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IDRI-TBVPX-120

Statistical Analysis Plan

Version 2

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INFECTIOUS DISEASE RESEARCH INSTITUTE

A PHASE 1, DOUBLE-BLIND, RANDOMIZED CLINICAL TRIAL TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF THE SINGLE-VIAL LYOPHILIZED ID93 + GLA-SE VACCINE ADMINISTERED INTRAMUSCULARLY IN HEALTHY ADULT SUBJECTS

Protocol IDRI-TBVPX-120

STATISTICAL ANALYSIS PLAN
Version 2.0

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LIST OF ABBREVIATIONS

Abbreviation	Full Wording
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CI	Confidence interval
DMSO	Dimethyl sulfoxide (ICS negative control)
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunosorbent spot assay
ICS	Intracellular cytokine staining
IM	Intramuscular
ITT	Intent-to-treat
MedDRA	Medical dictionary for regulatory activities
MEPT	Mean endpoint titer
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline (ICS negative control)
PID #	Participant identification number
PIMMC	Potential immune-mediated medical condition
SAP	Statistical analysis plan
SFU	Spot forming unit
Study Day	Actual day on study (e.g., Visit Day 84 happened on Study Day 90)
Visit Day	Scheduled visit day (e.g., Day 28, Day 84)
WBA	Whole blood assay

1 INTRODUCTION

Note: The safety analysis has already been performed. This amendment is to the immunology section only.

The purpose and scope of this document is to describe the statistical analysis plan (SAP) that will guide the analysis of the clinical safety, IgG ELISA, ELISpot, and ICS data (primary and secondary study objectives and endpoints) for the final study report for protocol IDRI-TBVPX-120.

The clinical study report will be prepared after all study subjects have completed all study visits, all data in the clinical study database have been reviewed, all data queries have been resolved, the clinical database has been locked, and all immunology laboratory assays have been completed. All individual subject data listings, summary tables, and statistical analyses described below will be provided in separate appendices to the study report. Unless otherwise indicated, all listings and summary tables will be provided by treatment group.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonization (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials[1] and the ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports[2].

2 STUDY OBJECTIVES

The ID93 + GLA-SE vaccine consists of the recombinant four-antigen *Mycobacterium tuberculosis* antigen ID93 in combination with the adjuvant formulation GLA-SE (Glucopyranosyl Lipid A – Stable Emulsion). This phase 1, double-blind, randomized clinical trial will evaluate the safety, tolerability, and immunogenicity of ID93 + GLA-SE in the lyophilized, single-vial presentation. The goal of this study is to evaluate the safety and tolerability of the new lyophilized single-vial presentation ID93 + GLA-SE and how immunological responses elicited by this product compare to ID93 + GLA-SE prepared from the two-vial presentation.

2.1 Primary Objectives

- To evaluate the safety and tolerability of single-vial lyophilized ID93 + GLA-SE following two intramuscular (IM) injections administered on Days 0 and 56.
- To compare the safety and tolerability of single-vial lyophilized ID93 + GLA-SE to the two-vial presentation consisting of lyophilized ID93 and liquid GLA-SE following two IM injections administered on Days 0 and 56.

2.2 Secondary Objectives

- To assess the immunogenicity of single-vial lyophilized ID93 + GLA-SE following two IM injections administered on Days 0 and 56 by quantifying cytokine production and IgG antibody responses to ID93 at specified time points.
- To compare the immunogenicity of single-vial lyophilized ID93 + GLA-SE to the two-vial presentation consisting of lyophilized ID93 and liquid GLA-SE following two IM injections administered on Days 0 and 56.

2.3 Exploratory Objective

- To further evaluate the immunogenicity of ID93 + GLA-SE following two IM injections administered on Days 0 and 56 by measuring mycobacterial growth inhibition, quantifying IgA levels in mucosal secretions, and measuring frequencies of circulating T follicular helper cells, antibody secreting cells, and memory B cells at specified time points.

Note that the scope of this SAP does not include the exploratory objectives.

3 STUDY DESIGN

This is a phase 1, double-blind, randomized clinical trial to evaluate the safety, tolerability, and immunogenicity of single-vial lyophilized ID93 + GLA-SE compared to the two-vial presentation consisting of lyophilized ID93 and liquid GLA-SE administered as two IM injections in healthy adult subjects (aged 18 - 55).

The two treatment groups are outlined in Table 1. Subjects will receive a total of two doses administered IM on Days 0 and 56. Subjects will be monitored for approximately 421 days (one year following the last study injection), including safety laboratory analyses done just prior to and 7 days following each study injection. Tears and nasal swabs will be obtained for exploratory antibody analysis at Days 0, 70, and 224. Blood samples will be obtained for immunological assays (secondary and exploratory) at Days 0, 7, 14, 56, 63, 70, 84, and 224).

Table 1: Treatment Assignments

Treatment Group	N	Treatment Assignment	Timing of Study Injections
1	25	ID93 (2 µg, lyophilized) + GLA-SE (5 µg, liquid) [two-vial presentation]	Days 0 and 56
2	23	ID93 + GLA-SE (2 µg ID93, 5 µg GLA) [lyophilized, single-vial]	Days 0 and 56

4 STUDY POPULATION

This study was conducted at one clinical site (Saint Louis University Center for Vaccine Development, located in St. Louis, Missouri).

Inclusion Criteria:

Subjects must meet ALL of the following criteria to be eligible for inclusion in the study:

1. Males and females 18 to 55 years of age.
2. In good general health as confirmed by a medical history and physical exam, vital signs*, and screening laboratories conducted no more than 30 days prior to study injection administration.

   **Temperature <38°C, respiratory rate < 17 breaths pm, heart rate ≤100 bpm and >54 bpm, systolic blood pressure ≤140 mmHg and >89 mmHg, diastolic blood pressure ≤90 mmHg and ≥60 mmHg. NOTE: Athletically trained subjects with a pulse ≥40 may be enrolled at the discretion of the principal investigator or designated licensed clinical investigator.*

3. Screening laboratory values within normal limits: sodium, potassium, ALT, AST, total bilirubin, alkaline phosphatase, creatinine, random glucose, total WBC count, hemoglobin, and platelet count.
4. Negative HIV 1/2 antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody.
5. Urine dipstick for protein and glucose (negative to trace protein are acceptable).
6. Women of childbearing potential* in sexual relationships with men must agree to practice acceptable contraception** for the 30-day period before Day 0 through 90 days after the last study injection.

   **Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or < 1 year of the last menses if menopausal). Post-menopausal defined as at least 12 months spontaneous amenorrhea and confirmed with FSH > 40 mIU/ml.*

   ***Includes, but is not limited to, sexual abstinence, monogamous relationship with vasectomized partner who has been vasectomized for 6 months or more prior to the subject receiving study product, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing ®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").*
7. Able to understand and comply with planned study procedures and willing to be available for all study-required procedures, visits and calls for the duration of the study.
8. Provide written informed consent before initiation of any study procedures.
9. Willing to abstain from donating whole blood or blood derivatives until 90 days after the final study injection.

Exclusion Criteria:

Subjects who meet any of the following criteria at screening/baseline will be excluded from study participation:

1. Previous exposure to ID93 vaccines or experimental products containing GLA-SE.
2. History of treatment for active or latent tuberculosis infection.
3. History or evidence of active or documented latent tuberculosis, or positive QuantiFERON®-TB Gold test.
4. Shared a residence within the last year prior to randomization with an individual on anti-tuberculosis treatment or with culture or smear positive tuberculosis.
5. Received a tuberculin skin test within 3 months (90 days) prior to randomization.
6. History of autoimmune disease or immunosuppression.
7. Used immunosuppressive medication (e.g., oral or injected steroids) within 3 months prior to randomization (inhaled and topical corticosteroids are permitted).
8. Received any investigational drug therapy or investigational vaccine within past 6 months prior to randomization, or planned participation in any other investigational study during the study period.
9. Received investigational TB vaccine at any time prior to randomization.
10. Received any vaccine within 30 days prior to the first study vaccination and no planned immunizations between Day 0-84 or Day 210-224 due to the washout period prior to immunology blood draws.
11. History or laboratory evidence of immunodeficiency state including but not limited to laboratory indication of HIV-1 infection at screening.
12. History of allergic disease or reactions, likely to be exacerbated by any component of the study vaccine.
13. History of allergic reaction to kanamycin-related antibiotics.

14. Subjects with a history of previous anaphylaxis or severe allergic reaction to vaccines or unknown allergens.
15. Previous medical history that may compromise the safety of the subject in the study, including but not limited to: severe impairment of pulmonary function from tuberculosis infection or other pulmonary disease; chronic illness with signs of cardiac or renal failure; suspected progressive neurological disease; or uncontrolled epilepsy or infantile spasms.
16. Known or suspected alcohol or drug abuse within the past 5 years.
17. Smokes 1 pack or more of cigarettes per day.
18. History of keloid formation or excessive scarring.
19. History or evidence on physical examination of any systemic disease or any acute or chronic illness that, in the opinion of the investigator, may interfere with the evaluation of the safety or immunogenicity of the vaccine, including axillary lymphadenopathy.
20. Received a blood transfusion or immunoglobulin within the past 3 months prior to randomization.
21. Donated blood products (platelets, whole blood, plasma, etc.) within past 1 month prior to randomization.
22. Presence of any febrile illness, oral temperature of >100.4 °F/38.0 °C within 24 hours of study injection administration. Such subjects may be re-evaluated for enrolment after resolution of illness.
23. Positive serum (at screening visit only) or urine pregnancy test at screening or within 24 hours prior to study injection for women of childbearing potential.
24. Breastfeeding at any time throughout the study.
25. Rash, tattoos, or any other dermatological condition on the upper anterolateral arm that could adversely affect the vaccine injection site or interfere with its evaluation.
26. BMI <18 or >35 kg/m2.
27. Any medical or neuropsychiatric condition which, in the Investigator's opinion, would render the subject incompetent to provide informed consent or unable to provide valid safety observations and reporting.
28. Cancer or treatment for cancer within 3 years of study injection administration. Persons with a history of cancer who are disease-free without treatment for 3 years or more are eligible. Persons with treated and uncomplicated basal cell carcinoma of the skin are eligible.
29. Subjects unlikely to cooperate with the requirements of the study protocol.

5 DEFINITIONS

The following definitions apply to the summary tables and statistical analyses planned for the study report:

Intent-to-treat (ITT) population: all subjects randomized to one of the treatment groups, classified according to the randomization assignment.

Safety population: all subjects who have received at least one dose of study injection and for whom any data on safety are available. Subjects will be classified according to the treatment received. The primary safety analysis will be done on this population.

Per-protocol (PP) population: all eligible subjects randomized to one of the treatment groups and who received both assigned study injections. Subjects will be classified according to the treatment received. Analysis of immunogenicity endpoints occurring after the second study injection will be performed on this population.

Immunogenicity population: all eligible subjects who have received at least one study injection, for whom data concerning post-baseline immunogenicity endpoint measures are available, and major protocol deviations are absent. The post-baseline immunogenicity endpoints may include humoral or cellular responses measured at the protocol-specified time points, or at the time of early termination.

Subjects will be classified according to the treatment received. Immunogenicity analysis will be done using this population or the Per Protocol Population, as appropriate

Baseline value: Most recent value recorded prior to the first study injection.

Treatment group: One of the two treatment assignments.

Visit Day: The scheduled Visit Day, for example Day 84.

Study Day: The day on study that a visit actually took place (date of visit minus date of enrollment, Day 0). For example, an adverse event onset might be on study day 5; or a scheduled Visit Day 84 evaluation could take place on study day 89.

Immunology laboratory assays/data/results: IgG antibody and cytokine assays/data/results.

6 DATA MANAGEMENT CONSIDERATIONS

Individual subject data will be collected on electronic case report forms (eCRFs) and stored in a proprietary database maintained by DF/Net. All reported adverse events will be coded to standard preferred terms and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA version 21.1 dictionary) terminology.

Immunologic assay data will be stored in a secure location by IDRI. Prior to analysis, the immunologic assay data will be transferred to the Biostatistician (or designee), who will convert it to a SAS dataset.

7 STATISTICAL CONSIDERATIONS

7.1 Determination of Sample Size

This study is a phase 1, randomized, double-blind clinical trial designed to evaluate the safety, tolerability, and immunogenicity of the single-vial and two-vial presentations of ID93 + GLA-SE. As such, the sample size of n=48 was determined by what is reasonable for a phase 1 trial and not based on statistical considerations of power, and will allow only preliminary safety and immunogenicity information relevant to progression to larger trials.

7.2 Plan for Statistical Summaries and Analyses

7.2.1 General Considerations

Statistical summaries and analyses of baseline and safety data will be provided for the safety population, defined as all subjects who receive at least one study injection. Summaries and analyses of immune response data will be provided for the per-protocol population. The objectives of the immune response statistical summaries and analyses are to assess the immunogenicity following intramuscular administration of study vaccine among the treatment groups. The goals are:

1. To describe the humoral and cellular response to both vaccine presentations in terms of magnitude and positive response rate
2. To determine if there is a significant difference in the immune response elicited by the two-vial and the single-vial lyophilized vaccine presentation

All individual subject data listings will be provided in appendices to the clinical study report. Data will be summarized and tabulated by treatment group and descriptive statistics will be provided where

appropriate. The immunology analysis will be performed using either SAS version 9.3 or higher or R version 3.5 or higher.

Categorical data will be analyzed using Fisher's exact test using 0.05 level of significance ($\alpha = 0.05$) with Holm-Bonferroni adjustments whenever pairwise comparisons are made between more than two categories.

Continuous data will be compared between two treatment groups using a Two-sample T-test if the normality assumption is met or Wilcoxon Rank-Sum Test, otherwise. Response rates will be compared using a Fisher's Exact test. Significant differences will be detected based on a two-sided test with $p \leq 0.05$ ($\alpha = 0.05$). Comparisons will be accompanied by the point-estimate and 95% CI for each group.

Missing data: The analysis plan assumes that missing data are missing completely at random (MCAR) and analysis will be performed on the available data.

Individual subject data listings will be provided as separate appendices in the CSR. All summaries, statistical analyses, and individual subject data listings will be analyzed by using either SAS version 9.3 or higher or R version 3.5 or higher.

7.2.2 Safety Assessments

The summaries and statistical analyses of the safety endpoints will be completed for the safety population. Safety endpoints include solicited adverse events, unsolicited adverse events, changes in laboratory values, and changes in vital signs. All solicited and unsolicited adverse events will be recorded by the Investigator in the AE eCRF, and all adverse event terms will be coded using MedDRA preferred term and system organ class.

7.2.2.1 Solicited Adverse Events

The severity rating (Grade 1, 2, or 3) assigned to each solicited adverse event will be summarized by the 7-day injection interval following each injection and by treatment group. The worst value (i.e., maximum severity) for each sign/symptom will also be summarized by injection interval and treatment group.

7.2.2.2 Unsolicited Adverse Events

All unsolicited adverse event terms will be coded using MedDRA. Summaries of the number (%) of subjects in each treatment group with at least one adverse event, classified according to MedDRA preferred term and system organ class, will be provided for:

- All adverse events
- Related adverse events
- Serious adverse events
- Potential immune-mediated medical conditions (PIMMCs)

Related adverse events are defined as adverse events that are possibly, probably, or definitely related to study injection, as assessed by the study Investigator. Fisher's exact tests will be used to compare the treatment groups with respect to the proportion of subjects with at least one adverse event.

The following additional summaries of unsolicited adverse events will also be provided:

- Incidence of adverse events after the first and second injections
- Incidence of adverse events by severity
- Incidence of adverse events by relationship to study injection

7.2.2.3 Reactogenicity Adverse Events

Local injection site reaction adverse events (pain, induration, and erythema) and solicited systemic reaction adverse events (headache, arthralgia, chills, loss of appetite, fever, fatigue, and myalgia) will be recorded at 60 minutes and seven days after each study injection (Days 0, 7, 56, and 63). The seven day period following each study injection is referred to as the 'post-injection period'. Study injection reactions will be classified as occurring during the corresponding 7-day post-injection period (Post Injection Period Day 0-7, and 56-63) if the onset date is on or after the date and time of injection and before the seventh day after injection.

Summaries of the number (%) of subjects in each treatment group with at least one reactogenicity adverse event reported in the CRF will be provided for:

- at least one local injection site reaction
- at least one systemic reaction
- each individual event

The distribution of the maximum severity grade for the 7-day post-injection period will be summarized for each adverse event. If a subject experienced an adverse event more than once, the event with the maximum severity grade will be included in this summary.

Primary analysis of reactogenicity adverse events will be conducted in an Intent to Treat manner, that is, subjects will be classified based on the treatment group to which they were assigned. If any subjects incorrectly received an injection of product they were not assigned to receive, secondary analysis will be conducted with subjects classified based on product actually received during the study. Any subject randomized (ITT) but not enrolled (did not receive a study injection) will not be included in any analyses.

Fisher's exact tests will be used to compare the treatment groups with respect to the proportion of subjects with at least one reactogenicity adverse event. Fisher's exact tests will also be used to compare the treatment groups with respect to local site of injection reactions and solicited systemic reactions separately.

Reactogenicity adverse events will be summarized by treatment group for each 7-day post-injection period. The treatment groups will be compared with respect to the number (%) of subjects with at least one reactogenicity adverse event during each 7-day post-injection period using Fisher's exact tests.

The following proportions of adverse events will be compared across treatment groups:

- Proportion of subjects with at least one local site of injection reaction
- Proportion of subjects with at least one systemic reaction adverse event
- Proportion of subjects with at least one local site of injection reaction in each post-injection period (Post Injection Periods Day 0-7 and 56-63)
- Proportion of subjects with at least one systemic reaction adverse event in each post-injection period (Post Injection Periods Day 0-7 and 56-63)

7.2.2.4 Incidence of Related Grade 3 AEs

After each study injection the Investigator or designee will record toxicity grade on the Adverse Event CRF.

The number (%) of subjects in each treatment group who experience at least one Grade 3 AE judged related to study injection during the study will be summarized. Brief narratives will be provided in the study report for each subject experiencing at least one Grade 3 AE.

7.2.2.5 Laboratory Data

Summaries of the actual value and change from baseline for all laboratory tests will be provided for each study visit. A tabulated summary of laboratory abnormalities by toxicity grade will also be provided.

7.2.2.6 Vital Signs

Summaries of the actual value and change from baseline for all vital signs will be provided for each study visit.

7.2.3 Immune Response

The following are the endpoints of interest:

Primary Analysis Endpoints:

- IgG antibody responses to ID93 by ELISA at Days 0, 14, 56, 70, 84, and 224
    - Mean endpoint titer of ID93-specific antibodies for total IgG on for each treatment group.
    - The proportion of subjects with at least a 4-fold increase in ID93-specific IgG antibody levels on Days 14, 56, 70, 84, and 224 relative to baseline (Day 0).
    - Mean fold change from baseline (Day 0) in IgG antibody responses to ID93.
- T cell responses to ID93 by PBMC ICS on Days 0, 7, 14, 56, 63, 70, 84, and 224
    - The proportion of cells expressing any two of IFN-γ, TNF, IL-2, IL-4, IL-21, or CD154 in response to ID93.
    - The polyfunctionality based on expression of IFN-γ, TNF, IL-2, IL-4, IL-21, or CD154.
- T cell responses to ID93 by ELISpot on Days 0, 14, 56, 70, 84 and 224
    - The number of IFN-γ cytokine-secreting cells in PBMC samples in response to the ID93 antigen relative to baseline (Day 0) at Days 14, 56, 70, 84, and 224, as assayed by ELISpot.

Secondary and Exploratory Analysis Endpoints:

- IgG subclass antibody responses to ID93 by ELISA at Days 0, 14, 56, 70, 84, and 224

- The proportion of subjects with at least a 4-fold increase in ID93-specific IgG1, IgG2, IgG3, and IgG4 antibody levels on Days 14, 56, 70, 84, and 224 relative to baseline (Day 0).
- Mean endpoint titer of ID93-specific antibodies for IgG1, IgG2, IgG3, and IgG4 on for each treatment group.

Immune responses for each treatment group will be evaluated by calculating the response rate and magnitude of response to ID93 at all timepoints for each immunologic endpoint. Comparisons of response rates for IgG and cytokine responses between treatment groups will be made using Fisher's exact tests.

The magnitude of IgG and cytokine responses will be compared between treatment groups using a Two-sample T-test if normality assumption is met or Wilcoxon Rank-Sum Test, otherwise.

Percentages of CD4+ cells producing selected immune markers will be summarized by time point and compared between treatment groups.

A boxplot with overlaid symbols (one per subject) will be used to summarize the magnitude of an immune response in a single group. Different colors and symbols will be used to indicate responders and non-responders. The boxplot will be used to summarize the responders with a horizontal line for the median, the extents of the box indicating the inter-quartile range (IQR) and whiskers indicating the lowest (highest) data point within 1.5 x IQR within the lower (upper) quartile.

7.2.3.1 IgG Antibody Response to ID93

IgG antibody responses (Total IgG, IgG1, IgG2, IgG3, and IgG4) to the ID93 antigen will be measured by ELISA using serum collected from a given subject. Specimens from all time points for a given subject will be analyzed on a single ELISA plate. The plate will contain standards and controls that will be used to determine plate validity based on criteria established during assay validation.

At each timepoint, the IgG mean endpoint titer (**Total IgG MEPT**) value calculated from duplicates or triplicates will be reported.

At each post-injection timepoint, the following **Post:Pre Ratio** will be calculated:

$$\text{Post:Pre Ratio} = \frac{\text{MEPT (Post-Injection Timepoint)}}{\text{MEPT (Baseline Timepoint)}}$$

A subject will be considered an antibody positive **Responder** at a given timepoint if:

a) System suitability criteria for a given ELISA are met; and
b) The Post:Pre Ratio at that timepoint is ≥ 4.

The following **listing** is planned:

- Humoral Response: IgG ELISA: IgG Antibody Response

- This listing will be organized by treatment group and will include: Subject ID, Visit Day, Collection Date, IgG MEPT, Post:Pre Ratio, Responder (Yes/No)

The following **tables** are planned:

- Humoral Response: IgG ELISA: IgG Antibody Response
  - This is a tabulation by Visit Day of the Total IgG MEPT, Post:Pre Ratio, and Responder status of each treatment group. The table will include descriptive statistics and a statistical comparison of each parameter between the two treatment groups.
- A Narrative Table will be prepared and will include
  - Percent responder status at each time point with p-value of the statistical comparison of the treatment groups

The following **figure** is planned:

- Humoral Response: IgG ELISA: IgG Antibody Response
  - IgG MEPT (log10) by study visit and treatment group.
  - Repeated for each IgG subclass
  - This will be a line plot with error bars representing +/- 95% CI.

7.2.3.2 T-cell Responses to ID93

PBMC samples will be assayed by ICS and ELISpot.

7.2.3.2.1 ICS

PBMC ICS will be performed on Days 0, 7, 14, 56, 63, 70, 84, and 224 to determine the proportion of single and multi-functional CD4 and CD8 T-cells producing various combinations of IFN-γ, TNF, IL-2, IL-4, IL-21, and CD154 in response to ID93 stimulation. A gate is applied for each functional marker, not accounting for co-expression of other markers. Boolean gates are later created based on the gates shown to identify cells expressing various combinations of markers. We will analyze the T cell population “Any Two”, which are positive for at least two immune markers among IFN-γ, TNF, IL-2, IL-4, IL-21, and CD154. We will also evaluate T cells that express selected combinations of the six immune markers. The selected combinations will be indicated in the data set. Any negative background-subtracted values will be set to zero.

The following **listing** is planned to be presented:

- Cellular Response: PBMC ICS: T cell Response
  - Percent immune marker-positive (“Any Two” and for selected combinations of the six), background-subtracted cytokine response, change from baseline, fold change from baseline, and responder status

Tabular summaries will present “Any Two” immune marker-positive and polyfunctional responses (only background-subtracted non-zero values will be presented). T-cell response frequencies will be summarized by treatment regimen. Percentages will be based on the total number of subjects within each treatment group. The following **tables** are planned for presentation:

- Cellular Response: PBMC ICS: T cell Response and Change from Baseline
  - Percent immune marker-positive background-subtracted T cell response (“Any Two” and for selected combinations of the six) and change from Baseline, including N, Median, and 95% CI for each parameter.
- A Narrative Table will be prepared and will include
  - Medians of the “Any Two” CD4 and CD8 T cell responses per treatment group and study visit with p-value of the statistical comparison of the treatment groups

- Responder Rate of the “Any Two” CD4 and CD8 T cell responses per treatment group and study visit with p-value of the statistical comparison of the treatment groups

The following **figures** are planned:

- Cellular Response: PBMC ICS: T Cell Response Magnitude by Study Day
  - Background-subtracted total T cell subject response (“Any two”) by visit and by T cell type.
  - This figure is also to include on separate pages the DMSO values as well to illustrate background for T cell results.
  - This is to be a **box and dot plot** of the background-subtracted with negative values set equal to zero ‘Any two’ with background-subtracted (or background values) values (y-axis) at each visit (x-axis) (study days plotted next to each other on one page). Box plots summarize by treatment group and dots represent individual values. The median values per treatment group are to be presented by means of a horizontal line, boxes outline first to third quartiles, and whiskers represent minimum to maximum values.
  - Different symbols are to be used to differentiate between responders (closed circles), non-responders (cross) and missing responder status (triangle).
- Cellular Response: PBMC ICS: Polyfunctional Profile Plot in CD4 and CD8 T Cells
  - Background-subtracted polyfunctional plot in T cells (selected combinations of IFN-γ, TNF, IL-2, IL-4, IL-21, and CD154) by visit and T cell type.
  - Separate pages for each visit day are to be prepared.
  - This is to be a **dot plot** of the background-subtracted single-gate T cell response (y-axis) versus each selected immune marker combination available (excluding where all included cytokines are negative) (x-axis).
  - The background-subtracted ANY TWO cytokine response in T cells is to be presented as a subplot on the right hand side.

7.2.3.2.2 ELISpot

IFN-γ and IL-10 ELISpot will be performed on Days 0, 14, 56, 70, 84 and 224 to determine the proportion of PBMCs producing IFN-γ or IL-10 in response to stimulation with ID93 or unstimulated (PBS).

The following **listing** is planned to be presented:

- By treatment group, each participant’s ELISpot response will be listed, including: Subject ID, Analyte, Stimulation condition, Visit Day, SFU-Background Scaled Result/<0 = 0 (SFU/10^6 PBMC), Change from Baseline, and Responder Status (Yes/No).

The following **tables** are planned for presentation (repeat each table for IL-10):

- PBS-subtracted antigen-specific IFN-γ ELISpot response (SFU/10^6 PBMC), change from Baseline, and Responder Status
  - This is to include descriptive statistics and 95% CI for the median for each visit and stimulation antigen.
  - Responder status is determined by the SCHARP method described in Section 7.2.2.3.
- A Narrative Table will be prepared and will include
  - Medians of the responses per treatment group and study visit with p-value of the statistical comparison of the treatment groups
  - Responder Rate of the IFN-γ responses per treatment group and study visit with p-value of the statistical comparison of the treatment groups

The following **figure** is planned for presentation (repeat figure for IL-10):

- PBS-subtracted IFN-γ ELISpot subject response (SFU/10^6 PBMC) by visit.
  - This is to be a box and dot plot of the DMSO-subtracted SFU/10^6 PBMC values (y-axis) at each visit (study days plotted next to each other on one page) per stimulation antigen (each stimulation antigen on a separate page, including DMSO). The median values per treatment group are to be presented by means of a horizontal line, boxes outline first to third quartiles, and whiskers represent minimum to maximum values.
  - Different dot symbols are to be used to differentiate between responders and non-responders (e.g., open vs closed symbols).

7.2.3.3 Defining Cut-Offs for Reactivity and Responder Rates

The resultant titer determination from assay of serum IgG is normalized to internal standards that are run on every plate in the low, middle, and high portions of the assay's dynamic range as well as multiple negative control samples. Values from control samples must be within established range for the plate to pass and for a valid endpoint titer determination. A qualification of the total IgG ELISA assay indicated that a Post:Pre Ratio of 4 for the antibody assay (IgG) is an appropriate cut-off value for distinguishing responders and non-responders.

ELISA responder rates are determined as follows. To determine the responder status (per subject and visit): If the calculated fold change (Post:Pre Ratio) is greater than or equal to 4, the subject is to be considered a responder at that specific post-Baseline visit. If the calculated fold change is less than 4, the subject is to be considered a non-responder at that specific post-Baseline visit. If the measured MEPT is below the limit of detection (LOD), the sample is assigned the LOD value.

ELISpot responder rates are determined using the method as follows:

- ELISpot responses per stimulation condition are provided.
- These results are then compared at each visit and stimulation antigen, using the Fisher's EXACT test.
- P-values are then to be adjusted for multiplicity, if necessary
- If both the DMSO and the active antigen values are below 27 SFU/10^6 PBMC, the result is to be regarded as non-responder. If the stimulation value is above 27 and the DMSO value is below 27, the normal procedure should follow to determine the responder status.
- Step 1: Determine whether the number of cells responding to the active antigen stimulation is statistically significantly more than the number of cells responding to DMSO stimulation at Baseline (Study Day 0).
  - One-sided adjusted p-value >= 0.05 indicates that the number of cells responding to the active antigen stimulation is not statistically more than the number of cells responding to the DMSO stimulation.
    - Subject is classified as a non-responder at Baseline (Study Day 0).
  - One-sided adjusted p-value < 0.05 indicates that the number of cells responding to the active antigen stimulation is statistically more than the number of cells responding to the DMSO stimulation.
    - Subject is classified as a responder at Baseline (Study Day 0).
- Step 2: Determine whether the number of cells responding to the active antigen stimulation is statistically significantly more than the number of cells responding to DMSO stimulation at Study Day 14, 56, 70, 84, and 224 respectively.

  - One-sided adjusted p-value >= 0.05 indicates that the number of cells responding to the active antigen stimulation is not statistically more than the number of cells responding to the DMSO stimulation.
    - Subject is classified as a non-responder at the relevant post-Baseline visit.
  - One-sided adjusted p-value < 0.05 indicates that the number of cells responding to the active antigen stimulation is statistically more than the number of cells responding to the DMSO stimulation.
    - Subject is classified as a responder at the relevant post-Baseline visit.

- Determine the responder status post-Baseline when taking Baseline (Study Day 0) into account:

Baseline (Determined in Step 1)	**Each post-Baseline visit (Determined in Step 2)**	**Responder Status**
Non-responder	Non-responder	Non-responder
Non-responder	Responder	Responder
Responder	Non-responder	Non-responder
Responder	Responder	Determine responder status by means of the odds ratio

- Determine the odds ratio for results at Baseline (Study Day 0). If the DMSO positive count is zero, the odds ratio will be undefined. In these cases the positive DMSO count should be imputed to 1 in order to determine an odds ratio.
- Determine the odds ratio for results at post-Baseline visits. If the DMSO positive count is zero, the odds ratio will be undefined. In these cases the positive DMSO count should be imputed to 1 in order to determine an odds ratio.
- Perform the Breslow-Day test:
  - Test for homogeneity of odds ratios at each relevant post-Baseline visit and Baseline (Study Day 0).
  - P-value >= 0.05 indicates that the odds ratio at Baseline (Study Day 0) and each post-Baseline visit do not differ statistically significantly.
    - Subject classified as a non-responder at the relevant post-Baseline visit.
  - P-value < 0.05 indicates that the odds ratio at Baseline (Study Day 0) and each post-Baseline visit differ statistically significantly.
    - Difference in log odds ratio should be evaluated in order to determine the responder status at the relevant post-Baseline visit.
- Calculate the difference in the log of the odds ratios at each relevant post-Baseline visit and Baseline (Study Day 0).
  - Difference in log odds ratio = log(odds ratio at Study Day x) – log(odds ratio at Baseline [Study Day 0]).
- Determine the responder status based on the Breslow-Day and difference in log odds ratio for cases where the Baseline (Study Day 0) and relevant post-Baseline visit was determined as responders:

Breslow-Day test p-value	**Difference in log odds ratio**	**Responder status**
>= 0.05	<0	Non-responder
>=0.05	>0	Non-responder
<0.05	<0	Non-responder
<0.05	>0	Responder

The aforementioned steps are to be implemented to determine the data record-level response per subject and stimulation antigen (excluding DMSO) and visit. In order to determine the response per

subject at a day-level, and therefore across T cell type and stimulation antigen, the following conventions are to be used:

- Per subject and visit, if all records have a record-level responder status of non-responder, the day-level responder status is determined as a non-responder.
- Per subject and visit, if at least one of the records has a record-level responder status of responder, the day-level responder status is determined as responder.

The overall responder status is determined as a single responder status per subject across T cell types, stimulation antigens and visit and the following conventions are to be used to determine the overall responder status:

- Per subject, if all days have a day-level responder status of non-responder the overall responder status is determined as non-responder.
- Per subject, if at least one of the days has a day-level responder status of responder, the overall responder status is determined as responder.

7.3 Exploratory Analyses Not Planned in Accordance with the Protocol

Not applicable.

7.4 List of Tables, Figures, and Listings

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NARRATIVE TABLES

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