Statistical Analysis Plan Cover Page

Study Title: Phase 2 Study to Assess the Safety, Efficacy and Immunogenicity of Na-GST-1/Alhydrogel® Co-administered with Different Toll-Like Receptor Agonists in Hookworm-Naïve Adults

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Phase 2 Study to Assess the Safety, Efficacy and Immunogenicity of *Na*-GST-1/ Alhydrogel® Co-administered with Different Toll-Like Receptor Agonists in Hookworm-Naïve Adults

Roman Ganzha
Roman Ganzha
Roman Ganzha (Jul 22, 2020 18:17 GMT+3)
Project Biostatistician
Quartesian
Date (MMM DD, YYYY)

David Diemert, MD
David Diemert
David Diemert (Jul 22, 2020 21:44 EDT)
Principal Investigator
Date (MMM DD, YYYY)

Maria Elena Bottazzi, PhD
Maria Elena Bottazzi
Maria Elena Bottazzi (Jul 22, 2020 20:45 CDT)
Sponsor Representative
Date (MMM DD, YYYY)

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REVISION HISTORY

Version	Date	Author	Reasons
NA			

LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
AE	Adverse Event
AESI	Adverse Event of Special Interest
BCM	Baylor College of Medicine
CHHI	Controlled Human Hookworm Infection
CpG	Cytosine-phosphate-Guanine Oligodeoxynucleotide
eCRF	Electronic Case Report Form
ELISA	Enzyme linked immunosorbent assay
GST-1	Glutathione S-Transferase
ICF	Informed Consent Form
MedDRA®	Medical Dictionary for Regulatory Activities
MBC	Memory B Cell
Na	*Necator americanus*
NC	Not Computable
PI	Principal Investigator
PD	Protocol Deviation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
SVI	Sabin Vaccine Institute (Albert B Sabin Vaccine Institute; Sabin)
WBC	White Blood Cell
WHO-DRL	World Health Organization-Drug Reference List

1. INTRODUCTION

This statistical analysis plan (SAP) provides the explicit guidance and describes the planned statistical and data handling methods to be followed during the final reporting and analyses for the study protocol SVI-CH-02.

The trial is intended to inform the design of a larger trial, as opposed to be used for seeking regulatory approval of a dose or regimen. Efficacy, tolerability, and safety outcomes will be used for decision-making in the clinical development planning for future studies and therefore, the hypothesis testing of efficacy in this trial is not vulnerable to type I errors in the sense of making available an ineffective vaccine.

This SAP should be read in conjunction with the study protocol (SVI-CH-02, Version 4.0 (25 SEP 2019) and electronic case report form (eCRF) (SVI-CH-02, Version 4.0 (February 13, 2020)).

2. STUDY DETAILS

2.1 Study Objectives

Primary:

1. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

2. To evaluate the safety and reactogenicity of *Na*-GST-1/ Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on Days 0, 56 and 112, in healthy, hookworm-naïve adults.

Secondary:

1. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on fecal egg counts, as determined by the McMaster method, after controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults.

2. To assess the relationship between antibody responses to *N*a-GST-1 induced by vaccination with *N*a-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

3. To assess the duration of antibody responses to *N*a-GST-1 induced by vaccination with *N*a-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104.

4. To assess the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on the affinity of antibody-antigen interactions, and how affinity relates to responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

5. To assess the relationship between *Na*-GST-1 specific memory B cells induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses

to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

6. To assess the relationship between innate immune responses to *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults.

Exploratory:

1. To assess the relationship between the functional capacity of vaccine-induced antibodies that neutralize the in vitro activity of native *Na*-GST-1 enzyme and responses to CHHI in healthy, hookworm-naïve adults.

2. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on levels of hookworm DNA as detected by real-time PCR in healthy, hookworm-naïve adults challenged with CHHI, as determined by the presence of eggs using a qualified flotation technique.

2.2 Study Design

The study will be a randomized, double blind, placebo-controlled Phase 2 clinical trial in healthy hookworm-naïve adult subjects. This study is designed to evaluate the efficacy, safety, reactogenicity, and immunogenicity of *Na*-GST-1/Alhydrogel® administered with or without the point-of-injection addition of the immunostimulants AP 10-701 or CpG 10104. To assess the impact of vaccination with these different vaccine formulations on infection, subjects will be challenged with 50 infectious *N. americanus* larvae by controlled human hookworm infection (CHHI) 4 weeks after the 3rd vaccination. Safety parameters will be monitored throughout the study.

Subjects will be invited to participate in the study by means of verbal and email announcements and poster, newspaper, and online advertisements. After providing written informed consent, subjects will undergo eligibility screening, including a complete medical history; physical examination; hematology testing; liver and renal function testing; anti-double stranded DNA (anti-dsDNA) and rheumatoid factor (Rf) testing; Human Immunodeficiency Virus (HIV), Hepatitis B, and C testing; fecal occult blood testing; fecal examination for ova and parasites; and, urinalysis (for protein and glucose). Urine pregnancy testing will be performed on all female subjects. All clinically significant abnormalities will be reviewed with subjects and they will be referred for follow-up care if appropriate. After screening, those subjects determined to be eligible, based on the inclusion and exclusion criteria described in Section 5 in the study protocol, will be invited to participate in the study. No exemptions from the inclusion or exclusion criteria will be granted on inclusion/exclusion.

In the study, 48 subjects will be enrolled and randomized into 4 groups. Groups will not be enrolled separately; rather, eligible subjects will be randomly assigned to a group upon enrollment in a double-blind fashion (i.e., neither the subject nor the investigators will know to which group the subject has been assigned). In the first group (n=12), subjects will receive 100µg *Na*-GST-1/Alhydrogel®. In the second group (n=12), subjects will receive 100µg *Na*-GST-1/Alhydrogel® co-administered with 500µg CpG 10104. In the third group (n=12), subjects will receive 100µg *Na*-GST-1/Alhydrogel® co-administered with 5µg AP 10-701. In the fourth group (n=12), subjects will receive sterile saline placebo.

Subjects will be followed until 10 months after their final vaccination, for a total of 14 months of study participation. See Appendix A of protocol for a detailed description of the scheduled clinical and laboratory evaluations during the Vaccination and CHHI phases of the trial.

The table below shows the detail of study Schedule of Events for Vaccination and CHHI Phase:

Table 1: Schedule of Events – Vaccination Phase

	Study Day	Pre[1]	0	3 (±1)	7 (±2)	14 (±2)	28 (±4)	56 (±7)	59 (±1)[3]	63 (±2)[3]	70 (±2)[3]	84 (±4)[3]	112 (±7)[3]	115 (±1)[4]	119 (±2)[4]	126 (±2)[4]
	Visit Number	Pre[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Blood Volume	**Procedures**															
	Complete History/Physical	X														
	Obtain Informed Consent	X														
	Interim Clinical Evaluation		X		X	X		X		X	X		X		X	X
	Telephone call			X			X		X			X		X		
	Urinalysis	X														
	Urine pregnancy test (females)	X	X					X					X			
2 mL	CBC[2]	X	X			X		X			X		X			X
5 mL	RF, anti-dsDNA	X	X			X		X			X		X			X
	Serum ferritin	X														
5 mL	ALT	X	X			X		X			X		X			X
	Creatinine	X	X			X		X			X		X			X
10 mL	HCV testing	X														
	HBsAg testing	X														
	HIV testing	X														
	Fecal sample collection	X														X
	VACCINATION		**1**					**2**					**3**			
10 mL	Anti-*Na*-GST-1 antibody assays		X			X		X			X		X			X
50 mL	Innate & Cellular immunity assays		X		X	X		X		X	X		X		X	X
	Blood Volume (mL)	22	72		50	72		72		50	72		72		50	72
	Total Blood Volume (mL)	22	94		144	216		288		338	410		482		532	604

[1]Completed within 90 days of first vaccination.
[2]CBC parameters to be assessed for safety: WBC, absolute neutrophil count, absolute eosinophil count, hemoglobin, and platelet count.
[3]Since Vaccination #2.
[4]Since Vaccination #3.

Table 1: Schedule of Events – CHHI Phase

	Study Day	140 (±7)[4]	143 (±1)[5]	147 (±2)	154 (±2)	175 (±3)	182 (±3)	189 (±3)	196 (±3)	203 (±3)	210 (±3)	217 (±3)	224 (±3)	231 (±3)	238 (±3)	245 (±3)	252 (±3)	259 (±3)	266 (±3)	273 (±3)	280 (±3)	290 (±3)	294 (±3)	297 (±3)	320 (±14)	380 (±14)
	Visit Number	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Blood Volume	**Procedures**																									
	Interim Clinical Evaluation	X	X	X	X	X	X	X		X		X		X		X		X		X	X	X	X	X	X	X
	Telephone Call								X		X		X		X		X		X							
2 mL	CBC[1]	X			X	X		X		X		X		X		X		X			X			X[2]		
5 mL	ALT, Creatinine	X			X																					
5 mL	RF, anti-dsDNA																								X	
	Urine pregnancy test (females)	X																			X			X		
	LARVAL INOCULATION	**X**																								
	Fecal sample collection[3]					X	X	X		X		X		X		X		X		X	X	X	X	X		
10 mL	Antibody assays	X		X	X	X		X						X							X			X	X	X
50 mL	Innate & cellular immunity assays	X			X	X		X						X							X			X		
	Anthelminthic Rx																				X					
	Blood Volume (mL)	67		10	67	62		62		2		2		62		2		2			62			62	15	10
	Total Blood Volume (mL)	671		681	748	810		872		874		876		938		940		942			1004			1066	1081	1091

[1]CBC parameters to be assessed for safety: WBC, absolute neutrophil count, absolute eosinophil count, hemoglobin, and platelet count.
[2]Subjects with laboratory evidence of eosinophilia post-larval inoculation only.
[3]Subjects that withdraw from the study prior to study Day 280 will be strongly encouraged to provide post-treatment fecal samples to ensure cure of the infection.
[4]Since Vaccination #3.
[5]Since Larval Inoculation.

3. DATA ANALYSIS CONSIDERATIONS

3.1 Determination of Sample Size

Sample size considerations are based on the proportion of subjects with detectable hookworm eggs at any time, as measured by the saturated saline flotation technique. The primary analysis will consist of comparing the proportion of subjects in each of the three *Na*-GST-1 vaccination groups (groups 1, 2 and 3) to the proportion of subjects in the placebo control group (group 4) using a two-sided Fisher exact test, for a total of three comparisons. Targeting a rate of 90% subject hookworm infection as observed in the 50 *Na*-L3 dose cohort and a greater than 80% rate of protection in at least one of 3 vaccination groups (e.g., different doses or adjuvant formulations) compared to controls that did not receive the hookworm vaccine, a sample size of 48 (12 per group) would be sufficient to demonstrate the efficacy of a prophylactic hookworm vaccine such as *Na*-GST-1 or *Na*-APR-1, with alpha ~ 0.1 and power of at least 80% [Diemert et al, OFID, April 2018].

3.2 Randomization

Eligible subjects will be asked to come to the clinic on their scheduled day of enrolment into the study (Day 0). After undergoing a clinical interview and directed physical examination to ensure that they remain eligible for participation in the study, that they have had blood collected for safety clinical laboratory and baseline immunogenicity assessments, and that females have had a negative urine pregnancy test, subjects will be randomized and enrolled in the order that they present for vaccination.

Within each group, randomization will be done through use of a randomization code using SAS software (Version 9.3 or higher), furnished to the study vaccine manager by the study statistician. Access to the randomization list will be exclusively limited to the study vaccine manager and assistant. Between vaccination days, the randomization list will be stored in a locked cabinet in the GW MFA Investigational Drug Service pharmacy or the office of the study vaccine manager. The study vaccine manager and assistant will be unblinded but will not be involved in further evaluation of study subjects or assessment of adverse events. Subjects will not be considered enrolled in the study until they have received their first dose of vaccine. In the event that a subject is randomized but not enrolled on the day of first vaccination, they will be replaced with an eligible alternate. Enrolled subjects that leave the study for any reason following first vaccination will not be replaced.

Vaccine/placebo assignment will be done by block randomization. That is, for each block of 4 subjects who are sequentially enrolled into the trial, 1 will be assigned to receive *Na*-GST-1/Alhydrogel®, 1 to *Na*-GST-1/Alhydrogel® plus CpG 10104, 1 to *Na*-GST-1/Alhydrogel® plus AP 10-701, and 1 to sterile saline placebo.

3.3 Blinding

Due to the double-blind nature of this Phase 2 clinical trial, neither study subjects nor study team personnel will know to which group an individual subject has been assigned. Investigators and subjects will be blinded to the vaccine/placebo allocation until all subjects have completed their final study visit (i.e., Visit #39 at Day 380), the primary (efficacy and safety) and secondary IgG immunogenicity outcomes (i.e., anti-*Na*-GST-1 IgG antibody results by ELISA) have been monitored and entered into the database, and the database has been locked for analysis.

The study vaccine manager will also prepare all investigational product doses (vaccine or sterile saline placebo) in a separate room and will hand filled syringes to the vaccinator(s). Since the doses of the *Na*-GST-1 formulations are of different opacity than that of sterile saline (slightly turbid vs. clear, respectively), the contents of all syringes will be disguised using opaque tape. As a further precaution, the vaccinator(s) will not be involved in assessments of reactogenicity or adverse events.

4. PRIMARY AND SECONDARY ENDPOINTS

4.1 Primary Outcome Measures

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Proportion of subjects with detectable hookworm eggs, at any time point, in fecal samples, as determined by microscopy using the qualified saline flotation technique.
2. Frequency of solicited injection site and systemic reactogenicity, graded by severity, on the day of each study vaccination through 14 days after each study vaccination.
3. Frequency of solicited adverse events, graded by severity, on the day of CHHI through study Day 280.
4. Frequency of study vaccine-related Serious Adverse Events from the time of the first study vaccination through approximately 10 months after the last study vaccination.
5. Frequency of clinical safety laboratory adverse events.
6. Frequency of unsolicited adverse events, graded by severity, from the time of each study vaccination through approximately 1 month after each study vaccination; and from the time of CHHI through treatment with albendazole (Day 297).
7. Frequency of new-onset chronic medical conditions through approximately 10 months after the third study vaccination.
8. Frequency of Adverse Events of Special Interest through approximately 10 months after the third study vaccination.

4.2 Secondary Outcome Measures

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Fecal egg counts during Weeks 5 through 20 post-CHHI.

2. The anti-*Na*-GST-1 IgG antibody response (ELISA) at approximately 14 days after each vaccination, and approximately 1, 2, 4, 6, 7, 8 and 10 months after the third dose.

4.3 Exploratory Outcome Measures

1. The functional capacity of vaccine-induced antibodies to neutralize the in vitro activity of the native *Na*-GST-1 enzyme.

2. Levels of *N. americanus* DNA in fecal samples, as measured by real-time PCR, during Weeks 5 through 20 post-CHHI.

5. STATISTICAL METHODOLOGY

5.1 General Considerations

The statistical analyses will be performed by Quartesian Clinical Research, using SAS Version 9.3 (or higher). All tables, figures and listings will be produced in landscape format. In general, all data will be listed by the subject and visit/time point where appropriate.

Data will be summarized by time-point where appropriate. The total number of subjects in the study group (N) under the stated population will be displayed in the header of summary tables.

Data will be summarized using descriptive statistics for continuous variables. Unless otherwise stated, descriptive statistics will include number of subjects, mean, standard deviation, minimum, median and maximum for all endpoints in case of n<2, where n indicates the number of evaluable subjects at the particular time point, only n, mean, minimum and maximum will be displayed. The statistic "Missing" will also be evaluated by enumerating the number of missing entries/subjects, if any at that visit, and presented along with summary statistic. Display NC if any value is not computable.

Decimal Precision Convention: The minimum and maximum statistics will be presented to the same number of decimal places as the original data. The mean and median will be presented to one more decimal place than the original data, whereas the standard deviation will be presented to two more decimal places than the original data.

For categorical variables, counts and percentages will be used. The count [n] indicates the actual number of subjects with non-missing value at particular visit or actual number of subjects with that event or category, which should always be less than or equal to the total number of subjects in the respective study group [N]. Percentage will be obtained by: % = (n/N) *100.

Unless otherwise stated, all percentages will be expressed to one decimal place.

Also, the distributions for continuous variables will be examined for normality using Shapiro -Wilk tests. If any deviation in the normality assumption is judged, then the data will be analyzed using appropriate non-parametric tests.

The following conventions will be applied to analyses/data presentation:

All dates will be displayed in DDMMMYYYY format.

In general, all data will be listed, sorted by cohort, treatment group, and subject ID, and when appropriate by parameter and then visit number within subject. All summary tables will be structured with a column for each treatment and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

Study Hypotheses:

This study is not designed to test statistically significant small differences in efficacy between different adjuvant formulations of the *Na*-GST-1 hookworm vaccine as assessed in the CHHI model. Rather, it is intended to provide an indication of an impact on infection as assessed by CHHI, and to detect large differences in efficacy with acceptable power. Furthermore, the study intends to assess the safety, reactogenicity, and immunogenicity of three novel adjuvant formulations of *Na*-GST-1: *Na*-GST-1

formulated on Alhydrogel, *Na*-GST-1/Alhydrogel® administered with AP 10-701, and *Na*-GST-1/Alhydrogel® administered with CpG 10104.

The chosen sample size principally facilitates the assessment of efficacy (as estimated by infection status following CHHI) as well as safety, as discussed in SAP Section 3.1, Sample Size Considerations. Given that, this study will attempt to assess the following hypotheses:

1. Administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will result in a reduced proportion of subjects infected with hookworm following CHHI compared to placebo controls.

2. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the IgG antibody response to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

3. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the affinity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

4. Co-administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG10104 will increase the induction of specific MBCs to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

5. Co-administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 increases the neutralizing capacity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

6. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the avidity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

Protocol Deviations:

If there are major protocol deviations, a per-protocol (PP) analysis may also be performed. Immunogenicity or parasitology data from any visit that occurs substantially out of window will be reviewed by the Principal Investigator for decisions regarding inclusion or exclusion of the data.

Handling of Missing Data:

For partial start dates:

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then:
    a. If the year matches the year of the first dose date, then impute the month and day of the dose date.
    b. Otherwise, assign “January”.
3. If the day is unknown, then:
    a. If the month and year match the month and year of the first dose date, then impute the day of the dose date.
    b. Otherwise, assign “01”.

For partial end dates:

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then assign "December".
3. If the day is unknown, then assign the last day of the month.

Vaccine Group Labelling in TFLs:

The table below outlines the Vaccine Groups labeling for all Tables, Listings and Figures (wherever as appropriate):

Group 1	Group 2	Group 3	Group 4
100µg *Na*-GST-1/Alhydrogel®	100µg *Na*-GST-1/Alhydrogel® plus 500µg CpG 10104	100µg *Na*-GST-1/Alhydrogel® plus 5µg AP 10-701	Placebo

5.2 Definition

Baseline: Baseline is defined as the latest non-missing assessment (whether from scheduled or unscheduled) prior to the first dose administration of the study vaccine, unless otherwise stated.

The **Post-baseline** values are defined as measurements taken after the first administration of study product.

Change from Baseline: The change from baseline values will be calculated as post baseline value minus the baseline value.

An Adverse Event is any untoward medical occurrence in a subject or clinical investigation in a subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this vaccination. An adverse event can be any unfavorable sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether considered related or not related to the investigational product.

A pre-vaccination condition is any event in a clinical study subject that occurs after he/she signed the Informed Consent Form (ICF) up until the first administration of study vaccine. All pre-vaccination conditions will be considered as medical history whereas any pre-vaccination condition that worsens after vaccination will be considered as an AE.

An SAE is any AE that results in death, is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe), requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, requires medical or surgical intervention to prevent any of the occurrences noted above.

Unexpected Adverse Event: Any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure.

5.3 Analysis Populations

All Subjects: All subjects will include the subjects who signed the informed consent form.

Randomized Population: The randomized population will include all subjects who were randomized to any one of the groups.

Safety Population: The safety population will include all randomized subjects who have received at least one dose of study vaccine. This population will be used to summarize safety variables and will summarize subjects as vaccinated.

Immunization Population: The immunization population will include all randomized subjects who received all three study vaccinations.

Per Protocol Population: The per protocol population will include all randomized subjects who received all three study vaccinations and CHHI and completed the study without any major protocol deviations or completed the study with a protocol deviation(s) having no significant impact according to Investigator's assessment.

5.4 Protocol Deviations

A separate listing will be provided with deviation type and description.

5.5 Coding Dictionaries Used

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 (or higher). Prior & concomitant medications will be coded using World Health Organization-Drug Reference List (WHO-DRL) (Version: WHODRUG GLOBAL B3 March 1, 2019) and by Anatomical Therapeutic Chemical (ATC) level 4 (or higher).

5.6 Study Product Administration and Accountability

The number and percentage of subjects that received the study product will be summarized by Vaccine Group using the Safety Population.

The exposure to study product will be summarized for total number of doses administered at vaccination and post CHHI visits, using the Safety Population.

A separate listing will be provided with study product administration information including date and time of dosing, dose administered and comment in case dose was not administered.

5.7 Analysis Methods

5.7.1 Disposition

The number and percentage of subjects who entered the study, were randomized and received the first dose of study product, and those who completed the study will be presented, together with the number and percentage of subjects who prematurely discontinued from the study along with reasons for study discontinuation. Additionally, subjects included in the Randomized Population, Safety Population, Immunization Population and Per Protocol Population will be summarized by Vaccine Group using all subjects.

A listing of each subject's analysis population (Randomized Population, Safety Population, Immunization Population and Per Protocol Population), disposition and reason for discontinuation will be listed.

5.7.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics (gender, ethnicity, race, age [years] will be summarized by Vaccine Group using descriptive statistics for subjects in the Randomized Population. Qualitative variables (gender, ethnicity, race) will be summarized using frequencies and percentages while quantitative variables (age) will be summarized using mean, standard deviation (SD), median, minimum, and maximum.

Individual subject listings of demographic and baseline data will be presented for the Randomized Population.

5.7.3 Prior and Concomitant Medications

Concomitant medications will be summarized by Vaccine Group using the Safety Population.

Concomitant Medications will be coded using World Health Organization-Drug Reference List (WHODRUG GLOBAL B3 March 1, 2019) and will be categorized by preferred name and ATC level 4 class per WHO.

Individual subject listings of all concomitant medications will be presented for the Safety Population.

5.7.4 Primary and Secondary Analysis

5.7.4.1 Primary Endpoint Analysis

The primary efficacy endpoint analysis of proportion of subjects with detectable hookworm eggs in fecal samples, at any time point post-CHHI, will be summarized using frequency counts and percentages as a descriptive measure at time points post-CHHI for all vaccination groups.

Along with the summaries for any time points post-CHHI, the proportion of subjects with detectable hookworm eggs in fecal samples will be summarized as a descriptive measure by study group at each time point.

The proportion of subjects with detectable hookworm eggs in each of the vaccination groups (Groups 1, 2 and 3) will be compared to the proportion of subjects in the placebo control group (Group 4) using a two-sided Fisher exact test. The p-values for the pairwise comparison of proportions of subjects in the vaccination groups (Group 1, 2 and 3) and placebo group (Group 4) will be reported. No adjustments for multiple comparisons will be made and therefore, the reported p-values will have to be interpreted with caution.

The proportion of subjects with at least one injection site AE and the frequency of the AE grouped by PT will be compared by *Na*-GST-1 vaccine formulation and placebo. The null hypotheses that the type and number of adverse events is the same across all groups will be tested using Fisher's exact test.

Laboratory results (haematological and clinical chemistry) will be descriptively examined and any clinically significant values for individuals will be reported.

5.7.4.2 Secondary Endpoint Analysis

Fecal egg counts of the subjects in each of the vaccination (Groups 1, 2 and 3) and placebo (Group 4) groups will be assessed for normality. If normality assumption is confirmed, the comparison between vaccination groups (Group 1, 2 and 3) and placebo group (Group 4) will be done using T-test. Otherwise, the vaccination groups (Group 1, 2 and 3) will be compared with the placebo group (Group 4) using a Wilcoxon-Mann-Whitney rank-sum test. Continuous data will be summarized using the descriptive statistics (mean, median, minimum, maximum and median 95% CI). Mean or Median differences and corresponding 95% CIs will be computed for each pairwise comparison.

Time to patent infection (i.e., the first timepoint at which hookworm eggs are detectable in a fecal sample) will be displayed using Kaplan-Meier estimates, with log-rank tests used to compare the distribution of time-to-patency between groups and censoring of early withdrawals or those failing to develop patent infection.

The detectable anti-*Na*-GST-1 IgG responses will be summarized as a descriptive measure using descriptive statistics (mean, standard deviation, median, minimum and maximum) for each observed measurement by vaccination groups and placebo. IgG levels will be displayed graphically by study group using time series and notched box plots at each time points.

Geometric mean antibody responses will be compared between vaccination groups and placebo at each time point using an ANOVA model. An ANOVA model will be fitted with IgG responses as the dependent variable and vaccination groups and placebo as independent variables. The estimated geometric least square means and geometric least square means difference from the model, 2-sided *p*-value and 95% CI for vaccination groups compared to placebo group will be constructed and presented for each time point.

Percent change in IgG antibody levels from days of vaccination to days 7, 14 and 28 post-vaccination, as well as percent change in IgG antibody levels from the peak following the third vaccination to 1, 2, 4, 6, 7, 8, and 10 months post-vaccination will be summarized using descriptive statistics (mean, standard deviation, median, minimum, maximum and 95% CI) for each observed measurement along with the percent change from baseline.

A longitudinal model will be built to describe the IgG levels over time. Using a longitudinal panel model, differences in antibody isotype levels by vaccination groups and placebo will be explored. The analysis will take account of the correlation between measurements on the same subject. A mixed model repeated measure (MMRM) will be fitted with IgG levels as the dependent variable and vaccination groups and placebo, visits and interaction between groups and visits as independent variables. the estimated geometric means Standard Error and geometric means difference from the model, 2-sided *p*-value and 95% CI for vaccination groups and placebo will be constructed.

Logistic regression will be explored for the binary response of hookworm infection post-CHHI as a function of antibody response. Dependant variable will be the hookworm infection status (Positive/Negative) and independent variable will be the IgG responses and vaccination groups and placebo. Estimated number of subjects, Standard Error and odds ratio from the model, 2-sided *p*-value and Wald 95% CI for vaccination groups and placebo will be constructed.

5.7.5 Safety Analysis

All safety assessments, including adverse events, solicited and unsolicited adverse events during the post-vaccination phase and post-CHHI phase, clinical laboratory test results, vital signs, (blood pressure [mmHg], heart rate [bpm], and oral temperature [Degrees Celsius]), abnormal physical examination

findings will be listed by subject and tabulated when applicable. The analysis will be performed on the Safety Population.

5.7.5.1 Adverse Events

Adverse Events (AEs) will be coded using the Medical Dictionary of Regulatory Activities (MedDRA version 23.0 or higher) AE coding system for purpose of summary tables.

An AE is any that is new in onset or was increased in severity following the first dose of study product, up to and including the last procedure of the study (i.e., Follow-up phone call).

Summary tables of the following AEs will be provided:

- An overview of AE summary will be presented by Vaccine Group and it will include number and percentages of subjects who had at least one AE, who had at least one SAE, who had at least one vaccine-related AE, who had at least one vaccine -related serious AE, who had at least one AESI, subjects who had at least one vaccine-related AESI and who had at least one AE leading to study discontinuation will be presented.

- A summary of the frequency (number and percentage of subjects) of AEs by Vaccine group will be presented by system organ class, preferred term.

- Similar summary results will be generated for AEs by severity (mild, moderate, severe) and by relationship (Definitely, Probably, Possibly, Unlikely and not related) to study product. AEs and SAEs associated with study discontinuation will be include in by subject data listings.

- A summary of the frequency (number and percentage of subjects) of SAEs by Vaccine Group will be presented by system organ class, and preferred term.

- A summary of the frequency (number and percentage of subjects) of vaccine related SAEs by Vaccine Group will be presented by system organ class, and preferred term.

- A summary of the frequency (number and percentage of subjects) of clinical safety laboratory AEs by Vaccine Group will be presented by system organ class, and preferred term.

- A summary of the frequency (number and percentage of subjects) of new-onset chronic medical conditions through approximately 10 months after the third study vaccination by Vaccine Group will be presented by system organ class, and preferred term.

- Similarly, a separate table will be provided for Adverse Events of Special Interest through approximately 10 months after the third study vaccination using number and percentage of subjects by Vaccine Group, and will be presented by system organ class, and preferred term.

- Additionally, the proportion of subjects with at least one injection site AE and frequency of AEs grouped by SOC term will be compared by *Na*-GST-1 vaccine formulation and placebo. The null hypotheses that the Adverse event type and number of adverse events is the same across all vaccination groups and placebo. 2-sided *p*-values will be obtained from the Fisher's exact test.

All AEs during the study will be summarized for the respective vaccination. A subject experiencing the same AE multiple times will be counted only once for that preferred term. Similarly, if a subject experience multiple AEs (preferred terms) within the same system organ class then that subject will be

counted only once for that system organ class. When summarizing by severity, only event with highest severity will be counted. All AEs will be listed in chronological order of the events occurred.

Individual subject listings containing all information pertaining to adverse events noted during the study will be presented by Vaccine Group, subject, verbatim and preferred terms, system organ class, start date, stop date, maximum severity, outcome, action taken and study vaccine relatedness.

5.7.5.2 Vaccine Reactogenicity

All subjects will be observed for at least 60 minutes after each study vaccination to detect and treat any immediate AEs. Subjects will also complete study memory aids after each vaccination beginning with the day of vaccination and through 14 days after vaccination. Injection site reactions including bruising, redness, induration (hardness)/swelling, pain, and tenderness will be recorded on the study aids. Systemic reactions including fever, myalgia (body aches/muscular pains exclusive of the injection site), arthralgia (joint pains exclusive of the injection site), headache, nausea, and vomiting will also be recorded. Vaccine reactogenicity events are AEs that are known to occur with types of vaccine similar in composition to those being tested in this study.

Solicited and Unsolicited events post-vaccination will be summarized by frequency counts and percentages will be tabulated by severity to Vaccine Group and placebo using Safety Population. The number of subjects reporting a solicited adverse event will be summarized by day post vaccination for each vaccination and for all vaccinations combined both in tables and graphically in bar charts.

5.7.5.3 CHHI Reactogenicity

All subjects will be observed for at least 60 minutes after application of the *N. americanus* larvae during CHHI to detect and treat any immediate AEs. Subjects will also complete study memory aids after CHHI beginning with the day of larval application and through Day 280 when they will be treated with albendazole to cure their infections. For the first 4 weeks after application of the *N. americanus* Larval Inoculum, the memory aids will be used to record pruritus, pain, tenderness, rash and swelling at the site of larval application, as well as cough and sore throat (to capture symptoms that may possibly be related to larval migration through the lungs). Starting on Day 175 (approximately 4 weeks after CHHI) of the study until the subject receives their first dose of albendazole, the memory aids will be used to record nausea, vomiting, diarrhea, abdominal pain, abdominal bloating and flatulence. CHHI reactogenicity events are AEs that are known to occur with hookworm infection.

Solicited and Unsolicited events post-CHHI will be summarized by frequency counts and percentages will be tabulated by severity to Vaccine Group using the Per-protocol Population.

5.7.5.4 Vital Signs

Vital Signs data will be presented in a Listing by Vaccine Group and subject for each parameter using the Safety Population.

5.7.5.5 Physical Examination

Physical examination will be presented in a Listing for the scheduled visits and which consist of assessments of the planned injection site, spleen & lymph nodes (LNs), arm movement and strength and any others.

5.7.5.6 Clinical Laboratory Parameters

Laboratory evaluation results and changes from baseline will be summarized descriptively by visit using the Safety Population. Unless otherwise specified, all continuous laboratory data will be summarized using descriptive statistics (n, mean, standard deviation (SD), median, minimum, and maximum) for each scheduled study assessment as well as change from baseline and percentage change from baseline [if applicable] by parameter class (hematology and biochemistry).

Hematology, chemistry, rheumatoid factor (RF) and anti-double strand DNA (anti-dsDNA) antibody data will be summarized by displaying shifts from baseline value (Normal/Abnormal) to subsequent assessment after administration of study product.

Safety laboratory data will be listed by visit for each parameter and subject and summarized by Vaccine Group and placebo. All abnormal values that met the definition of an AE as per the protocol will be flagged on the listing.

Hematology parameters includes WBC, Hemoglobin, Platelet Count, Absolute Neutrophil Count and Absolute Eosinophil Count

Biochemistry parameters includes ALT and Creatinine.

6. INTERIM ANALYSES

Not Applicable

7. CHANGES OF ANALYSIS FROM PROTOCOL

The analysis point no. 2 in the analysis plan section secondary outcome #2 and #3 measures performs the ANOVA. Hence analysis Section “e” (Protocol Version 4.0 Page no.72) is not required if it is not specific to three-way ANOVA.

8. REFERENCES

Not Applicable

9. APPENDICES

Not Applicable

10. TABLES, LISTINGS AND FIGURES

The list of Tables, Listings and Figure to be prepared for this study will be provided as a separate document.