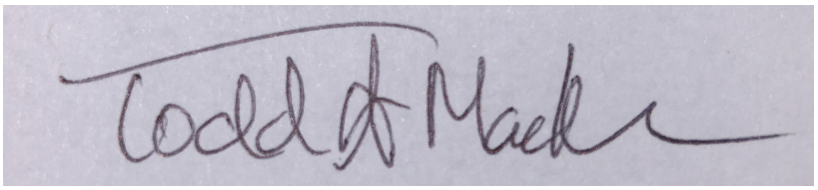
DAR-PIAT

STATISTICAL ANALYSIS PLAN

Study Title	A Phase 2, Randomized, Placebo-Controlled, Double-Blind, Study of the Prevention of Infection with Mycobacterium tuberculosis Among Adolescents Who Have Previously Received BCG (DAR-PIAT)
Investigational Drug:	DAR-901
IND Number:	15838
Sponsor:	Charles Fordham von Reyn MD Geisel School of Medicine
Date:	25 November, 2019
Version:	3.0 updated - added - randomization check (3.2) - clarification - study populations (4.0) - clarification – no interim efficacy analysis (6.0) - clarification - classification by IGRA results (7.5) - clarification – efficacy analyses (7.6) - clarification – safety analyses (7.7) - clarification – repeated lab values will be analyzed (7.7.2) - clarification – vital signs listed, not compared (7.7.3)

SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in DAR-PIAT.



18 Nov 2019

Todd MacKenzie PhD, Trial Statistician

Signature *Date*

C. Fordham von Reyn, MD, Sponsor, DAR-PIAT

18 Nov 2019

Signature *Date*

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1. INTRODUCTION

DAR-PIAT is a randomized, controlled trial of the DAR-901 vaccine booster for the prevention of tuberculosis infection among healthy adolescents in Tanzania. The statistical analysis plan (SAP) is outlined in the DAR-PIAT study protocol. Additional detail and corrections are provided in the SAP.

2. STUDY OBJECTIVES

Primary objective:

- To determine the safety and efficacy of a 3 dose series of DAR-901 for the prevention of infection with M. tuberculosis (TB) among healthy adolescents in Tanzania previously immunized with BCG.

Secondary objectives:

- To identify risk factors for infection with TB among adolescents in Tanzania.
- To identify subject characteristics associated with vaccine induced protection against infection with TB.

3. STUDY SUMMARY AND DESIGN

This is a Phase II, 3-injection, randomized, controlled trial of DAR-901 to be conducted in 13-15 year old adolescents in Tanzania previously immunized with BCG.

All subjects will be screened by the T-spot® IGRA (Oxford Immunotec, Oxford, England) for evidence of TB infection. All screened subjects will have a structured interview to identify risk factors for TB infection (=positive IGRA). IGRA-positive subjects will be referred for further evaluation and will not be entered in the immunization phase of the trial.

A total of 650 IGRA-negative adolescents will be enrolled in the immunization phase of the trial. Subjects will be randomized 1:1 (block size = 4) to intradermal injections of DAR-901 or saline control at 0, 2 and 4 months. IGRA testing will be repeated before dose 2, at 14 months, and again at 24 and 36 months. Subjects who are IGRA negative at baseline and at 2 months will comprise the efficacy cohort. Subjects who are IGRA negative at baseline but are IGRA positive, borderline or invalid at 2 months will not be included in the efficacy cohort.

3.1 Sample Size

Our data from Tanzania indicate that rates of TB infection defined by a positive tuberculin skin test increase from 0% at birth to approximately 70% in adulthood. Data from South Africa using an IGRA to define TB infection indicate that approximately 30% of 12-14 year olds already have TB infection, and that 7-14% develop new TB infection each year. Since adult rates of TB infection as measured by tuberculin skin test surveys are similar in Tanzania and South Africa, we estimate conservatively that Tanzanian adolescents have an annual rate of new TB infection of 7% per year as defined by conversion of an IGRA from negative to positive. We hypothesize that a DAR-901 booster regimen will be 50% effective in preventing new TB infection among adolescents in Tanzania.

Assuming a 7% annual rate of infection, and 5% loss to follow-up per year, 650 subjects randomized to vaccine or placebo and followed for an average of 1.75 years will provide 80% power to detect vaccine efficacy of 50%.

3.2 Randomization

Subjects will be randomized 1:1 to DAR-901 or placebo. A randomization list with block sizes of 4 will be prepared by the data management contractor (Axiom Real Time Metrics, Toronto, CN). After unblinding the study database will be reviewed for discrepancies between treatment assignments and treatment administration. Any errors found will be managed as detailed below.

4. STUDY POPULATIONS

DAR-PIA is designed to determine if DAR-901 reduces the risk of new infection with M tuberculosis. Infection will be defined as a positive T spot interferon gamma release assay (IGRA). Subjects need to be free of TB infection to be eligible for the vaccine efficacy cohort. Since it is estimated to take 4-8 weeks from TB exposure to conversion of an IGRA test to positive, the study design requires 2 negative IGRA tests separated by 8 weeks to ensure that the subject is not already infected with TB.

Subjects eligible for safety analysis:

- All subjects who receive at least one dose of study vaccine.

Safety analysis sets are defined as follows, regardless of randomization assignment.

- Placebo set: All subjects who received *only* Placebo vaccine
- DAR-901 set: All subjects who received at least one (1) dose of DAR-901

Subjects eligible for efficacy analysis: Subjects who meet the ***both*** of following criteria:

- Received at least one dose of study vaccine, regardless of whether treatment was received as assigned.
- Had negative IGRA tests both at baseline and at approximately eight (8) weeks after the first dose of vaccine (i.e., when Dose 2 is scheduled). Subjects with positive, invalid or borderline IGRA results at *either* timepoints are not eligible for efficacy analysis.

Efficacy analysis populations.

- Subjects will be analyzed as randomized using the two populations defined below .

Intention to Treat (ITT) Population

- All subjects eligible for the efficacy analysis.

Per Protocol (PP) Population

- All subjects who meet ***one*** of the following criteria
  - ITT subjects who received all three doses as assigned
  - Subjects randomized to DAR-901 who received at least two (2) doses of DAR-901. Subjects who received only a single dose of DAR-901 will be excluded from the PP population, regardless of whether the remaining scheduled doses were missed or were administered as Placebo (in error)
  - Subjects randomized to Placebo who received at least two (2) doses of Placebo *and* only Placebo (i.e., no DAR-901). Subjects who received only one dose of Placebo and subjects who received even a single dose of DAR-901 will be excluded from the PP set.

5. DEFINITIONS

Age: the difference in years between the informed consent date and the birth date.

Partial dates: will be treated as missing, unless specified elsewhere in the SAP for specific endpoints.

Treatment-Emergent Adverse Events: those events with an onset after the administration of the first dose of study drug.

Treated subjects: those who receive at least one dose of vaccine.

6. CONFIRMATION OF ADEQUATE POWER

The rate of primary endpoints at 24 months was lower than expected. Consequently follow-up was extended to 36 months in Protocol 1.4. No efficacy analyses were performed.

7. STATISTICAL METHODS

Study data are entered in the proprietary Fusion database of Axiom Real Time Metrics (Toronto, CN). All data processing, summarization, and analyses will be performed using SAS® Version 9.4 or higher.

All tables will be displayed by treatment assignment. Tables and listings will be further broken down as deemed necessary.

Descriptive statistics will include the number of subjects (n), mean, standard deviation, median, minimum and maximum. Categorical variables will be summarized using absolute and relative frequencies (i.e. counts and percentages).

7.1 Subject Disposition

The number and percentage of subjects randomized and treated in the study will be presented, together with the number and percentage of subjects who withdrew from the study prematurely and a breakdown of the corresponding reasons for withdrawal.

The number and percentage of subjects in each analysis population will be presented by treatment assignment.

7.2 Protocol Deviations

All collected protocol deviations will be listed only.

7.3 Treatments and Concomitant Medications

Number of doses of administered as scheduled and assigned will be summarized and presented in listing. Compliance will be determined based on the number of doses received compared to the number scheduled and will be summarized by treatment group.

Concomitant medications will be listed only.

7.4 Demographic and Baseline Characteristics

All pertinent demographic and baseline assessments will be summarized and listed; medical history details will only be listed.

7.5 Classification based on IGRA results

The efficacy analysis is based on IGRA testing, which (a) will be performed at screening, 2 months, and subsequently as scheduled and (b) will be reported as negative, positive, indeterminate, or invalid.

Testing at Screening: Only subjects who are IGRA negative at screening will be eligible for randomization and initiation of study treatment.

Testing at 2 Months: Treated subjects will be eligible for efficacy analysis if, and only if, they are IGRA negative ***both*** at screening and again at approximately 2 months (visit #2, administration of study dose #2). Subjects who convert from IGRA negative at screening to positive, indeterminate, or invalid at 2 months will be assumed to represent TB infection acquired before administration of study dose #1 and will be excluded from the endpoint analysis.

Testing after 2 months:

- For subjects who are eligible for efficacy analysis as defined above, ***new TB infection*** is defined as conversion to IGRA positive at any time thereafter.
- ***Persistent new TB infection*** is defined as subjects who *both* meet criteria for new TB infection *and* have a second positive IGRA on any sample obtained ≥3 months later.

7.6 Efficacy analyses

The primary endpoint is time to new TB infection using the ITT population, subject to right censoring. The primary test statistic will be a log-rank test comparing the two study arms (intention-to-treat), with $p < 0.05$ defined as significant. An estimate of vaccine efficacy will be calculated as the hazard ratio comparing vaccine to control using Cox's proportional hazards model. Point and interval estimate (95% confidence level) will be reported.

The proportion of subjects converting over time will be calculated using Kaplan-Meier statistic. To account for the interval censoring in the capture of IGRA conversion, we will apply methods for discrete time-to-events.

The secondary endpoint using the ITT population is time to persistent new TB infection as defined above and analyzed using the same methods as detailed for the primary endpoint.

Additional secondary analyses will be performed using the two endpoints defined above applied to the Per Protocol population.

Although the rate of active tuberculosis disease (pulmonary and extra-pulmonary) is expected to be very low, it will be analyzed as an exploratory endpoint. All participants with suspect active TB or treatment for active TB will be categorized as active TB by a panel of 3 experts blinded to treatment assignment. The consensus decision will be recorded on the SAE form.

7.7 Safety Analyses

All subjects in the Safety Population defined above will be followed throughout the trial. Safety endpoints will include adverse events, concomitant medications, clinical observations (e.g. vital signs, physical examination), and complete blood count.

All safety parameters will be summarized for the Safety Population using descriptive statistics.

7.7.1 Adverse Events

Only treatment-emergent adverse events (TEAEs) will be included in summary tables. These will include those produced by system organ class, preferred term and treatment presenting the number and percent of subjects with AEs, the number and percent of subjects with AEs by severity, the number and percent of subjects with AEs by relationship to treatment assignment, the number and percent of subjects with serious AEs and the number and percent of subjects with AEs that led to discontinuation of participation in the study. Subjects may have more than one

AE per system organ class and preferred term. At each level of subject summarization, a subject will be counted once if they reported 1 or more events coding to the same Preferred Term.

All adverse events (including non-treatment-emergent events) in the study database will be listed.

7.7.2 Laboratory Data

For hemoglobin, white blood count and platelet count (CBC) results, actual values and changes from baseline to treatment-emergent values will be summarized in shift tables using low, normal and high categories (relative to the normal range).

7.7.3 Physical Examinations, Vital Signs, and Other Observations Related to Safety

These data will be listed only.

7.8 Immunology

Blood collected 7 days after dose 3 in Paxgene tubes will be analyzed to compare RNA expression in vaccine versus placebo groups. This is an exploratory analysis.

7.9 Visit Windowing

Study events will be recorded using the calendar date and (where applicable) the time to the nearest minute.

For purposes of post-study analysis (e.g., tables and listings), study days will be designated as follows:

- Day 0 is defined as the calendar day of the first dose of study treatment.
- The days prior to Day 1 are designated Day –1, Day –2, etc;.
- The days following the day of the first dose of study drug are designated Day 1, Day 2, etc.

The times of events related to dosing of study drug will be designated as minutes or hours before or after the time of dosing, which is designated as t = 0 (zero). Thus, 15 minutes prior to dosing is t = –15 min; 2 hour after dosing is designated t = 2 h.

8. VALIDATION

Derived datasets will be independently reprogrammed by a second programmer. The separate datasets produced by the two programmers must match 100%.

Tables will be independently reprogrammed by a second programmer for numeric results. Statisticians will be involved in the process of programming and validating tables that include inferential statistical results.

Figures will be checked for consistency against corresponding tables and listings, or independently reprogrammed if there are no corresponding tables or listings.

Listings will be checked for consistency against corresponding tables, figures, and derived datasets.

9. REFERENCES

Not applicable.