) to ~20%. Based on a mixed effects logistic regression we looked at 200 simulations with an expected reduction in infectivity of 90%, an expected baseline proportion of infectivity of 20% for the average participant, and an expected intra-cluster correlation of 0.4. We estimated 91.5% empirical power to detect >80% reduction in infectivity with a one-tailed test with a 0.025 level of significance when including 30 participants (of whom 20 are included in the analysis, i.e. after excluding non-infectious individuals) and dissecting 40 mosquitoes per participant at each timepoint. Importantly, these analyses are based on DMFA where venous blood is offered to mosquitoes. We and others have previously demonstrated that mosquito infection rates are at least 2-fold higher when allowing mosquitoes to feed directly on the skin of participants. When the expected baseline proportion infected mosquitoes is updated to account for higher infection rates by skin feeding (30% for the average participant) and we conservatively increase intra-cluster correlation to 0.8, we estimated 96% empirical power to detect >80% reduction in infectivity with a one-tailed test with a 0.025 level of significance when including 30 participants and dissecting 40 mosquitoes per participant.

Statistical interim analyses and stopping guidance

There are no pre-defined criteria for study termination in this clinical trial but safety and reactogenicity data will be evaluated after each mAb TB31F administration before proceeding to the next group. A safety report including a list of all reported adverse events and any clinically significant safety laboratory values outside the normal ranges will be prepared:

- After completion of day 14 follow-up after IP administration from groups 1-2 AND before start of groups 3 and 4.
- After completion of day 14 follow-up after IP administration from group 3-4 AND before start of group 5.
- After completion of day 7 follow-up after IP administration from group 5 AND before start of group 6.
- Upon completion of the study.

Statistical Software

- Safety data analysis will be performed in STATA (latest version available), or R.
- SMFA, DMFA and DSF data analysis will be performed in R.
- The pharmacokinetic and pharmacokinetic/pharmacodynamic analyses will be performed by means of compartmental non-linear mixed effects modelling, using the software package NONMEM V7.5 (Icon, Dublin, Ireland) using R and the Perl modules package Perl Speaks NONMEM v5.4.0 for data processing.

Statistical Principles

Confidence intervals and P values

Confidence intervals and P values will be based on 0.05 as significance level. Adjustments for multiple comparisons will be implemented where appropriate but not for pre-defined study endpoints.
Where applicable, normally distributed continuous outcomes will be presented as mean with 95% CI or standard deviations. Continuous outcomes that are not normally distributed will be transformed using

a suitable transformation before reporting 95% CIs. Where a suitable transformation cannot be found, medians will be presented with interquartile ranges. For discrete (or count) data we will find interquartile ranges. Binary data will be presented as counts and proportions.

Population pharmacokinetic/pharmacodynamic modelling

Population pharmacokinetic/pharmacodynamic modelling will be performed in line with best practice (Byon et al CPT Pharmacometrics Syst Pharmacol. 2013 Jul 3;2(7):e51.). Internal evaluation of the developed model will be performed using standard goodness-of-fit plots, prediction-corrected visual predictive checks and absence of parameter correlation. Parameter imprecision will be assessed using the covariance step in NONMEM or the sampling importance resampling procedure as implemented in Perl Speaks NONMEM. Nested models will be compared using the objective function, that follows approximately a chi-square distribution. Non-nested models will be compared using the Akaike Information Criterion. Covariates will be included based on 1) physiological plausibility and 2) statistical significance as assessed by the objective function value and 3) improvement of goodness-of-fit plots. From the final model, individual empirical bayes estimates for traditional pharmacokinetic endpoints like the area under the concentration time curve (AUC), maximum concentration (Cmax), time of maximum concentration (Tmax) and elimination half-life (T½) will be obtained.

Adherence and protocol deviations

Missing data will not be imputed due to the small number of participants in each group. Participants who miss an appointment date will be retained in the study and available data will be analysed. If human errors are made during TB31F administration, the actual administered dose and time will be used in analyses.

A best-worst case sensitivity analysis may be performed. Lack of precision is expected in some outcome measures, for instance estimates of (low) transmission reducing activity (TRA) by SMFA. For some analyses, we may thus use TRA estimates that are inferred from antibody concentration based on the best fit model for the association between antibody concentration and TRA. Details are provided on page 16.

Multiple comparisons

Primary outcomes are numerous and often related (e.g. TRA and TBA are closely related). We will account for the risk of false-discovery by recalculating p-values using permutation tests. As part of this approach, we will generate an empirical distribution from study data, automatically adjusting p-values for the complexity and dependence structure in the data. This obviates the need for traditional multiple testing correction amongst the primary aims. Secondary outcomes are hypothesis-generating, and therefore, not adjustments for multiple comparisons will be applied.

Study Outcome Measures

Outcome Definitions

(Protocol section 2).

Safety outcome measures:

1. Occurrence of at least possibly related
    a) solicited local and systemic adverse events (AEs) within 7 days of mAb TB31F administration
    b) unsolicited AEs within 28 days of mAb TB31F administration
    c) serious adverse events during the entire study period

Pharmacokinetic/pharmacodynamic outcome measures:

2. Terminal serum half-life (t½)
3. Maximum observed serum concentration (Cmax)
4. Time to reach maximum serum concentration (tmax)
5. Accumulation index (Racc), and area under the serum concentration-time curve (AUC0-τ, AUC0-t and AUC)
6. The impact of the potential covariates total serum IgG, serum leptin, total protein, albumin, and pre-albumin as surrogates for malnutrition and protein metabolism, as well as age, weight, height, and nutritional status on the TB31F pharmacokinetics.
7. The parameter estimates for the fixed and random effects, as well as their imprecision, describing the integral model for the pharmacokinetics and pharmacodynamics of TB31F in adults and children.

A large number of efficacy outcome measures are listed in the protocol (section 2). These outcome measures are related in terms of the endpoints (oocyst prevalence, oocyst density, proportion infectious individuals) and comparisons (within group, between group, between assays) are grouped as such.

Efficacy outcome measures related to within-group % reduction in proportion infected mosquitoes:

8. Group averaged within-individual percent reduction in the proportion of mosquitoes infected at day 5 post-treatment relative to baseline (day 0), assessed through direct membrane feeding assays (DMFA) and measured as oocyst prevalence (timeframe: day 0 [baseline] & 5)
9. Group averaged within-individual percent reduction in the proportion of mosquitoes infected in DSF, at all feeding timepoints relative to baseline (day 0). Comparisons of these reductions between groups at each post-baseline timepoint and comparisons of the reductions between post-baseline timepoints for each group (timeframe: day 0 [baseline], 1, and 5)
10. Group averaged within-individual percent reduction in the proportion of mosquitoes infected in DMFA, at all feeding timepoints relative to baseline (day 0). Comparisons of these reductions between groups at each post-baseline timepoint and comparisons of the reductions between post-baseline timepoints for each group (timeframe: day 0 [baseline], 1, 5, and 14)

Efficacy outcome measures related to mosquito infection prevalence (oocyst prevalence):

11. Mosquito infection prevalence, assessed by DSF and measured as the proportion dissected with any number of oocysts, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, and 5)
12. Mosquito infection prevalence, assessed by DMFA and measured as the proportion dissected with any number of oocysts, compared within groups between baseline and all feeding

timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, 5, and 14)

13. The association between naturally acquired anti-gametocyte immune responses and naturally acquired functional TRA on mosquito infection prevalence.

Efficacy outcome measures related to mosquito infection intensity (oocyst density):

14. Mosquito infection intensity, assessed by DSF and measured as the number of oocysts in dissected mosquitoes, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, and 5)
15. Mosquito infection intensity, assessed by DMFA and measured as the average number of oocysts in dissected mosquitoes, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, 5, and 14)
16. The association between naturally acquired anti-gametocyte immune responses and naturally acquired functional TRA on mosquito infection intensity.

Efficacy outcome measures related to participant infectivity:

17. Participant infection prevalence, assessed by DSF as the proportion of individuals infectious to any number of mosquitoes, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, and 5)
18. Participant infection prevalence, assessed by DMFA as the proportion of individuals infectious to any number of mosquitoes, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 1, 5, and 14)
19. The association between naturally acquired anti-gametocyte immune responses and naturally acquired functional TRA on participant infectivity.

Efficacy outcome measures related to transmission reducing activity (reductions in oocyst intensity):

20. Transmission-reducing activity in children and adults, measured as the percent reduction in mean oocyst intensity compared to experimental controls, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 5, 14, 28, 56, and 84)
21. Transmission-blocking activity in children and adults, measured as the percent reduction in mosquito infection prevalence compared to experimental controls, compared within groups between baseline and all feeding timepoints, and between groups at all feeding timepoints (timeframe: day 0 [baseline], 5, 14, 28, 56, and 84)

Outcome measures related to the correlation between transmission assays:

22. Correlation between transmission measures assessed by SMFA and DMFA in Malian adults and children
23. Correlation between transmission measures assessed by SMFA and Direct Skin Feed (DSF) in Malian adults and children
24. Correlation between transmission measures assessed by DMFA and DSF in Malian adults and children

Outcome measures related to estimated inhibitory concentrations:

25. The concentration of TB31F in serum that provides >80% reduction in the average proportion of infected mosquitoes as measured in DSF after TB31F administration
26. The concentration of TB31F in serum that provides >80% reduction in the average proportion of infected mosquitoes as measured in DMFA after TB31F administration
27. The concentration of TB31F in serum that provides >80% transmission blocking activity relative to experimental controls as measured in SMFA after TB31F administration
28. The concentration of TB31F in serum that provides >80% reduction in the average mosquito infection intensity (oocyst number) as measured in DSF after TB31F administration
29. The concentration of TB31F in serum that provides >80% reduction in the average mosquito infection intensity (oocyst number) as measured in DMFA after TB31F administration
30. The concentration of TB31F in serum that provides >80% transmission reducing activity relative to experimental controls as measured in SMFA after TB31F administration

Analysis Methods

Trial population

The primary safety and reactogenicity data will include all participants who meet the eligibility criteria, receive study product mAb TB31F, and for whom safety, efficacy and immunogenicity data are available.

Recruitment

Recruitment data will be summarized in a flow diagram.

Screening data

Screening data will be summarized for each study group.

Withdrawal and follow-up

Should reasons for withdrawal be known, these will be described, including the timing of withdrawal.

Baseline patient characteristics

Demographic data will be summarized by descriptive statistics per dose group and will include the total number of observations (n), plus the mean, standard deviation (SD) and range for normally distributed continuous variables and number and percentages for dichotomous variables. This data will be tabulated. Where applicable, continuous outcomes that are not normally distributed will be transformed using a suitable transformation before reporting means and SDs. Where a suitable transformation cannot be found, the mean and SDs will be substituted with the median and interquartile ranges. For discrete (or count) data we will report the medians and interquartile ranges. Binary data will be presented as counts (n/N) and proportions.

Analyses in relation to endpoints

Safety outcome analyses

- For each solicited (local or systemic) adverse event type, the number and proportion of participants experiencing that AE within the Protocol-defined timeframe will be tabulated by severity grade for each dose group and for the entire study population. This will also be tabulated for participants experiencing any solicited local, any solicited systemic and any unsolicited AE. Where applicable, we will compare these proportions between groups.

Relatedness (possibly, probably or definitely) of solicited AEs to TB31F administration may also be presented.

- For each related unsolicited adverse event type (categorized by MedDRA), the number of events per dose group and overall study population will be described and categorized by severity grade and relation to TB31F administration. (Outcome measure 1).
- The proportion of individuals with grade 1, 2 and 3 adverse events may be compared between groups.
- The total number of grade 1, 2 and 3 adverse events as count data may be compared between groups.
- Individual serious adverse events (SAEs) will be summarized, including relatedness to TB31F administration.
- Withdrawals due to AEs/SAEs will be summarized per group.

Clinical Laboratory Data Analysis

- All clinically significant laboratory abnormalities will be analyzed by participant and will include details of onset time, duration, severity and relationship to the study dose.
- Any clinically significant deviations in routine laboratory test results, as determined by the investigator, will be summarized per group.
- Isolated laboratory abnormalities will be reported as unsolicited AEs if they are considered clinically relevant by the investigator.

Pharmacokinetic outcome measures

PK measures are dependent on quantification of TB31F antibody levels by ELISA. The ELISA will be conducted in Mali and/or the Netherlands. For this, serum samples are collected at the following time-points:

	0	1	5	7	14	21	28	42	56	84
Safety cohorts*	X	X	X	X	X	X	X	X	X	X
Efficacy cohorts	X	X	X		X		X		X	X

*safety cohort group 1 only has follow-up until day 28; safety cohort groups 2-5 have follow-up until day 84.

Analysis approach:

- For all ELISA samples, TB31F antibody density and prevalence will be determined by ELISA. All samples will be processed in duplicate alongside blank wells (for background correction), a standard curve (for concentration interpolation), and non-treated local controls (for seropositivity calculation). Optical density values from duplicate observations will be compared using the coefficient of variation (CV) and will be re-run if the CV exceeds 30%. OD values in agreement will be converted to concentrations using ADAMSEL software based on plate specific control wells; the mean of duplicate concentration estimates will be used for analyses. Antibody density will be expressed in µg/mL.
- Seropositivity will be calculated using either a) the pooled concentration values of non-treated local controls (including trial specific controls), defined as the mean of these values plus 2 standard deviations, or b) using a two-normal compartmental mixture model, using all values from the control and treated samples, in which the mean of the lower normally distributed population of values plus 2 SD defines the threshold for positivity.

- TB31F antibody density will be plotted over time per individual and either as a mean or geometric mean per group. Mixed effects models may be used to investigate the interaction of timepoints and group on TB31F antibody concentration, allowing for participant specific random intercepts to control for the correlation within individuals.
- TB31F pharmacokinetics will be analyzed using standard non-compartmental methods to establish the maximum concentration (C_{max}), terminal half-life ($t_{1/2}$), area under the curve (AUC 0-∞) and Volume of Distribution (V_d) for each participant who received mAb TB31F. Additionally, the absorption rate constant (Ka) will be determined. Geometric mean and geometric coefficients of variation of $T_{1/2}$ and V_d, as well as of dose-adjusted C_{max} and AUC will be reported per dose group and overall.

Efficacy outcome measures

Efficacy outcome measures related to within-group % reduction in proportion infected mosquitoes:

We will determine averaged within-individual percent reduction in the proportion of mosquitoes infected at days post-treatment (1, 5, 14) compared to baseline (day 0) for each group. This will be assessed through DMFA and DSF and outcomes for these different feeding assays will be presented separately as percentage reduction (with 100% as total reduction of transmission, and negative values as enhanced transmission) with 95% confidence interval. Data will be analysed for all individuals who received the product per protocol and only for those individuals who are infectious at baseline (i.e. infecting at least one mosquito with any number of oocysts). All analyses on within-individual percentage reduction in the proportion of infected mosquitoes will use a mixed effects generalized linear model assuming a binomial error structure and a log-link, using individual mosquito data with day of follow-up and dose of TB31F and their interaction as fixed effects. A random intercept will be added to models to allow for participant-level variation in pre-intervention transmissibility and thus account for the correlation between outcomes for mosquito samples from the same participant (i.e. intra-cluster correlation).

In exploratory analyses, these analyses will be performed following stratification for naturally acquired transmission reducing immunity, as quantified by SMFA at baseline (pre-TB31F administration) samples.

The impact of naturally acquired transmission reducing immunity, assessed in baseline samples by SMFA (TRA>50%; TRA>80%) and by antibody prevalence to known gametocyte antigens Pfs230 and Pfs48/45, on infecting mosquitoes will also be determined. For this, TRA as categorical variable and antibody prevalence (binary variable), will be added as fixed effects to mixed effects logistic regression models on proportion infected mosquitoes at baseline (pre-TB31F administration). These models may be expanded to include human genetic factors (e.g. a fixed effect for HbAS or HbAC carriage as compared to wild-type HbAA).

Efficacy outcome measures related to mosquito infection prevalence (oocyst prevalence):

In this analysis, we do not assess reductions in infection rates but present the average prevalence of infected mosquitoes (percentage of mosquitoes with any oocyst density) at each timepoint for each arm. Data will be presented for DMFA and DSF separately.

If, despite randomization, there are considerable differences in baseline infectivity, we may adjust for baseline infectivity. We will fit a mixed effects logistic regression with fixed factors time and study arm and their interaction and individual-specific random intercepts to estimate proportions (or adjusted proportions) at each timepoint for each arm and compare prevalence estimates between time-points for each arm and between arms for each time-point using odds ratios (and 95% CIs).

In exploratory analyses, these analyses will be performed following stratification for naturally acquired transmission reducing immunity, as quantified by SMFA at baseline (pre-TB31F administration) samples.

The impact of naturally acquired transmission reducing immunity, assessed in baseline samples by SMFA (TRA>50%; TRA>80%) and by antibody prevalence to known gametocyte antigens Pfs230 and Pfs48/45, on oocyst prevalence will also be determined. For this, TRA as categorical variable and antibody prevalence (binary variable), will be added as fixed effects to mixed effects logistic regression models on mosquito infection prevalence at baseline (pre-TB31F administration). These models may be expanded to include human genetic factors (e.g. a fixed effect for HbAS or HbAC carriage as compared to wild-type HbAA).

Efficacy outcome measures related to participant infectivity:

The prevalence of infectiousness (i.e. the proportion of individuals infecting at least one mosquito) will be presented per arm and timepoint and presented for DMFA and DSF separately. We will fit a logistic regression with fixed factors time and study arm and their interaction to estimate proportions (or adjusted proportions if baseline proportions differ quite significantly between arms) at each timepoint for each arm and compare prevalence estimates between time-points for each arm and between arms for each time-point using odds ratios (and 95% CIs).
Similar to the paragraph on oocyst prevalence, naturally acquired transmission reducing immunity and human genetic factors may be added as fixed effect in logistic regression models on participant infectivity as binary dependent variable.

Efficacy outcome measures related to mosquito infection intensity (oocyst density):

In this analysis, we do not assess reductions in infection intensity but present the average oocyst density at each timepoint for each arm. Data will be presented for DMFA and DSF separately.
We will fit a mixed effects negative binomial regression model with fixed factors time and study arm and their interaction and individual-specific random intercepts to estimate average oocyst densities at each timepoint for each arm and compare prevalence estimates between time-points for each arm and between arms for each time-point using oocyst density ratios (and 95% CIs).

Efficacy outcome measures related to transmission reducing activity (reductions in oocyst intensity):

We will determine averaged within-individual percent reduction in the density of oocysts at days post-treatment (1, 5, 14) compared to baseline (day 0) for each group. This will be assessed through DMFA, DSF and SMFA and outcomes for these different feeding assays will be presented separately as percentage reduction (with 100% as total reduction of transmission, and negative values as enhanced transmission) with 95% confidence interval. For DMFA and DSF, these data will be analysed for all individuals who received the product per protocol without restrictions to baseline infectivity, and for those individuals who are infectious at baseline (i.e. infecting at least one mosquito with any number of oocysts). For SMFA, these data will be analysed for all individuals who received the product per protocol without restrictions to baseline infectivity in DMFA or DSF, as well as for the subset of individuals who are infectious at baseline. SMFA, an exploratory outcome, will be conducted after unblinding to allow testing of a realistic sub selection of test and control samples. Participants may have naturally acquired TRA due to exposure to gametocytes prior to enrollment; SMFA on baseline samples will allow us to identify these individuals. Additional samples that are most informative for determining the IC80 will be selected based on the ELISA data and tested in the SMFA. For this, one person not involved in data analysis will be unblinded and select samples based on ELISA values and treatment arm.
All analyses on within-individual percentage reduction in oocyst density will use a mixed effects negative binomial regression model with day of follow-up and dose of TB31F as fixed effects. A random intercept will be added to models to allow for participant-level variations (i.e. intra-cluster correlation).

Outcome measures related to the correlation between transmission assays:

The association between DMFA and DSF will be analysed across common timepoints for the two outcomes: (1) whether or not an individual is infectious and (2) the proportion of infected mosquitoes. A mixed effects logistic regression with logit-link will be used to express the association for (1) using the risk ratio. For (2) a mixed effects linear model will be used and the association will be expressed in terms of the slope. We use individual-specific random intercepts in both models to account for the within-individual correlation.

Outcome measures related to estimated inhibitory concentrations:

Transmission blocking activity (TBA, the reduction in the proportion of infected mosquitoes) and transmission reducing activity (TRA, the reduction in oocyst numbers/infection intensity) will be determined for DMFA and DSF separately. Baseline infection prevalence and intensity estimates will be used to calculate relative reductions in these transmission measures during follow-up. For SMFA experiments, TRA will be quantified as the relative reduction in oocyst intensity for test samples (one feeder per test sample) compared to pooled naïve serum controls (two feeders). Samples may be tested in one or two independent SMFA experiments.

TRA values for each participant and time-point will be estimated using mixed effect negative binomial regression models with random intercepts for each experiment to account for between experiments differences in oocyst intensity that is achieved in control experiments (SMFA) or baseline measurements (DMFA/DSF).

TBA values for each participant and time-point will be estimated using mixed effect logistic regression models with random intercepts for each experiment to account for between experiments differences in oocyst prevalence that is achieved in control experiments (SMFA) or baseline measurements (DMFA/DSF). Of note: for SMFA TBA estimates are deemed to be less informative than TRA estimates due to the design of these experiments that aim for high oocyst prevalence and density in control experiments.

TRA and TBA estimates will be correlated with TB31F antibody measures to obtain the best possible fit for DMFA, DSF and SMFA separately. This will allow us to estimate IC50 and IC80 values for TB31F for each assay separately and model TRA and TBA estimates based on antibody concentration. TRA/TBA modeling is relevant because the precision of these estimates decreases with decreasing activity.[7,8] Samples with lower levels of TRA (typically below 80%) and TBA may thus have relatively imprecise estimates.[8] For some analyses, we may thus use TRA or TBA estimates that are inferred from antibody concentration based on the best fit model for the association between antibody concentration and TRA or TBA. For this fit, will use mixed effects models, similar to methods described by Miura et al.[9] and Ramjith et al, adjusting TB31F antibody concentrations measured in serum to reflect whole blood concentrations. These models will estimate Inhibitory Concentration values (e.g. IC_{80}, IC_{50}) based on the best fit.

TB31F concentration in serum at which 80% TRA is expected (IC80) is calculated through (mixed-effects) linear regression by regressing the square root of serum concentration on the log-mean oocyst ratio, i.e. $IC80 = \left(\frac{\left(\log\left(\frac{100}{100-80}\right)-\hat{\beta}_0\right)}{\hat{\beta}_1}\right)^2$ where $\hat{\beta}_0$ and $\hat{\beta}_1$ are the estimated regression coefficients for the intercept and slope of the linear model respectively. The delta method will be used to estimate the standard error for the IC80 value and thus a 95% confidence intervals for the IC80 can be calculated. To compare assays (SMFA/DMFA/DSF) we can include a categorical variable in the model as an interaction variable to allow different slopes ($\hat{\beta}_1$) to be estimated for the different assays.

Thereafter comparisons can be drawn for the IC80 values between assays. A similar calculation is made for IC50 values.

Modeled TRA and TBA estimates (based on antibody concentration) will be plotted against observed estimates and may be used to determine the duration of TB31F activity at time-points when no feeding assays were performed. This will be achieved by analysing pharmacokinetic and pharmacodynamic data by means of non-linear mixed effects modeling. We will fit single and multicompartmental methods to the obtained pharmacokinetic data and investigate both linear and non-linear elimination and disposition. Thereafter, we will investigate whether the pharmacokinetics relate with TBA/TRA. The developed pharmacokinetic-pharmacodynamic model will be used for *in silico* exploration of:

- TB31F dosing regimens needed to achieve at least 80% TRA during an epidemiological relevant time frame (e.g. a typical transmission season of three months)
- the effect of monoclonal antibody engineering, resulting in a longer circulation half-life due to a more favorable pH-dependent binding to human neonatal receptor, on the dose needed to achieve at least 80% TRA during an epidemiological relevant time frame (e.g. a typical transmission season of three months)

We may calculate the percentage of individuals per group that have at least 50%, 80% and 90% TRA per timepoint and per study arm and the percentage of individuals with statistically significant TRA at each timepoint. Mean TRA levels may also be calculated per study group per timepoint and compared between groups.

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