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Phase 1 study to evaluate the safety, tolerability, and pharmacokinetics and pharmacodynamics of monoclonal antibody TB31F in healthy malaria-naive adults in the Netherlands

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
CMV	Cytomegalovirus
CL	Drug clearance
ELISA	Enzyme-Linked Immuno Sorbent Assay
GMT	Geometric mean titers
HIV	Human immunodeficiency virus
mAB	Monoclonal Antibody
PD	Pharmacodynamics
PK	Pharmacokinetics
SAE	Serious adverse event
SAP	Statistical analysis plan
SMC	Safety Monitoring Committee
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 of this trial. 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 standard membrane feeding assay (SMFA) data of participants of the trial.

Introduction

TB31F is a first-in-human phase I, open-label, single-site, dose-escalation study to determine the safety, tolerability and pharmacokinetics and pharmacokinetics of the monoclonal antibody (mAb) TB31F. MAb TB31F will be administered intravenously (i.v.) (groups 1-4) or subcutaneously (group 5) as a single dose to 25 healthy adults, 5 groups of 5 volunteers. The study participants are followed for a 12-week period after infusion; samples are collected following a scheme defined in the study protocol.

Method

Study design

TB31F is a first in human safety trial in healthy volunteers, designed to identify safety concerns associated with TB31F administration at different dosages. A total of twenty-five (n=25) healthy adults (males and females) aged 18 – 35 will be divided over five groups. Each group will include five subjects. Each group will receive a single dose of mAb TB31F administered by intravenous or subcutaneous infusion. Group 1 will receive 0.1 mg/kg TB31F i.v., Group 2 will receive 1 mg/kg TB31F i.v., group 3 will receive 3 mg/kg TB31F i.v., group 4 will receive 10 mg/kg mAb TB31F i.v. and group 5 will receive 100mg mAb TB31F subcutaneously. A step-wise approach will be taken.

Randomization

Given the dose-escalating nature of the trial, volunteers will not be randomized but can choose in which group they wish to participate. Samples will be analyzed as they become available without any pre-defined order.

Sample size

Sample size calculations are based on assessing safety parameters. A total of twenty-five healthy adults (males and females) aged 18 – 35 will be divided over four groups. Each group will include five subjects. With the study's original sample size of n=20 (four intravenous groups of n=5), there is a 90% chance of observing at least 1 event if the true rate of such an event is 10.87% or more; and there is a 90% chance of observing no events if the true rate is 0.52% or less. The 5^{th} (subcutaneous) group was pragmatically chosen to have the same size. Sample size for each group is appropriate for mAb infusion phase 1 trials based on similar trial designs for HIV, CMV, and anthrax monoclonal antibody prophylactic studies. Comparisons of the occurrence of adverse events and efficacy endpoints (e.g. antibody prevalence, density and transmission reducing activity) between study arms will be performed but the study is not specifically powered for this. Instead, TB31F pharmacokinetic parameters will be assessed by standard non-compartmental methods, using multiple observations per group and per study participant, and related to functional transmission reducing activity estimates (TRA).

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 summary report of adverse events (AEs) will be provided to the SMC after all five groups have completed 84 days follow-up.

Statistical Software

- Safety data analysis will be performed in IBM SPSS (latest version available), or R.
- SMFA data analysis will be performed in R.
- mAb TB31F pharmacokinetics will be analyzed using standard non-compartmental methods using the Phoenix WinNonlin software package.
- The relationship between plasma mAb TB31F concentrations and transmission reducing activity in SMFA will be assessed using standard compartmental pharmacokinetic models and empirical and mechanistic pharmacokinetic-pharmacodynamic models using the non-linear mixed effects modelling software package NONMEM 7.4, using Piraña 2.9 as an interface and R for data processing.

Statistical Principles

Confidence intervals and P values

Confidence intervals and P values are specified in protocol section 10 and will be based on 0.05 as significance level. Adjustments for multiple comparisons will be implemented where appropriate.
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.

Adherence and protocol deviations

Accounting for missing, unused and spurious data is described in protocol section 10.5. Briefly,
missing data will not be imputed due to the small number of volunteers in each group. Subjects who miss an appointment date will not be removed from the study. Rather, their appointment and laboratory values will be recorded by appropriate missing value notation in the clinical database. Non-analyzable data will be documented in the deviations. A best-worst case sensitivity analysis may be performed.

Study Outcome Measures

Outcome Definitions

(Protocol section 2.3).

Safety outcome measures:

1. Number and severity of solicited local adverse events from the moment of first product administration through day 7
2. Number and severity of solicited general AEs from the moment of first product administration through day 28
3. Number and severity of clinically significant hematological and biochemical laboratory abnormalities from the moment of first product administration through day 28
4. Number and severity of unsolicited adverse events from first product administration through end of study
5. Number of serious adverse events from first product administration through end of study

Efficacy outcome measures:

6. TB31F mAb concentration in serum at different time-points post administration
7. Pharmacokinetic parameters of the TB31F mAb determined by non-compartmental analysis (e.g. maximum concentration (C_{max}), terminal half-life ($t_{1/2}$), area under the concentration versus time curve (AUC), volume of distribution (V_d) and the absorption rate constant (Ka) when TB31F is administered subcutaneously)
8. Transmission reducing activity (TRA) of participant serum at different time-points post administration, quantified as percentage reduction of oocyst densities in the presence of test serum as compared to control serum in standard membrane feeding assays (SMFA)
9. Transmission blocking activity (TBA) of participant serum at different time-points post administration, quantified as percentage reduction in oocyst prevalence in the presence of test serum as compared to control serum in standard membrane feeding assays (SMFA)

Other outcome measures:

10. Anti-drug antibody (ADA) levels in study subjects at different time-points post administration

Analysis Methods

Trial population

The primary safety and reactogenicity data will include all subjects 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 to for each study group. Eligibility criteria are specified in protocol section 4.2 and section 4.3.

Withdrawal and follow-up

- Withdrawal of individual subjects is described in protocol section 8.4. 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 and proportions.

Analyses in relation to endpoints

Safety outcome analyses:

- For each solicited (local or general) adverse event type, the number and proportion of subjects 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 subjects experiencing any solicited local, any solicited general and any solicited AE (see example table below). Where applicable, we will compare these proportions between groups. Relatedness (possibly, probably or definitely) of solicited AEs to TB31F administration may also be presented. (Outcome measures 1 and 2).

- For each unsolicited adverse event type (categorized by ICD-10), the number of events per dose group and overall study population will be described categorized by severity grade and relation to TB31F administration. (Outcome measure 4).

- The proportion of individuals with grade 1, 2 and 3 adverse events may be compared between groups (Outcome measures 1,2,4) .

- 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 (Outcome 5).

- Withdrawals due to AEs/SAEs will be summarized per group.

Table - Descriptive statistics for subjects experiencing solicited AEs

		Group 1		Group 2		Group 3		Group 4		Group 5		All	
		#Subjects	%	#Subjects	%	#Subjects	%	#Subjects	%	#Subjects	%	#Subjects	%
Local AE1	Grade 1												
	Grade 2												
	Grade 3												
	Any grade												
Local AE2	Grade 1												
	Grade 2												
	…												
	….												
Any local AE*	Grade 1												
	Grade 2												
	Grade 3												
	Any grade												
Systemic AE1	Grade 1												

	Grade 2												
	…												
Any general AE*	Grade 1												
	Grade 2												
	…												
Any AE*	Grade 1												
	Grade 2												
	…												

* List (only) highest grade AE per subject

Clinical Laboratory Data Analysis

- All clinical laboratory abnormalities will be analyzed by participant and will include details of onset time, duration, severity and relationship to the study infusion dose (outcome measure 3).

- Any clinically significant deviations in routine laboratory test results, as determined by the investigator, will be summarized per group (outcome measure 3).

- Isolated laboratory abnormalities will be reported as unsolicited AEs if they are considered clinically relevant by the investigator.

Efficacy outcome analyses:

- TB31F antibody prevalence and density will be determined by ELISA; the mean of duplicate concentration estimates is used for analyses. Antibody density will be expressed in µg/mL (outcome measure 6).

- 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 subject specific random intercepts to control for the correlation within individuals (outcome measure 6).

- 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 subject who received mAb TB31F. Additionally, the absorption rate constant (Ka) will be determined for group 5 (subcutaneous administration). 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 (outcome measure 7).

- 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 generalized linear models, as previously described[1] (outcome measure 8).

- We may calculate the percentage of individuals per group that have at least 50%, 80% and 90% TRA per timepoint and the percentage of individuals per group with statistically significant TRA at each timepoint. Mean TRA levels may also be calculated per study group per time point and compared between groups (outcome measure 8).

- The precision of TRA estimates by SMFA decreases with decreasing TRA[1,2]. Samples with lower levels of TRA (typically below 80%) may thus have relatively imprecise TRA estimates [2]. 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. For this fit, will use mixed effects models, similar to methods described by Miura et al. [3], adjusting TB31F antibody concentrations measured in serum to reflect whole blood concentrations. These models will be used to find the best fit model for the association between TB31F antibody concentration and TRA and to estimate Inhibitory Concentration values (e.g. IC_{80}, IC_{50}) (outcome measures 7, 8).

Modeled TRA estimates (based on antibody concentration) will be plotted against observed TRA estimates (outcome measure 8).

- TBA will be quantified as the reduction in oocyst prevalence in the presence of test serum as compared to control serum[1] and presented per group and time-point (outcome measure 9).

- The obtained pharmacokinetic and pharmacodynamic data will be further analyzed integrally by means of non-linear mixed effects modeling (outcome measure 7, 8). Using an exploratory approach, 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 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)
    - the effect of subcutaneous administration 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)

Other outcome analyses:

- ADA will be measured in terms of seroprevalence and geometric mean titers (GMTs) with 95% CI per time-point and summarized by group and in the overall study population. ADA prevalence and densities will be compared between groups (outcome measure 10).

- We will explore whether mAb concentration is associated with ADA seropositivity and concentration. ADA results may be incorporated into the PK/PD model to test whether it is a relevant predictor of pharmacokinetic and/or pharmacodynamic parameters.

References:

1. Churcher TS, Blagborough AM, Delves M, et al. Measuring the blockade of malaria transmission--an analysis of the Standard Membrane Feeding Assay. *International journal for parasitology* 2012; **42**(11): 1037-44.
2. Miura K, Stone WJ, Koolen KM, et al. An inter-laboratory comparison of standard membrane-feeding assays for evaluation of malaria transmission-blocking vaccines. *Malar J* 2016; **15**: 463.
3. Miura K, Deng B, Tullo G, et al. Qualification of standard membrane-feeding assay with Plasmodium falciparum malaria and potential improvements for future assays. *PLoS One* 2013; **8**(3): e57909.