Arcturus Therapeutics, Inc.

ARCT-021-04

A PHASE 2 RANDOMIZED, OBSERVER-BLIND, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY, REACTOGENICITY, AND IMMUNOGENICITY OF THE SARS-CoV-2 VACCINE ARCT-021 IN HEALTHY ADULT PARTICIPANTS

Statistical Analysis Plan

Version 5.0 for Final Analysis
Version Date of SAP: 09JUN2022

Prepared by:

PPD
929 N Front Street
Wilmington, NC 28401

PPD	PPD Biostatistics and Programming
	Statistical Analysis Plan (SAP) Client Approval Form

Client:	**Arcturus Therapeutics**
Protocol Number:	**ARCT-021-04**
Document Description:	**Statistical Analysis Plan**
SAP Title:	Statistical Analysis Plan for Study of A Phase 2 Randomized, Observer-Blind, Placebo-Controlled Study To Assess The Safety, Reactogenicity, And Immunogenicity Of The SARS-CoV-2 Vaccine ARCT-021 In Healthy Adult Participants
SAP Version Number:	**Version 5.0 for Final Analysis**
Effective Date:	**09JUN2022**
Version History:	**Final Version 5.0 09JUN2022**

Author(s):

[redacted] **PPD**

Approved by:

[redacted]

Arcturus Therapeutics

Date *(DD-MMM-YYYY)*

10-Jun-2022

[redacted]

Date *(DD-MMM-YYYY)*

10-Jun-2022

Table of Contents

List of Abbreviations

Abbreviation	Definition
ACE-2	angiotensin-converting enzyme 2 receptor
AE	adverse event
BMI	body mass index
CESI	clinical events of special interest
CFR	Code of Federal Regulations
CI	confidence interval
CMI	cell-mediated immune
COVID-19	Coronavirus Disease 2019
CRF	case report form
CRO	contract research organization
CSR	clinical study report
CTMS	Clinical Trial Management System
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
EDC	Electronic Data Capture
eCRF	electronic case report form
FDA	US Food and Drug Administration
FSH	follicle-stimulating hormone
GMFR	geometric mean fold-rise
GMR	geometric mean ratio
GMT	geometric mean titer
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
IcEv	intercurrent event
ICH	International Council for Harmonisation
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IRT	interactive response technology
LLOQ	lower limit of quantification
LOD	limit of detection
MAAE	medically attended AE
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition
mITT	modified intent-to-treat
MSD	Meso Scale Discover
NAb	neutralizing antibody
NOCD	new onset of chronic disease
PBMC	peripheral blood mononuclear cells
PRNT	plaque reduction neutralizing titer
RBD	receptor binding domain
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SUSAR	suspected unexpected serious adverse reaction
TNF	tumor necrosis factor
VAERD	vaccine-associated enhanced respiratory disease
WOCBP	women of childbearing potential

1. Introduction

This document outlines the statistical methods to be implemented in the analysis of data collected within the scope of Arcturus Therapeutics, Inc., protocol ARCT-021-04 (A Phase 2 Randomized, Observer-Blind, Placebo-Controlled Study to Assess the Safety, Reactogenicity, and Immunogenicity of the SARS-CoV-2 Vaccine ARCT-021 in Healthy Adult Participants) for the final study analysis based on Protocol Version 6.0, dated March 9, 2022.

ARCT 021 is a self-replicating RNA vaccine being developed for prevention of Coronavirus Disease 2019 (COVID-19), caused by SARS CoV 2. This is a multiregional, multicenter, Phase 2, randomized, observer-blind study designed to evaluate the safety, reactogenicity, and immunogenicity of the study vaccine in younger and older adult participants. Enrolled participants will be randomly assigned to receive either study vaccine ARCT 021 or placebo (sterile saline) as the priming vaccination series. The study also includes administration of booster doses of vaccine, which are to be given 6 months after completion of the priming vaccination series.

The PPD Biostatistics and Programming team will perform the final statistical analysis. Version 3 of the SAP outlined the final safety and reactogenicity analyses and indicated a separate analysis plan will be created for the final analysis of immunogenicity data. In order to consolidate all final analyses into one document, SAP version (Version 4) outlined all (safety, reactogenicity, and immunogenicity) final analyses. This document, SAP Version 5, contains additional clarifications to Version 4 discussed at the blinded Data Review Meeting on June 7, 2022. Separate analysis plans were created for the interim analyses. Statistical Analysis System (SAS) Version 9.4 or higher will be used. If the methods in this SAP differ from the methods described in the protocol, the SAP will prevail.

References throughout this SAP to specific visits by study day (e.g., Day 0) refer to study day as defined in schedule of events in Appendix 1. Study day (also referred to as Analysis Day) is relative to the first vaccination date as:

- Day 0 is defined as the first vaccination dose
- Study Day = Assessment Date – First Vaccination Date

In order to be compliant with the Clinical Data Interchange Standards Consortium (CDISC), study day within the analysis database is calculated as Study Day = Assessment Date – First Vaccination Date + 1.

Prior to the finalization of SAP Version 3.0, Arcturus Therapeutics had determined to early terminate the study prior to the planned visits up to Day 570. In notifications sent to the Singaporean Health Authority (HSA) and US Food and Drug Administration (FDA), it was specified that final study vaccinations would conclude in December 2021 and safety would be followed through 56 days after final vaccination or February 2022, whichever came first. This decision was taken based on refocusing development efforts on other COVID-19 vaccine candidates. However, it was also recognized that due to the study participation attrition, the significant risk of receipt of other COVID-19 vaccines by participants the study may be limited in its interpretation at timepoints beyond Day 56.

2. Study Objectives and Endpoints

2.1. Primary Objectives and Endpoints

The primary objectives and endpoints are presented in Table 2-1 (For estimands, see Appendix 3.).

Table 2-1 Primary Objectives and Endpoints

Objective(s)	Endpoint Descriptions
Primary	
• To assess the safety and reactogenicity of ARCT-021 compared to placebo for priming vaccination	Safety will be evaluated in all participants receiving at least 1 administration of study vaccine (ARCT-021 or placebo) and will be summarized for each vaccination as number and percentage of participants with: • Any unsolicited adverse event (AE) initiating within 28 days after each study vaccine administration, by severity and relationship to study vaccine • Any medically attended adverse event (MAAE), new onset of chronic disease (NOCD), AE leading to discontinuation/withdrawal or serious adverse event (SAE) through Early Termination • Safety laboratory assessment before and 7 days after each study vaccine administration, by toxicity grade Reactogenicity will be evaluated in all participants receiving at least a single administration of study vaccine and will be summarized for each vaccination as number and percentage of participants with: Any solicited local or systemic AE initiating within 7 days after each study vaccine administration, by toxicity grade

• To assess the neutralizing antibody responses of ARCT-021 compared to placebo for priming vaccination	Neutralizing antibody (NAbs) responses will be evaluated in all participants receiving at least 1 administration of study vaccine (ARCT-021 or placebo). • Geometric Mean Titer (GMT) measured at all time points • Geometric Mean Fold-rise from baseline (GMFR); measured at all time points after baseline (= Day 0) • Percentages of participants with ≥2- and 4-fold increase in titer from baseline (Seroconversion [SC]); measured at all time points after baseline • GMT ratio (ARCT-021/placebo) measured at all time points
• To select the dose and schedule for use in the Phase 3 study in adult participants	Safety and reactogenicity data after each priming vaccine administration and immunogenicity response measured 28 days after each priming vaccination will be evaluated in younger (18 to <56 years) and older (≥56 years) adults

2.2. Secondary Objectives and Endpoints

The secondary objectives and endpoints are presented in Table 2-2. (For estimands, see Appendix 3.)

Table 2-2 Secondary Objectives and Endpoints

Objective(s)	Endpoint Descriptions
Secondary	
• To assess the binding antibody responses of ARCT-021 compared to placebo for priming vaccination	Binding antibody (BAb) responses will be evaluated in all participants receiving at least 1 administration of study vaccine (ARCT-021 or placebo). • GMC and GMC ratio at all time points • GMFR and SC at all time points after baseline

2.3. Exploratory Objectives and Endpoints

The exploratory objectives and endpoints are presented in Table 2-3.

Table 2-3 Exploratory Objectives and Endpoints

Objective(s)	Endpoint Descriptions
Exploratory	
• To evaluate cell-mediated immune (CMI) responses in participants receiving ARCT-021 versus placebo for priming vaccination	CMI responses to SARS-CoV-2 spike antigen may be measured in a subset of participants (CMI subset): • Cytokine-producing SARS CoV-2 Spike protein-specific T-cells o as measured by flow cytometry and intracellular cytokine (Tumor necrosis factor (TNF) α, Interleukin-2 (IL-2), Interferon-γ (IFN- γ), IL-4, and IL-13) staining assay at all time points

• To evaluate safety, reactogenicity, and immunogenicity responses in participants receiving ARCT-021, ARCT-154, ARCT-165 versus placebo as booster vaccination given 6 months after priming vaccination	Safety: • Any unsolicited AE initiating within 28 days after booster dose of study vaccine administration, by severity and relationship to study vaccine (ARCT- 021, ARCT-154, ARCT-165, or placebo) • Any MAAE, NOCD, AE leading to discontinuation/withdrawal or SAE through Early Termination • Safety laboratory assessment prior to and 7 days after booster vaccine administration by toxicity grade Reactogenicity: • Any solicited local or systemic AE initiating within 7 days after booster vaccine administration, by toxicity grade Immunogenicity: • Binding and neutralizing antibody responses (GMFR, SC, GMC, GMT, NAbs/BAbs ratio) before and after booster administration • CMI responses before and after booster administration (CMI subset)
• To evaluate the incidence of confirmed COVID-19 in participants vaccinated with ARCT-021, ARCT-154, ARCT-165 versus placebo	Confirmed COVID-19 will be assessed in all participants starting 7 days after first study vaccine (ARCT-021, ARCT-154, ARCT-165, or placebo) administration and will include: • Confirmation of COVID-19 symptoms • Confirmation of SARS-CoV-2 infection by reverse transcriptase-polymerase chain reaction (RT-PCR) • Collection of COVID-19 diagnoses made outside of study-related procedures
• To evaluate the incidence of asymptomatic SARS-CoV-2 infection in participants vaccinated with ARCT-021, ARCT-154, ARCT-165 versus placebo	Asymptomatic SARS-CoV-2 will be assessed in all participants starting 7 days after first study vaccine and will include: • Confirmation of SARS-CoV-2 infection by RT-PCR • Confirmation of seroconversion of SARS-CoV-2 nucleocapsid (N) antibody at timepoints after Day 14
• To assess for vaccine-associated enhanced respiratory disease (VAERD) following vaccination with ARCT 021, ARCT 154, ARCT 165 versus placebo	Confirmed COVID-19 cases will be evaluated by an unblinded Data Safety Monitoring Board (DSMB) for frequency and severity in ARCT 021-, ARCT 154-, ARCT 165-, and placebo-vaccinated participants
• To assess the immune responses of ARCT-021, ARCT-154, ARCT-165 compared to placebo using other exploratory assays (eg, plaque reduction neutralizing titer [PRNT], or assays evaluating for responses to SARS-CoV-2 variants)	If additional assays and the corresponding blood samples are available, additional immune antibody responses may be evaluated in some or all participants receiving at least 1 administration of study vaccine (ARCT-021, ARCT-154, ARCT-165 or placebo).

3. Investigational Plan

3.1. Overall Study Design and Plan

The overall study design is described below. For the final analysis, data for all participants through early termination will be included.

This is a multiregional, multicenter, Phase 2, randomized, observer-blind study designed to evaluate the safety, reactogenicity, and immunogenicity of the study vaccine in younger and older adult participants. Enrolled participants will be randomly assigned to receive either study vaccine ARCT-021 or placebo (sterile saline) as the priming vaccination series and ARCT-021, ARCT-154, ARCT-165, or placebo as the booster administration.

Approximately 600 participants (300 each in younger [≥18 to <56 years of age in United States or ≥21 to <56 years of age in Singapore] and older [≥56 years of age] participants) will be enrolled. At Day 0, participants will be stratified by age and then randomly assigned to one of the 4 groups (3 ARCT-021:1 placebo) to receive 2-doses of study vaccine and/or placebo (see Table 3-1) separated by 28 days and, at Day 208, participants will be randomly assigned to active booster vaccine or placebo for a third (booster) dose of study vaccine given at 180 days after second study vaccination (Day 208). At a subset of clinical sites, all enrolled participants will also undergo blood sampling for evaluation of CMI responses. For further details relating to the booster assignments, please refer to the protocol.

Participants who complete study procedures through Day 98 will be offered the opportunity to determine if they are eligible for study re-enrollment to ensure dosing with ARCT-021.

After Day 98 and until Day 198, participants will be contacted to determine whether they are interested, if eligible, in re-enrolling in the study in order to receive active study vaccine. Only participants who are assigned to Study Group 4 (placebo) may re-enroll into the study. Participants who received ARCT-021 of any dose (Study Groups 1, 2, 3) are asked to remain on the study schedule. Those participants who choose to re-enroll into the study complete early termination procedures and are assigned a new participant number when they re-enter the study. For this interim analysis, the data from re-enrolled participants is not included.

Vaccine doses for primary vaccination will be assigned as shown in Table 3-1.

Table 3-1 Study Group Assignments

<table>
<tr><th>Stratification Groups</th><th>Study Groups</th><th>Priming Vaccination 1 (Day 0)
Randomized 1:1:1:1</th><th>Priming Vaccination 2 (Day 28)</th><th colspan="3">Booster Vaccination (Day 208)
Booster randomization ratio: ARCT-021 : ARCT-154 : ARCT-165 : Placebo 1:1:1:1</th></tr>
<tr><td rowspan="6">Younger adults[a]

AND

Older adults[b]</td><td>1</td><td>ARCT-021 7.5 µg</td><td>Placebo</td><td colspan="3" rowspan="3">ARCT-021 5.0 µg
ARCT-154 5.0 µg
ARCT-165 5.0 µg
Placebo</td></tr>
<tr><td>2</td><td>ARCT-021 5.0 µg</td><td>ARCT-021 5.0 µg</td></tr>
<tr><td>3</td><td>ARCT-021 7.5 µg</td><td>ARCT-021 7.5 µg</td></tr>
<tr><td rowspan="6">4</td><td rowspan="6">Placebo</td><td rowspan="6">Placebo</td><td colspan="3">If <u>not</u> re-enrolled at Day 98-198[c]: Booster Vaccination (Day 208)
Booster randomization ratio:
ARCT-021 : ARCT-154 : ARCT-165 1:1:1 (No Placebo)</td></tr>
<tr><td colspan="3">ARCT-021 5.0 µg
ARCT-154 5.0 µg
ARCT-165 5.0 µg</td></tr>
<tr><td>If re-enrolled (Day 98-198)
Vaccination 1 (Day 0)
Groups 1, 2, and 3 (No Placebo)
Randomized 1:1:1</td><td>Vaccination 2 (Day 28)</td><td>Booster Vaccination (Day 208)
1:1:1:1
(Includes placebo)</td></tr>
<tr><td>ARCT-021 7.5 µg</td><td>Placebo</td><td rowspan="3">Placebo
ARCT-021 5.0 µg
ARCT-154 5.0 µg
ARCT-165 5.0 µg</td></tr>
<tr><td>ARCT-021 5.0 µg</td><td>ARCT-021 5.0 µg</td></tr>
<tr><td>ARCT-021 7.5 µg</td><td>ARCT-021 7.5 µg</td></tr>
</table>

a Younger adults: 18 to <56 years of age in US and 21 to <56 years of age in Singapore
b Older adults: ≥ 56 years of age
c See Section 3.1

4. General Statistical Considerations

Data from participants excluded from an analysis will be included in the applicable data listings but not included in the calculation of summary statistics for that analysis set.

For categorical variables, frequencies, percentages, and 95% CIs (where applicable) will be presented. All percentages will be rounded to one decimal place. The number and percentage will be presented in the form XX (XX.X), where the percentage is displayed in parentheses.

Continuous variables will be summarized using descriptive statistics (i.e., number of participants, mean, median, SD, minimum, maximum, and 95% CIs [where applicable]). All mean and median values will be formatted to one more decimal place than the actual value. Standard deviation values will be formatted to two more decimal places than the actual value. Minimum and maximum will be formatted to the same decimal place as the actual value.

When count data are presented, the percentage will be suppressed when the count is zero in order to draw attention to the non-zero counts. The denominator for all percentages will be the number of participants in the given treatment group within the analysis set of interest and stratification level (if applicable) unless otherwise specified in a footnote.

All CIs will be 2-sided and performed using a 5% significance level. No multiplicity adjustment will be made for this study where multiple treatment groups and endpoints are being evaluated.

All analyses adjusting for age group (≥ 18 to <56 years; ≥ 56 years) will use the values derived from age reported in the interactive response technology (IRT) which was used for randomization stratification.

Early termination visits will be summarized separately for the priming vaccination period and the booster vaccination period, respectively.

Analysis will be presented by study phase and study treatment group where applicable as per Table 4-1 below:

Table 4-1 Study Phase and Study Treatment Groups

Study Phase	Study Treatment Groups	Shell Presentation of Treatment Group
Priming Vaccination	ARCT-021 7.5 µg - Placebo	A
	ARCT-021 5.0 µg - ARCT-021 5.0 µg	B
	ARCT-021 7.5 µg - ARCT-021 7.5 µg	C
	Placebo	D
Booster Vaccination	ARCT Priming - ARCT-021 Booster	E1
	Placebo Priming - ARCT-021 Booster	E2
	ARCT Priming - ARCT-154 Booster	F1
	Placebo Priming - ARCT-154 Booster	F2
	ARCT Priming - ARCT-165 Booster	G1
	Placebo Priming - ARCT-165 Booster	G2
	ARCT Priming - Placebo/No Booster	H1
	Placebo Priming - No Booster	H2
Priming Vaccination (Re-enrolled Subjects Only)	ARCT-021 7.5 µg - Placebo	A
	ARCT-021 5.0 µg - ARCT-021 5.0 µg	B
	ARCT-021 7.5 µg - ARCT-021 7.5 µg	C

For some summary tables of booster vaccination period, active boosters will be pooled, and treatment groups will be presented like the following:

Booster Vaccination	ARCT-021 7.5 µg - Placebo - ARCT Booster	A1
	ARCT-021 7.5 µg - Placebo - Placebo Booster/ No Booster	A2
	ARCT-021 5.0 µg - ARCT-021 5.0 µg - ARCT Booster	B1
	ARCT-021 5.0 µg - ARCT-021 5.0 µg - Placebo Booster/No Booster	B2
	ARCT-021 7.5 µg - ARCT-021 7.5 µg - ARCT Booster	C1
	ARCT-021 7.5 µg - ARCT-021 7.5 µg - Placebo Booster/No Booster	C2
	Placebo - ARCT Booster	D1
	Placebo - No Booster	D2

4.1. Baseline Definition

For both the priming vaccination phase and booster vaccination phase, the baseline is defined as the last non-missing assessment prior to the first priming study vaccine administration. Both scheduled and unscheduled visits and assessments will be used in determining baseline. The change from baseline is calculated as the post-baseline value minus the baseline value.

4.2. Sample Size

The sample size is based on clinical considerations to provide sufficient safety information for the analysis of the primary safety objective. With 75 participants randomly assigned to each priming vaccination dose group but with as few as 25 participants randomly assigned to individual booster vaccine groups Table 4-2 presents estimated probability to detect at least one adverse event at various incidence rates by possible sample size in this study. The estimated probability was calculated using a formula as below:

$$p = 1 - (1 - R)^N$$

where R = incidence rate and N = sample size.

If the incidence rate of an adverse event is 1%, the probability to detect one event in 75 vaccinated participants is 52.9%, and the probability of detecting one event in 25 vaccinated participants is 22.2%; however, this probability will be 95.1% in a sample size of 300 in each Age Cohort. See more details and scenarios in the table below.

Table 4-2 Probability to Detect at Least One Adverse Event by Incidence Rate of Event and Sample Size

Rate (%)	N=25	N=75	N=150	N=225	N=300
0.01	0.002497002	0.007472	0.014889	0.02225	0.029556
0.1	0.024702287	0.072291	0.139357	0.201574	0.259293
1.0	0.222178641	0.529413	0.778548	0.895788	0.950959
2.0	0.39653527	0.780236	0.951704	0.989386	0.997667
5.0	0.722610427	0.978656	>0.999	>0.999	>0.999
10.0	0.928210201	>0.999	>0.999	>0.999	>0.999

The study plans to randomize 75 participants to each Study Group within each Age Cohort. Assuming a geometric coefficient of variance (CoV) of 180% (SD of 1.2 on $\log_e$ scale), a sample size of 75:75 will provide a >99% power to detect 2-fold increase in antibody titers in ARCT-021 Study Groups versus placebo Study Groups. These power analyses were performed using PASS Version 15 software.

4.3. Study Blinding and Unblinding

The study is blinded to study site staff, participants, CRO staff and the sponsor up to Day 98 and again from Day 208 (administration of the booster) up to end of study. Between Day 98 and Day 208, a partial unblinding may occur as follows:

Between Day 98 and Day 198, certain site staff may become unblinded to some participants' treatment assignment to determine eligibility for re-enrollment (Section 3.1). At this point, these site staff will know whether the participants in question received active study vaccine or placebo during the initial priming vaccinations. The blinded CRO team will also be unblinded to who received active study vaccine or placebo for re-enrolled participants due to the ability to link the new re-enrolled participant ID with the previous participant ID in the database. At Day 208, participants are re-randomized to booster vaccination, which will be administered in a blinded fashion. The blinded site and CRO staff, the Sponsor team providing direct oversight of the study, and the SRC will remain blinded to individual participant vaccine assignments for the duration of the booster phase of the study. The identification of Sponsor team members and blinding status will be documented in an unblinding memo.

The study vaccines will be administered in an observer-blind fashion.

Each of the study vaccines will be prepared by an unblinded pharmacist and in accordance with the Pharmacy Manual.

Unblinded personnel (of limited number) will be assigned to vaccine accountability procedures and will prepare study vaccine for all participants. These personnel will have no study functions other than study vaccine management, documentation, accountability, preparation, and administration. They will not be involved in participant evaluations and will not reveal the identity of study vaccine to either the participant or the blinded study site personnel involved in the conduct of the study unless this information is necessary in the case of an emergency.

- Unblinded health care providers will administer the study vaccine. They will not be involved in assessments of any study endpoints.
- Unblinded site monitors, not involved in other aspects of monitoring, will be assigned as the study vaccine accountability monitors. They will have responsibilities to ensure that sites are following all proper study vaccine accountability, preparation, and administration procedures.
- An unblinded statistical and programming team will perform the pre-planned interim analyses.
- A DSMB will review the interim data to safeguard the interests of clinical study participants and to help ensure the integrity of the study. The DSMB will review unblinded statistical outputs and interim analysis results, provided by the unblinded statistician, and make recommendations to the Sponsor.

In order to maintain an observer-blind design, investigators, site staff, participants, and CRO staff with oversight of study conduct will remain blinded to vaccine assignments for the study

duration with the exception of the partial unblinding of required site staff, participants and CRO staff for re-enrollment purposes.

The plan to protect the blind and identification of unblinded Sponsor and PPD team members are documented in the following blinding plans:

- Arcturus Sponsor Blinding Plan - Unblinding Plan for Interim Analyses, V3.0, 15Mar2022
- PPD B&P Blinding Plan – ARCT-021-04_Biostatistics and Programming Blinding Plan_V2.0 dated 20Jul2021.

4.4. Study Phases and Analysis Sets

There are two study phases defined:

- Priming Vaccination Period: All timepoints up through Day 208 (prior to booster vaccination).
- Booster Vaccination Period: All timepoints from Day 208 through Early Termination.

The following analysis sets are defined for the priming vaccination period: Randomized, Intent-to-Treat, Modified Intent-to-Treat, Per-protocol, CMI Subset, Safety, and Reactogenicity. A Randomized set, Reactogenicity Set, Safety, Modified Intent-to-Treat, CMI Subset set are defined for the booster vaccination period.

4.4.1. Randomized Analysis Set

The Randomized analysis set consists of all participants who are randomized, regardless of the participant's study treatment status in the study. Participants will be analyzed according to the study treatment group to which they were randomized.

- Note: The Randomized analysis set for re-enrolled participants consists of all re-enrolled participants who were re-randomized, regardless of the participant's study treatment status in the study.

4.4.2. Intent-to-Treat Analysis Set

The Intent-to-Treat (ITT) analysis set includes all participants who receive at least 1 dose of priming study vaccine (ARCT-021 or placebo). Participants will be analyzed according to the vaccine to which the participant was randomly assigned.

- Note: The ITT analysis set for re-enrolled participants consists of all re-enrolled participants who receive at least 1 dose of study vaccine while as a re-enrolled participant.

4.4.3. Modified Intent-to-Treat Analysis Set

The Modified Intent-to-Treat (mITT) analysis set includes all participants who received at least one dose of study vaccine and who have pre- and at least one post-vaccination immunogenicity data evaluable by the assay in use with valid results for the relevant timepoint(s). The mITT analysis set will be analyzed according to vaccine assigned.

- Note: The mITT analysis set for re-enrolled participants consists of all re-enrolled participants who received at least one dose of study vaccine and who have pre- and at least one post-vaccination immunogenicity data evaluable by the assay in use with valid results while as a re-enrolled participant. Immunogenicity data for re-enrolled participants will only be listed (i.e., will not be included in the summary tables).

4.4.4. Per-Protocol Analysis Set

The Per-Protocol analysis set includes all eligible randomized participants who receive the correct assigned dose(s) of assigned study vaccine within the predefined window (see Table 4-3), have blood collection with valid immunogenicity results within the predefined window (see Table 4-4) and have no other major protocol deviations expected to affect immunogenicity, as determined by the Sponsor Medical Monitor in a blinded manner.

Table 4-3 Study Vaccine Dosing Windows – Priming Vaccination Period

Visit	Window
Dose 1 Administration (Day 0)	0
Dose 2 (Day 28)	+/- 3 days

Table 4-4 Blood Sampling for SARS-CoV-2 Antibodies and CMI Windows – Priming Vaccination Period

Visit	Window
Day 56	+/- 7 days

4.4.5. CMI Subset

The CMI subset includes all participants who have evaluable pre- and at least one post-vaccination CMI data available. The data sets include: CD4+ T-cell responses (background subtracted), CD8+ T-cell responses (background subtracted), and Th1/Th2 CD4+ T-cell responses (background subtracted).

Analyses on the CMI subset of the mITT (mITT-CMI) and PP (PP-CMI) analysis sets will be presented (see section 8.4 for more details).

4.4.6. Safety Analysis Set

The Safety analysis set includes all participants who receive at least 1 dose of priming study vaccine (ARCT-021 or placebo). Participants will be analyzed according to the vaccine received.

- Note: The Safety analysis set for re-enrolled participants consists of all re-enrolled participants who receive at least 1 dose of study vaccine while as a re-enrolled participant.

4.4.7. Reactogenicity Analysis Set

The Reactogenicity analysis set (RAS) includes all participants who receive any dose of priming study vaccine (ARCT-021or placebo) and provide at least 1 reactogenicity diary report for the time period evaluated. Participants will be analyzed according to the vaccine received.

- Note: The RAS for re-enrolled participants consists of all re-enrolled participants who receive any dose of study vaccine while as a re-enrolled participant and provide at least 1 reactogenicity diary report for the time period evaluated.

The following analysis sets will be applicable for the Booster Vaccination Period:

4.4.8. Booster Randomized Analysis Set

The Booster Randomized analysis set consists of all participants who are randomized to a booster vaccination, regardless of the participant's study treatment status in the study. Participants will be analyzed according to the study vaccine to which they were randomized for receipt of booster vaccine.

- Note: The Booster Randomized analysis set for re-enrolled participants consists of all re-enrolled participants who were randomized to a booster vaccination, regardless of the participant's study treatment status in the study.

4.4.9. Booster Safety Analysis Set

The Booster Safety analysis set includes all Safety Set participants who receive at least 1 dose of booster study vaccine (ARCT-021, ARCT-145, ARCT-156 or placebo) or have safety data collected in the period following booster administration. If a participant did not receive a booster dose but has safety data collected for the booster vaccination period, the participant is summarized with the placebo booster group. Participants will be analyzed according to the vaccine actually received.

- Note: The Booster safety analysis set for re-enrolled participants consists of all re-enrolled participants who receive at least 1 dose of booster study vaccine while as a re-enrolled participant or have safety data collected in the booster vaccination period while as a re-enrolled participant.

4.4.10. Booster Reactogenicity Analysis Set

The Booster Reactogenicity analysis set includes all participants who receive any dose of booster vaccination (ARCT-021, ARCT-145, ARCT-156 or placebo) and provide at least 1 reactogenicity diary report for the time period evaluated. Participants will be analyzed according to the vaccine actually received.

- Note: The Booster RAS for re-enrolled participants consists of all re-enrolled participants who receive any dose of booster vaccination while as a re-enrolled participant and provide at least 1 reactogenicity diary report for the time period evaluated.

4.4.11. Booster Intent-to-Treat Analysis Set

The Booster Intent-to-Treat (Booster ITT) analysis set includes all ITT participants who receive at least 1 dose of booster study vaccine (ARCT-021, ARCT-145, ARCT-156 or placebo) or have immunogenicity data collected in the period following booster administration. If a participant did not receive a booster dose but has immunogenicity data collected for the booster vaccination period, the participant is summarized with the placebo booster group. Participants will be analyzed according to the vaccine assigned.

4.4.12. Booster Modified Intent-to-Treat Analysis Set

The Booster Modified Intent-to-Treat (Booster mITT) analysis set includes all mITT participants who has at least one immunogenicity data evaluable by the assay in use with valid results during the booster vaccination period. Participants will be analyzed according to the vaccine assigned. If a participant was not randomized to a booster dose on the Day 208 visit but has immunogenicity data collected during the booster vaccination period, the participant will be summarized with the placebo booster group.

- Note: The Booster mITT analysis set for re-enrolled participants consists of all re-enrolled participants who has at least one immunogenicity data evaluable by the assay in use with valid results during the booster vaccination period while as a re-enrolled participant. Immunogenicity data for re-enrolled participants will only be listed (i.e., will not be included in the summary tables).

4.4.13. Booster Per-Protocol Analysis Set

The Booster Per-Protocol (Booster PP) analysis set includes all eligible randomized participants who receive the correct assigned dose(s) of assigned study vaccine (including booster vaccine) within the predefined window (see Table 4-5), have blood collection with valid immunogenicity results within the predefined window (see Table 4-6) and have no other major protocol deviations expected to affect immunogenicity, as determined by the Sponsor Medical Monitor in a blinded manner. All participants in the Booster per-protocol analysis set will be analyzed according to the study vaccine that was received.

Table 4-5 Study Vaccine Dosing Windows – Booster Vaccination Period

Visit	Window
Booster (Day 208)	No Window

Table 4-6 SARS-CoV-2 Antibodies and CMI Blood Sampling Windows – Booster Vaccination Period

Visit	Window
Day 236 (28 days post-booster vaccination)	+/- 7 days

4.4.14. Booster Cell-Mediated Immunity Analysis Subset

The Booster CMI analysis subset includes all priming period CMI analysis subset participants who has at least 1 CMI data evaluable during the booster vaccination period. The data sets include: CD4+ T-cell responses (background subtracted), CD8+ T-cell responses (background subtracted), and Th1/Th2 CD4+ T-cell responses (background subtracted).

Analyses on the Booster CMI subset of the Booster mITT (Booster mITT-CMI) analysis set will be presented (see section 8.4 for more details).

4.5. Treatment Misallocation

In a blinded fashion, it was determined that there are 4 scenarios observed for 5 participants in the final data with treatment misallocation where the vaccine combination/sequence received differs from the randomized treatment group:

Randomized Treatment Group (Priming Vaccination)	Treatment Misallocation Details	Treatment Group to be Used for Safety Set and Reactogenicity Set for Priming Vaccination
7.5 µg ARCT-021 Single Dose	Non-placebo received at Dose 2	7.5 µg ARCT-021 Two Dose
7.5 µg ARCT-021 Two Dose	Placebo received at Dose 2	7.5 µg ARCT-021 Single Dose
5.0 µg ARCT-021 Two Dose	Placebo received at Dose 2	7.5 µg ARCT-021 Single Dose
7.5 µg ARCT-021 Single Dose	Placebo received at Dose 1*	Placebo

* Note that it was confirmed placebo was received at Dose 2 as planned.

4.6. Missing Data Handling and Imputation Rules

No missing safety and reactogenicity results will be imputed. Incomplete AE onset and end dates will be imputed according to the rules specified in Appendix 2. For safety analysis, percentages will be based on the analysis set of interest (e.g., Safety Set) within each treatment group and overall for the relevant vaccination/period of interest. For reactogenicity analysis, the denominator will be number of participants in the reactogenicity analysis set for the relevant vaccination/period of interest.

Any immunogenicity results that are below the limit of detection (LOD) or lower limit of quantification (LLOQ) will be imputed as half of the corresponding limit and results above the upper limit of quantification will be imputed as that limit for analysis purposes.

5. Participant Disposition and Protocol Deviations

5.1. Participant Disposition

The number and percentage of participants in the following categories (analysis sets defined in Section 4.4) will be summarized based on the following (where applicable):

- Randomized Set
- Intent-to-Treat Set
- Modified Intent-to-Treat Set (mITT)
- Per-Protocol Set (PP)
- Cell Mediated Immunity Analysis Subsets (CMI)
    - mITT-CMI
    - PP-CMI
- Safety Set

- Reactogenicity Set (Dose 1)
- Reactogenicity Set (Dose 2)
- Booster Randomized Set
- Booster Safety Set
- Booster Reactogenicity Set
- Booster Intent-to-Treat Set (Booster ITT)
- Booster Modified Intent-to-Treat Set (Booster mITT)
- Booster Per-Protocol Set (PP)
- Booster Cell Mediated Immunity Subset (Booster CMI)
    - Booster mITT-CMI
    - Booster PP-CMI

The number of participants in the following categories will be summarized based on participants screened:

- Number of participants screened
- Number and percentage of screen failure participants and the reason for screen failure

The number and percentage of participants in each of the following disposition categories will be summarized based on the Randomized set for the priming vaccination period:

- Received each dose of study vaccine for priming vaccination dose 1 and dose 2
- Prematurely discontinued before receiving the second dose of study vaccine and the reason for discontinuation
- Prematurely discontinued before receiving the booster dose of study vaccine and the reason for discontinuation
- Completed study
- Prematurely discontinued the study and the reason for discontinuation

A participant disposition listing will be provided, including informed consent, participants who completed the study injection schedule, participants who completed study, participants who discontinued from study vaccine or who discontinued from participation in the study, with reasons for discontinuation. Randomization data will be also provided in a listing.

A table summarizing the frequencies and percentages for the following will be provided by the primary vaccination treatment groups for Safety Analysis set:

- Participants who have completed booster vaccination and who are followed for 56 days post booster vaccination or less

- Participants who have completed booster vaccination and who have been followed longer (> 56 days but < 180 days after booster dose)

- Participants who have completed primary vaccination and who have been followed < 12 months

A table summarizing the frequencies and percentages for the following will be provided by the primary vaccination treatment groups for re-enrolled Safety Analysis set:

- Participants who have completed primary vaccination and who have re-enrolled and with follow-up < 12 months

- Participants who have completed primary vaccination, who have re-enrolled and who have received a booster dose with follow-up > 56 days but < 180 days.

A separate listing will be provided for screen failure participants with reasons for screen failure.

For re-enrolled subjects, disposition will be summarized and listed separately based on the Randomized set. For the booster vaccination period, the number of participants randomized to booster and the number of participants in the Booster Safety and Booster Reactogenicity sets will be summarized.

5.2. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. Deviations are classified as significant or non-significant based on the following definition:

- A significant protocol deviation affects primary efficacy and safety assessments, the safety or mental integrity of a participant, or the scientific value of the trial/project.

- A major protocol deviation is defined as a deviation that affects primary immunogenicity assessments and results in subject being removed from per-protocol analysis set.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The investigator will be notified in writing by the monitor of deviations. The human research ethics committee/IRB should be notified of all protocol deviations, if

appropriate, in a timely manner. Protocol deviations will be reviewed prior to any formal analyses.

Significant and major protocol deviations will be summarized (separately) by deviation type (e.g., missed assessment) and subtype for the Randomized analysis set. Only significant and major PDs are summarized. Reasons for exclusion from PP Set and Booster PP Set (e.g., major protocol deviations, no assigned dose of assigned study vaccine within the predefined window, no blood collection with valid immunogenicity results within the predefined window) will also be summarized for the Randomized analysis set and Booster Randomized analysis set, respectively.

All protocol deviations will be presented in a data listing. A separate listing of all protocol deviations for re-enrolled Randomized analysis set will also be provided. Details of admission criteria deviations will be presented in a separate data listing.

6. Demographics and Baseline Characteristics

6.1. Demographics

Descriptive statistics will be calculated for the following continuous demographic and baseline characteristics: age (years), weight (kg), height (cm), body mass index (BMI) (kg/m^2). The number and percentage of participants will be provided for categorical variables such as age group, sex, race, ethnicity, country, site, baseline SARS-CoV-2 serostatus (positive/negative/missing) defined by nucleocapsid testing at Day 0, and evidence of viral shedding as measured by a saliva RT-PCR test at Baseline.

Baseline SARS-CoV-2 serostatus is defined as SARS CoV-2 nucleocapsid (N) antibody level of greater or equal to 5000 (AU/mL). This threshold is defined by BioA laboratories based on levels for a clinical definition of PCR-confirmed COVID-19 infection.

The number and percentage of participants will be summarized by site and by stratification factor at randomization (i.e., ≥ 18 to <56 years; ≥ 56 years) separately based on the Safety set for the priming vaccination period, re-enroll subjects, and booster vaccination period.

Summaries will be provided separately based on the Safety set, mITT set, PP set, mITT-CMI subset by study treatment group. The summaries will be repeated by study treatment group for re-enrolled subjects based on Safety set and for the booster vaccination period based on the Booster Safety set, Booster mITT set, Booster PP set, Booster mITT-CMI subset. Summary of demographics by ≥ 18 to <65 years vs. ≥ 65 years will also be provided for the priming vaccination period.

For screened failure participants, age (years), gender, race and ethnicity will be presented in a listing.

6.2. Medical History

Medical history will be coded using MedDRA version 23.0 or higher.

The number and percentage of participants with any medical history will be summarized by SOC and PT based on Safety Set. A participant will be counted only once for multiple events within each SOC and PT. SOC will be displayed in internationally agreed order. Medical history will also be presented in a data listing.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Prior and concomitant medications and non-study vaccinations will be coded using the WHO drug dictionary (WHODrug-Global-B3 202003). The summary of concomitant medications will be based on the Safety set (separate summary will also be provided for the Booster Safety Set). Categorization of prior, concomitant, and post medications is summarized in Appendix 2.

The number and percentage of participants using concomitant medications will be summarized by study treatment group as follows:

- All concomitant medications overall for priming vaccination period, concomitant medications within 7 days after either priming dose of study vaccine, and concomitant medications within 28 days after either priming dose of study vaccine
- All concomitant medications taken through 7 days after each priming dose of study vaccine separately
- All concomitant medications overall for booster vaccination period, concomitant medications within 7 days after booster dose of study vaccine, and concomitant medications within 28 days after booster dose of study vaccine.

For re-enrolled subjects, similar summaries as above will be performed for the priming vaccination period only.

Prior, concomitant and post medications will be presented in a listing.

7.2. Medical and Surgical Treatment Procedures

The number and percentage of participants with at least one medical and surgical treatment procedure during the priming vaccination period will be summarized for the Safety Analysis set.

Indication (i.e., Adverse Event vs. Other) and whether the treatment/procedure is ongoing or not will also be summarized by frequency.

All medical and surgical treatment procedures will also be presented in a data listing; for re-enrolled subjects, a separate data listing will be provided.

7.3. Vaccine Administration and Exposure

Study vaccinations will be summarized including the number and percentage of participants receiving each dose, whether administered per protocol or not, or not administered for the Safety set.

All study drug administration data will also be presented in a data listing.

Vaccine administration will be summarized and listed separately for re-enrolled subjects.

8. Immunogenicity Analysis

8.1. Primary and Secondary Immunogenicity Endpoints

For both primary and secondary endpoints blood sampling for immunogenicity testing will be drawn according to the schedule of events (Appendix 1: Schedule of Study Procedures).

8.1.1. Primary Immunogenicity Endpoint

To address the primary objective of the study the neutralizing antibody responses of ARCT-021 will be compared to placebo for the priming vaccination period. SARS-CoV-2 neutralizing antibody (NAbs) titer will be tested using pseudoviral microneutralization assay (Wuhan-Hu-1), and, if available, PRNT assay. If assays are available, additional strain-specific neutralizing antibody testing may be performed. Neutralizing antibody (NAbs) responses will be evaluated on the mITT analyses set using the following estimands:

- Geometric Mean Titer (GMT) measured at all time points;
- Geometric Mean Fold-rise from baseline (GMFR); measured at all time points after baseline (= Day 0) (Estimand 2)
- Percentages of participants with ≥2- and 4-fold increase in titer from baseline (Seroconversion [SC]) measured at all time points after baseline (Estimand 3).

8.1.2. Secondary Immunogenicity Endpoint

To address the second objective of the study the binding antibody responses of ARCT-021 will be compared to placebo for the priming vaccination period. SARS-CoV-2 binding antibodies

(BAbs) will be tested using an MSD multiplex assay which measures total immunoglobulin G (IgG) against the full-length spike, RBD and N antigens of the Wuhan strain.

- IgG measured against the Nucleocapsid (N) antigen of the Wuhan strain SARS-CoV-2 (VAC72_ECL_CoV-2_N)
- IgG measured against the RBD antigen of the Wuhan strain SARS-CoV-2 (VAC72_ECL_CoV-2_RBD)
- IgG measured against the Spike antigen of the Wuhan strain SARS-CoV-2 (VAC72_ECL-CoV-2_S)

Similar estimands will be used as for the neutralizing antibody (NAbs).

8.2. Analysis of Immunogenicity Primary and Secondary Endpoints

8.2.1. Analysis of primary and secondary immunogenicity endpoints

Total NAbs and BAbs titers will be summarized using descriptive statistics (number of subjects (n), median, minimum and maximum) by each time point in priming vaccination period (Baseline, Days 14, 28, 42, 56, 208) and booster vaccination period (Day 208, 236, Early Termination). Summaries of priming vaccination and booster vaccination periods based on their corresponding mITT and PP analysis sets will be presented separately using the applicable treatment groups (Table 4-1). For the analysis of booster mITT analysis set, summary tables with active boosters pooled (as presented in Table 4-1; e.g., A1, A2, B1, B2, etc.) will also be presented.

The following statistics will be provided in the summary tables and/or figures:

- Geometric mean titer (GMT) with corresponding 95% CI will be provided at each time point. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale for presentation. GMT level and corresponding 95% CI will be plotted at each timepoint in priming vaccination period for mITT analysis set. Plots of the reverse cumulative distribution curves also will be provided by treatment group and visit in priming vaccination period for mITT analysis set.
- Geometric mean fold rise (GMFR) with corresponding 95% CI will be provided at each post-baseline timepoint over baseline. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale for

presentation. GMFR and corresponding 95% CI will be plotted at each timepoint in priming vaccination period for mITT analysis set.

- The number and percentage of subjects with fold-rise ≥ 2 and fold-rise ≥ 4 from baseline (Seroconversion) at each post baseline time points will be tabulated with the 2-sided 95% CI using the Clopper Pearson method at each post-baseline timepoint.

- Seropositivity at a participant level is defined as any detectable antibody level above the lower limit of quantification (LLOQ) for the specific assay and antibody measured at timepoints after baseline. Seropositivity rates will be provided with 2-sided 95% CI using the Clopper-Pearson method at each post-baseline time point.

The definition of seroconversion/seropositivity may depend on assay-specific performance characteristics. The conventions are as follows:

- Seropositivity is defined as any detectable antibody level above the lower limit of quantification (LLOQ) for the specific assay and antibody measured.

- For the MesoScaleDiscovery nucleocapsid antibody test (VAC72_ECL_CoV-2_N), seroconversion will be assessed in 2 ways:

    - Defined as a 2- or 4-fold increase above baseline (pre-vaccination antibody levels).

    - Based on detection of an antibody level ≥5000 AU/mL, a threshold associated with evidence of SARS-CoV-2 clinical infection/viral exposure.

- For all other assays and antibodies, seroconversion will be defined as a 2- or 4-fold increase above baseline (pre-vaccination antibody levels).

- For seronegative individuals, one half of the value of the LLOQ will be calculated as the baseline antibody level

The LLOQs for each assay are shown below.

- VAC62_MN_CoV-2 (IU/mL): 7.8431372549

- VAC72_ECL_CoV-2_N (BAU/mL): 0.06372

- VAC72_ECL_CoV-2_RBD (BAU/mL): 0.5168

VAC72_ECL_CoV-2_S (BAU/mL): 0.20723The GMT will be calculated using the following formula:

$$\text{GMT} = e^{\left\{\frac{\sum_{i=1}^{n} log_e(t_i)}{n}\right\}}$$

where t_1, t_2, … t_n are observed immunogenicity titers for n participants.

The GMFR measures the changes in immunogenicity titers within participants. The GMFR will be calculated using the following formula:

$$\text{GMFR} = e^{\left\{\frac{\sum_{i=1}^{n} \log_e {}^{v_{ij}}/_{v_{ik}}}{n}\right\}} = e^{\frac{\{\sum_{i=1}^{n}(\log_e v_{ij} - \log_e v_{ik})\}}{n}}$$

where, for n participants, v_{jj} and v_{ik} are observed immunogenicity titers for participant i at time points j and k=0 (Baseline, Day 0).

8.2.2. Supportive Analyses of Primary and Secondary Immunogenicity Endpoints

8.2.2.1. Supportive Analyses of Estimand 2

To assess the magnitudes of the differences between the treatment groups (ARCT-021 vs. Placebo) in SARS-CoV-2 neutralizing antibody (NAbs) and SARS-CoV-2 binding antibody (BAbs) endpoints, an analysis of covariance (ANCOVA) model will be fitted to the natural log transformed neutralizing antibody titers measured against SARS CoV 2 with terms for treatment group, age group (≥18 to <56 and ≥ 56 years) (stratification factor at randomization) and natural log baseline titer by each post-vaccination immunogenicity visit. No imputation of missing data will be done.

The geometric least squares mean (GLSM) and corresponding 2-sided 95% CI for the antibody titers for each treatment group will be provided by visit. The GLSM and corresponding 95% CI results in log-transformed scale estimated from the model will be back-transformed to obtain these estimates in the original scale. Geometric mean ratio (GMR), estimated by the ratio of GLSM and the corresponding 2-sided 95% CI will be provided to assess the treatment difference between ARCT-021 group vs. placebo group at each visit.

ANCOVA analyses will be performed for the priming vaccination period on mITT analysis set only.

8.2.2.2. Supportive Analyses of Estimand 3

The common difference of seropositivity and seroconversion rates and 95% CIs between each vaccine dose group and the placebo will be estimated using the Miettinen-Nurminen method with age group (≥18 to <56 and ≥ 56 years) as stratification factor for each time point.

Miettinen-Nurminen analyses will be performed for the priming vaccination period on mITT analysis set only.

8.2.2.3. Other Supportive Analyses

Subgroup analyses will be performed by gender, age group (≥ 18 to <56 years; ≥ 56 years), and country (US versus Singapore) for selected analyses as noted in Section 8.4. Age group will not be included in the model for ANCOVA analyses by age group. Subgroup analyses will be performed by "≥ 18 to <65 years vs. ≥ 65 years" age groups for selected analyses as noted in Section 8.4.

Box plots of titers and fold rise as well as plots of reverse cumulative distribution curves will be provided for each assay by treatment and by each of the subgroups as noted in Section 8.4.

A scatterplot for each of the SARS-CoV-2 binding antibodies and the neutralizing antibody levels at Day 56 will also be produced, both overall and by the subgroups as noted in Section 8.4.

8.3. Exploratory Immunogenicity Analyses

8.3.1. Assays for Response to SARS-CoV-2 Variants

To address the exploratory immunogenicity objective to evaluate responses to SARS-CoV-2 variants, the binding antibody responses of ARCT-021/ARCT-154/ARCT-165 will be compared to placebo for the booster vaccination period. SARS-CoV-2 binding antibodies (BAbs) will be tested using an MSD multiplex assay which measures total immunoglobulin G (IgG) against the full-length spike, RBD and N antigens of the beta strain. Neutralizing antibodies against the beta strain of SARS-CoV-2 will also be measured:

- IgG measured against the Nucleocapsid (N) antigen of the beta variant (B1.351) of SARS-CoV-2 (VAC83_ECL_CoV-2_N)
- IgG measured against the RBD antigen of the beta variant (B1.351) of SARS-CoV-2 (VAC83_ECL_CoV-2_RBD)

- IgG measured against the Spike antigen of the beta variant (B.1.351) of SARS-CoV-2 (VAC83_ECL-CoV-2_S_B.1.351)

- Microneutralization antibody concentration against the beta variant (B.1.351) of SARS-CoV-2 (VAC85_MN_CoV-2)

Similar estimands will be used as for the secondary objective.

The LLOQ for each assay is shown below:

- VAC83_ECL_CoV-2_N (AU/mL; 1:500 dilution): 27

- VAC83_ECL_CoV-2_RBD (AU/mL; 1:500 dilution): 19

- VAC83_ECL_CoV-2_S_B.1.351 (AU/mL; 1:500 dilution): 18

- VAC85_MN_CoV-2 (AU/mL; 1:5 dilution): 14

Assays for other variants may be analyzed as available as part of the exploratory analysis.

8.3.2. CMI Analysis

CMI responses will be evaluated for CMI subset using parametric flow cytometry for quantification and phenotyping and cytokine staining for TNF-α, IL-2, IFN-γ, IL-4, and IL-13. Blood sampling will be performed at Days 0, 14, 28, 42, 56, 208, 215, 236, and Early Termination in participants enrolled at centers identified with capabilities for isolation of peripheral blood mononuclear cells (PBMCs). CMI testing will not be performed on participants re-enrolling at Day 98-198 (i.e., placebo participants who re-enter the study to receive ARCT-021 priming vaccinations).

Descriptive statistics (N, mean, SD, min, Q1, median, Q3, max) will be provided for each visit including change from baseline at each timepoint, where CMI results are available for the priming vaccination period (mITT and PP analysis CMI subsets) and booster vaccination period (Booster mITT CMI subset). When summarizing the Early Termination visit, only include observations within the booster vaccination period post day 236. The following CMI responses will be summarized:

- CD4+ T-cell responses background subtracted (combined pools): IFN-γ, TNF-α, IL-2, IFN-γ+ TNF-α+, IFN-γ+ TNF-α+ IL-2+
- CD8+ T-cell responses background subtracted (combined pools): IFN-γ, TNF-α, IL-2, IFN-γ+ TNF-α+, IFN-γ+ TNF-α+ IL-2+

- Th1/Th2 CD4+ T-cell responses background subtracted (combined pools): IFN-γ, IL-4, IL-4+ IL-13+, IFN-γ+ / IL4+ IL13+

Box plots will be provided for the following for priming vaccination period (mITT CMI subset): CD4+/CD8+ IFNγ, IL4+IL13+, IFNγ+/IL4+IL13+, Th1/Th2.

Analyses will be performed overall and by age group (younger adults (≥18 - <56 years), older adults (≥56 years)) for priming vaccination period (mITT CMI subset).

Summary tables and figures will exclude any data points collected at or after the first occurrence of the following intercurrent events: death, missed second ARCT-021 (if applicable) dose, use of immune-modifying drugs, and study infection. Summary tables and figures will exclude all data from subjects with early (before Day 7), active or prior SARS-CoV-2 infection at first vaccination.

8.3.3. ACE2 Receptor Exploratory Analysis

Exploratory analysis of the following sVNT ACE2 receptor binding inhibition for three strains will be performed: VAC114_ECL_CoV-2_S, VAC114_ECL_CoV-2_S_B1.351, VAC114_ECL_CoV-2_S_B1.617.2. The analysis will be similar to the analysis specified for the primary and secondary immunogenicity endpoints (section 8.2.1) and will be performed for Priming (mITT analysis set) and Booster vaccination period (Booster mITT analysis set).

The LLOQ for each assay is shown below:

VAC114_ECL_CoV-2_S, VAC114_ECL_CoV-2_S_B1.351, and VAC114_ECL_CoV-2_S_B1.617.2: 0.78125 units/mL at the 1:10 dilution and 7.8125 units/mL at the 1:100 dilution

8.3.4. NAbs/BAbs Ratio Analysis

NAbs/BAbs ratio at each visit during priming vaccination period and booster vaccination period will be summarized using descriptive statistics by treatment group for the mITT and Booster mITT analysis sets, respectively. Binding antibody test results for both the spike protein and RBD will be included in this analysis. Neutralizing and binding antibody tests for two strains may be performed, but only NAbs/BAbs ratios derived from tests of the same strain of SARS-CoV-2 will be presented:

NAbs/BAbs Ratio	Parameter Codes	Analysis Period
Wuhan Neutralizing/Spike Binding	VAC62MN/VAC72S	Priming and Booster
Wuhan Neutralizing/RBD Binding	VAC62MN/VAC72R	Priming and Booster

Beta Neutralizing/Beta Spike Binding	VAC85MN/V83B1351	Booster Only
Beta Neutralizing/Beta RBD Binding	VAC85MN/VAC83RBD	Booster Only

8.4. Summary of Immunogenicity Endpoints Analyses

Parameter	Statistics[1]	Analysis set	Vacc. Period[2]	Age group <56 and ⩾ 56	Age group <65 and ⩾ 65	Gender	Country
Primary: SARS-CoV-2 Neutralizing Antibody (Estimands 2 and 3)	Table: Summary GMT/GMFR/SC/SP	mITT	P	x	x	x	x
		PP	P	x			
		Booster mITT	B	x	x		
		Booster PP	B	x			
	Figure: GMT Over Time	mITT	P	x		x	x
	Figure: GMFR Over Time	mITT	P	x		x	x
	Figure: RCDF at each time point	mITT	P	x		x	x
	Table: ANCOVA Model	mITT	P	x		x	x
	Table: Miettinen-Nurminen for SC/SP Rates	mITT	P				
Secondary: SARS-CoV-2 Binding Antibody (Estimands 2 and 3)	Table: Summary GMT/GMFR/SC/SP	mITT	P	x	x	x	x
		PP	P	x			
		Booster mITT	B	x	x		
		Booster PP	B	x			
	Figure: GMT Over Time	mITT	P	x		x	x
	Figure: GMFR Over Time	mITT	P	x		x	x
	Figure: RCDF at each time point	mITT	P	x		x	x
	Figure: scatterplot binding/neutralizing antibody	mITT	P	x		x	x
	Table: ANCOVA Model	mITT	P	x		x	x
	Table: Miettinen-Nurminen for SC/SP Rates	mITT	P				
Exploratory: CMI	CMI responses	mITT-CMI	P	x			
		PP-CMI	P	x			
		Booster mITT-CMI	B	x			

	Box plots: CD4+/CD8+ IFNγ, IL4+IL13+, IFNγ+/IL4+IL13+, Th1/Th2	mITT-CMI	P	x			
Exploratory sVNT (ACE2 receptor binding inhibition)	Table: Summary GMT/GMFR/SC/SP	mITT	P				
		Booster mITT	B				
NAbs/BAbs ratio	Table: Summary NAbs/BAbs Ratio	mITT	P				
		Booster mITT	B				

[1]BAbs = Binding Antibodies, NAbs = Neutralizing Antibodies, GMT=Geometric mean titers, GMFR=Geometric Mean Fold-rise, SC=Seroconversion, SP=Seropositivity, RCDF=Reverse Cumulative Distribution Function.
[2]P=Priming Vaccination Period: All timepoints up through Day 208 (prior to booster vaccination). B=Booster Vaccination Period: All timepoints from Day 208 through Early Termination.

9. Safety Analysis

All safety summaries and analyses will be conducted for the Safety analysis sets. All safety data collected will be summarized by defined duration relative to each vaccination, as well as for overall study by treatment group.

Safety and reactogenicity will be assessed by clinical review of all relevant parameters including solicited local and systemic AE, unsolicited AEs, new onset of chronic disease (NOCD), medically attended adverse events (MAAEs), AEs leading to next vaccine delayed or vaccine will not be re-dosed and SAE through early termination. Any AE that leads to discontinuation of the vaccine and/or withdrawal from the study will be regarded as an AE leading to discontinuation/withdrawal.

9.1. Adverse Events

Adverse Event (AE) collection will begin after the signing of informed consent; however, AEs that occur prior to the administration of first dose of study vaccine (i.e., non-treatment-emergent AEs) will be listed in the clinical study report. MedDRA version 23.0 or higher will be used for coding to System Organ Class (SOC) and Preferred Term.

Any case of confirmed COVID-19 or asymptomatic SARS-CoV-2 infection will be regarded as a Clinical Event of Special Interest (CESI) and entered on the CRF as an Adverse Event. Because CESI events are typically associated with the disease under study, they will not be reported as suspected unexpected serious adverse reaction (SUSAR) but on the CESI eCRF page.

Summaries of number of participants (%) with solicited AEs (by toxicity grade), safety laboratory assessments (by toxicity grade), unsolicited AEs (by severity and relatedness to study vaccine), MAAEs, NOCDs, and AEs leading to discontinuation/withdrawal will be presented and

also with a focus on those related to vaccine administration. All summaries will be evaluated by study treatment group as well as vaccine administration (first dose, second dose, booster dose).

Incomplete AE onset and end dates will be imputed according to the rules specified in Appendix 2.

9.1.1. Reactogenicity

Any solicited local or systemic AE initiation within 7 days after each priming vaccination will be summarized by vaccination and by toxicity grade as number and percentage of participants. A summary by grade group (Grade 0 through 4 and Grade 1 through 4) will also be presented with 2-sided 95% CI using the Clopper-Pearson method. Grade 0 is a reported symptom lower than Grade 1. The duration of solicited local and systemic AEs after each vaccination will also be summarized. For injection site erythema, injection site induration/swelling, and diarrhea the duration for events graded 0 to 4 will be summarized. For all other solicited events, the duration for events graded 1 to 4 will be summarized.

Solicited local and systemic AEs through 7 days following each study vaccination will be evaluated by the participant and recorded in the diary as the following:

- Solicited local AEs: injection site erythema, injection site pain, injection site induration/swelling, injection site tenderness
- Solicited systemic AEs: arthralgia, chills, diarrhea, dizziness, fatigue, fever (categorized by measured body temperature), headache, myalgia, nausea/vomiting

Data will also be summarized by age group (≥ 18 to <56 years; ≥ 56 years).

The toxicity grade will be categorized by application of the DHHS CBER Toxicity Grading Scale. Participants will be counted once for the most severe event in cases where the participant reported more than one event per solicited AE category (e.g., pain).

For re-enrolled subjects and the booster vaccination period, summary of solicited AEs within 7 days post each vaccination by toxicity grade and age group will also be performed. In addition, a summary of solicited adverse events within 7 days post booster vaccination by grade and age group with active boosters pooled (as presented in Table 4-1; e.g., A1, A2, B1, B2, etc.) will be presented.

In addition, summary of solicited adverse events within 7 days post each priming vaccination by grade group and age group (≥ 18 to <65 years; ≥ 65 years) for Reactogenicity analysis set will provided.

9.1.2. Unsolicited Adverse Events

Unsolicited AEs that meet the protocol definition of MAAE, NOCD, SAE, AEs leading to delay in dosing of the next vaccine, or AEs leading to termination of vaccine dosing will be captured for the duration of study participation. NOCD are defined as a subset of MAAEs that are specific to new onset of chronic diseases not otherwise suggested or suspected prior to entry into the study. Continuing solicited AEs after 7 days will be captured as unsolicited AEs and followed until stabilization/resolution.

Only treatment emergent adverse events (TEAEs) will be included in summary tables. Percentages will be based upon the number of participants in the Safety analysis set within each treatment group. A participant with 2 or more AEs within the same level of summarization (e.g., SOC or PT) will be counted only once in that level.

9.1.2.1. Unsolicited AEs through 28 Days Post Each Vaccination

Summaries of unsolicited AEs through 28 days post each vaccination dose will be presented using the following definition:

- **Vaccination 1**: Time period from first vaccination through prior to the second vaccination for participants that received the second vaccination and through 28 days following the first vaccination for participants that did not receive the second vaccination
- **Vaccination 2**: Time period from second vaccination through 28 days following the second vaccination
- **Booster Vaccination**: Time period from booster vaccination through 28 days following the booster vaccination.

The summaries for Vaccination 1 and Vaccination 2 will be by the 4 priming vaccination treatment groups for the priming vaccination period while the summaries for booster vaccination period will be by the 8 priming-booster vaccination treatment groups and for the combined E1+E2+…+G1+G2 and H1+H2 groups (Table 4-1).

For re-enrolled subjects, the above summary for the priming vaccination phase will be repeated.

An overall summary of unsolicited AEs through 28 days post vaccination will be produced for all participants and by age group including the number and percentage of participants with any of the following (including 2 sided 95% CIs using the Clopper-Pearson method):

- Any unsolicited TEAEs
- Any severe unsolicited TEAEs
- Any unsolicited TEAEs that are treatment related

- Any severe unsolicited TEAEs that are treatment related
- Any serious TEAEs
- Any treatment-emergent MAAEs
- Any treatment-emergent NOCDs
- Any TEAEs leading to discontinuation/withdrawal
- Any TEAEs leading to death

In addition, incidence of unsolicited AEs through 28 days post each vaccination will be summarized by SOC and PT including number and percentage of participants with an event for all participants and by age group for the following:

- All unsolicited AEs
- All unsolicited AEs by severity
- All unsolicited AEs by relationship to treatment

For re-enrolled subjects, the above summaries will be repeated for the Safety set. A summary of incidence of unsolicited adverse events up to 28 days post booster vaccination with active boosters pooled will be presented.

Incidence tables will be presented by SOC and PT with counts of participants included. SOC will be displayed in internationally agreed order. PT will be displayed in descending order of frequency of treatment group and then alphabetically within SOC. When summarizing the number and percentage of participants with an event, participants with multiple occurrences of the same AE or a continuing AE will be counted once. Only the maximum severity level will be presented in the severity summaries, and the strongest relationship level will be presented in the relationship summaries. If the severity information is missing, the AE will be considered severe in the summary, but the severity will be presented as missing in the data listings.

9.1.2.2. Unsolicited AEs by Time Period

Summaries of unsolicited AEs by time period will be presented using the following definition:

- **Prior to Booster Vaccination** summary tables include the following time periods with data summarized by the 4 priming vaccination treatment groups:
    - **Vaccination 1 through prior to Vaccination 2:**

        - For participants who receive vaccination 2, this is the time period from first vaccination through prior to second vaccination
        - For participants who do not receive vaccination 2 but do receive a booster vaccination, this is the time period from first vaccination through prior to the booster vaccination
        - For participants who only received the first vaccination, this is the time period from first vaccination until early termination or end of the priming period (Day 207), whichever is earlier
    - **Vaccination 2 through prior to Booster Vaccination:**
        - For participants who receive the booster vaccination, this is the time period from the second vaccination through prior to the booster vaccination
        - For participants who do not receive the booster vaccination, this is the time period from second vaccination until early termination or end of the priming period (Day 207), whichever is earlier
    - **Prior to Booster Vaccination - Overall**
        - This is the time period from first vaccination through prior to booster vaccination. For participants who do not receive a booster vaccination, this is the time period from first vaccination through early termination or end of the priming period (Day 207), whichever is earlier.
- **Post Booster Vaccination through Early Termination** summary tables include data summarized by the 8 priming-booster treatment groups as well as a summary for the combined E1+E2+…+G1+G2 and H1+H2 groups. For subjects who do not receive a booster vaccination, this is the time period from the start of the booster period (Day 208) through early termination.
    - An overall summary of unsolicited adverse events post booster vaccination with active boosters pooled (as presented in Table 4-1; e.g., A1, A2, B1, B2, etc.) will also be presented.

The summaries described in Section 9.1.2.1 will be produced by time period and by time period and age group. In addition, the following summaries will be presented by time period and by time period and age group:

- Overall summary and incidence of treatment emergent MAAE
- Overall summary and incidence of treatment emergent NOCDs
- Incidence of treatment emergent serious AEs
- Incidence of treatment emergent AEs leading to discontinuation/withdrawal

Individual participant adverse event data will be provided in data listings for the following:

- All unsolicited adverse events
- Serious unsolicited adverse events
- Medically attended adverse events (MAAE)
- New onset of chronic disease (NOCD)
- Adverse events leading to discontinuation/withdrawal

In addition, overall summary and incidence of unsolicited adverse events prior to booster vaccination by time period and age group (≥ 18 to <65 years; ≥ 65 years) for Safety analysis set will provided.

9.2. Vital Sign Measurements

The vital signs, body temperature and pulse oximetry will be collected at scheduled timepoints through the trial. Actual values, changes from baseline and abnormal toxicity grading will be summarized by study treatment group and age group for the priming vaccination period using descriptive statistics. Shift in toxicity grades from baseline will be summarized by visit and treatment group for all participants using the frequency count and percentage of participants in each category.

For re-enrolled subjects and the booster vaccination period, separate summaries will be produced for abnormal vital sign assessments by toxicity grading and age group. All data will be included in data listings.

9.3. Clinical Laboratory Evaluations

Clinical chemistry and hematology will be collected at scheduled timepoints. Actual values, changes from baseline, and abnormal toxicity grading for clinical safety laboratory assessment results will be summarized by study treatment group and age group for the priming vaccination period at each timepoint using descriptive statistics. Abnormal toxicity grading is defined as a toxicity grade equal or greater than Grade 1. Shift in toxicity grades from baseline will be summarized by visit and treatment group for all participants using the frequency count and percentage of participants in each category. Shift table for Neutrophils only will also be presented by visit and treatment group for all participants and by race using the frequency count and percentage of participants in each category.

For re-enrolled subjects and the booster vaccination period, separate summaries will be produced for abnormal chemistry and hematology by toxicity grading and age group. All data will be included in data listings.

9.4. COVID-19 exposure and symptoms

The number and percentage of participants reporting symptoms of potential COVID-19 (Yes/No/missing) from CRF page SARS-CoV-2 or COVID-19 Symptoms Assessment will be summarized by treatment groups for the Safety analysis set. For participants reporting 'Yes', all symptoms will also be summarized by frequency.

For participants identified as on-study infection, the number and percentage of participants reporting 'Yes' to the question Any Symptom of Confirmed COVID-19 from CRF page Clinical Event of Special Interest will be reported by treatment groups and by each priming vaccination period. All symptoms will also be summarized by frequency.

Incidence of COVID-19 and incidence of asymptomatic infection based on the Adverse Event CRF page will be summarized for the following time periods: after first study vaccine dose to 14 days after first study vaccine dose, between 14 days after first study vaccine dose to 2nd study vaccine dose, and after 2nd study vaccine dose to booster vaccine / early termination prior to Day 208 visit. This will also be summarized for re-enrolled subjects separately.

Incidence of COVID-19 and incidence of asymptomatic infection after booster vaccine to early termination after booster vaccine based on the Adverse Event CRF page will be summarized. For subjects not receiving a booster vaccination, events from Day 208 through early termination will be summarized under the Booster Placebo/No Booster group.

9.5. Physical Examinations and Emergency Room and/or Hospitalization for COVID-19 Assessment

Listings of complete physical examinations and symptom directed physical examination will be provided. Listings of emergency room visits and/or hospitalization for COVID-19 assessment will be provided.

10. Interim analyses

Three interim analyses will be performed.

- The first interim analysis will focus on safety and will evaluate available safety data for all participants who were initially enrolled as of 05 February 2021 when they have completed Day 7 or Early Termination visit procedures.

- The second interim analysis will evaluate available safety and immunogenicity data for all participants who were initially enrolled as of 05 February 2021 and when they have completed Day 28 or Early Termination visit procedures.

- The third interim analysis will evaluate available safety and immunogenicity data for all participants after they have completed Day 56 or Early Termination visit procedures.

At each of these interim analyses, the datasets from immunogenicity assessments may not contain all participants' data. However, the data cuts outlined above are intended to inform additional clinical development considerations, including selection of ARCT-021 dose for booster administration and selection of final dose and schedule for ARCT-021 administration in the Phase 3 study. The data will be monitored prior to and the database will be locked after the third interim analysis to inform an interim clinical study report.

All available safety and immunogenicity data will be summarized. Blinded data will be shared with the SRC and unblinded data will be shared with the DSMB and sponsor.

Please note that a dry run of safety analysis TLFs will be delivered unblinded to sponsor before the final analysis (i.e., prior to final database lock).

11. Change from Planned Analysis

1. Protocol Section 2.3 states the following exploratory objective: *"To evaluate cell-mediated immune (CMI) responses in participants receiving ARCT-021, ARCT-154, and ARCT-165 versus placebo for priming vaccination"*. This exploratory objective has been revised in SAP section 2.3. to the following: *"To evaluate cell-mediated immune (CMI) responses in participants receiving ARCT 021 versus placebo for priming vaccination."*
    - Justification: ARCT-154 and 165 are not given as priming vaccinations. This update is to clarify the intent of this exploratory analysis.

2. Protocol Section 7.6.4.2 states the following: *"Seropositivity at a participant level: is defined as a change of NAb titer from below the LOD or Lower Limits of Quantification (LLOQ) to equal to or above LOD or LLOQ (respectively), or a 4-times or higher log-transformed titer ratio in participants with pre-existing NAb titers."* This definition has been revised in SAP Section 8.2.1 to the following: *"Seropositivity at a participant level is defined as any detectable antibody level above the lower limit of quantification (LLOQ) for the specific assay and antibody measured at timepoints after baseline."*
    - Justification: Clarify the clinical definition of seropositivity.

3. Protocol Section 7.6.4.2 states the following: *"The common difference of seroconversion rate and 95% CI between each vaccine dose group and the placebo will be estimated using CMH method with stratification factors for each time point from Day 14 to Day 208 for the mITT."* The Miettinen-Nurminen method will be used instead of the CMH method as stated in SAP Section 8.2.2.2.
    - Justification: CMH uses normal approximation, Miettinen-Nurminen has better coverage property than CMH.

4. Protocol Table 2.3 states that BAbs/NAbs ratio will be summarized. This was revised to have the neutralizing antibodies as the numerator and the binding antibodies as the denominator instead because neutralizing antibodies are a subset of binding antibodies.

12. References

Arcturus Therapeutics, Inc., protocol ARCT-021-04 (A PHASE 2 RANDOMIZED, OBSERVER-BLIND, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY, REACTOGENICITY, AND IMMUNOGENICITY OF THE SARS-CoV-2 VACCINE ARCT-021 IN HEALTHY ADULT PARTICIPANTS) Version 6.0, dated 09 March 2022.

SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc.

13. Appendices

13.1. Appendix 1: Schedule of Study Procedures

Table 13-1 Schedule of Events: Priming Vaccinations

Visit Type (Study Day)	Screen-ing	Dose 1 Administration	Follow-up 1	Follow-up 2	Study Calls	Dose 2 Administration	Follow-up 3	Follow-up 4	Study Calls	Follow-up 5	Study Calls	Un-scheduled Visit	Early Termin-ation
		Clinic	Clinic[a]	Clinic[a]	TC	Clinic	Clinic[a]	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	Clinic[a]
Study Day	−14 to −1	0	7	14	21	28	35	42	49	56	70, 98[m], 126[m], 156[m], 198	n/a	n/a
Procedure Visit Window (in days)	0	0	0	±1	±2	±3	0	±1	±2	±3	±2	n/a	n/a
Informed consent	X												
Inclusion/exclusion	X												
Medical history	X												
Weight/Height	X												
Randomization[b]		X											
Physical examination[c]	X	X	X	X		X	X	X		X		X	X
Vital signs, pulse oximetry, and body temperature[d]	X	X	X	X		X	X	X		X		X	X

Visit Type (Study Day)	Screen-ing	Dose 1 Administration	Follow-up 1	Follow-up 2	Study Calls	Dose 2 Administration	Follow-up 3	Follow-up 4	Study Calls	Follow-up 5	Study Calls	Un-scheduled Visit	Early Termin-ation
		Clinic	Clinic[a]	Clinic[a]	TC	Clinic	Clinic[a]	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	Clinic[a]
Pregnancy test[e]	X	X				X							
Urine sampling for drugs/ alcohol	X												
Blood sampling for eligibility[f]	X												
Blood sampling for SARS-CoV-2 antibodies[g]		X		X		X		X		X		X	X
Blood sampling for safety[f]	X	X	X			X	X						
Blood sampling for CMI (CMI subset only)[h]		X		X		X		X		X			X
Saliva sample for SARS-CoV-2[i]		X	X	X		X	X	X		X		X	X
Study vaccine administration[j]		X				X							

Visit Type (Study Day)	Screen -ing	Dose 1 Adminis tration	Follow -up 1	Follow -up 2	Study Calls	Dose 2 Administ ration	Follow-up 3	Follow-up 4	Study Calls	Follo w-up 5	Study Calls	Un-scheduled Visit	Early Termin-ation
		Clinic	Clinic[a]	Clinic[a]	TC	Clinic	Clinic[a]	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	Clinic[a]
Post vaccination observation [k]		X				X							
Adverse Events [l]		X	X	X	X	X	X	X	X	X	X	X	X
Concomitant meds collection	X	X	X	X	X	X	X	X	X	X	X	X	X
Recording of symptoms of COVID 19, and exposure to COVID 19 cases		X	X	X	X	X	X	X	X	X	X	X	X

[a] Visits will be performed in person unless the participant is otherwise directed to remain at home or be seen at a hospital. Follow-up visits and unscheduled visits may be performed by telemedicine visits or in a hospital setting if warranted by clinical circumstances (e.g., COVID-19 lockdown in the vicinity) and permitted by local regulation. The target dates for the Day 7 - Day 28 visits are based on the actual date of the Day 0 visit. The target dates for the Day 35-Day 208 visits are based on the actual date of the Day 28 visit. The target dates for visits after Day 208 are based on the actual Day 208 visit.
[b] On Day 0, participants will be randomly assigned to one of four Study Groups (3 ARCT-021:1 placebo), where they will receive 2 doses of study vaccine on Day 0 and Day 28 and also for participants in Study Groups 1, 2, and 3 further randomly assigned to receive a single booster dose of study vaccine (1 ARCT-021:1 placebo) on Day 208. Study Group 4 will not be randomized to receive booster but will receive 1 dose of placebo at Day 208.
[c] Complete physical examination will be performed at Screening and Final Visit (Day 570 or Early Termination); symptom-directed examination (if any symptoms) will be performed at other timepoints as indicated to assess changes from Screening.
[d] Blood pressure, heart rate, respiratory rate, body temperature, and pulse oximetry will be measured. On days of study vaccine administration, these will be measured before and after vaccine administration.
[e] Pregnancy testing will be performed on women who are not surgically sterile or postmenopausal. Pregnancy testing by urine dipstick is acceptable. Pregnancy testing will be performed and evaluated prior to study vaccine administration.
[f] Analytes for eligibility and safety assessments are listed in Appendix 4: Clinical Laboratory Tests. Repeat safety laboratory testing at Screening will not be permitted, unless the result is uninterpretable, or Screening assessments are repeated due to lapse of the 14-day screening visit window. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. Throughout the study, additional laboratory analyses may be performed for enrolled participants if relevant to understanding participant safety. The decision to conduct these analyses will be based on discussion between the Medical Monitor and the Investigator and subject to Medical Monitor approval.

[g] Immunogenicity samples will include anti-SARS-CoV-2 neutralizing and binding antibodies. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. On unscheduled visits that are performed for assessment of non-COVID-19-related AEs, a blood sample for immunogenicity is not required.
[h] At each timepoint blood will be drawn in the participants assigned to the CMI subset. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. Participants assigned to the CMI subset and who re-enroll into the study will not undergo blood sampling for CMI after re-enrollment.
[i] Nasal swabs and nasal turbinate swabs may replace saliva testing with alternate means of confirmatory testing of SARS-CoV-2. Participants will receive Home Test Kits if the participant reports possible COVID-19 symptoms or risk of exposure to SARS-CoV-2.
[j] Study vaccine will be administered by intramuscular injection into the deltoid muscle of the non-dominant arm. Study vaccine administration will not be performed at these visits if the participant has not met reasons for study vaccine withdrawal.
[k] Vaccinated participants will be observed at the site for at least 30 minutes following vaccine administration or until clinically stable.
[l] Review of AEs will include surveillance for solicited and unsolicited AEs, SAEs, MAAEs, NOCDs, AEs leading to next vaccine delayed or vaccine will not be re-dosed; data will be gathered by Diary and telephone contacts.
[m] At telephone contacts performed from Day 98 through Day 198, participants will be asked if they are interested in re-enrolling into the study to receive ARCT-021 vaccine. Participants eligible to re- enroll to receive ARCT-021 vaccine (placebo group only), will complete the Early Termination visit.

Schedule of Events: Booster Vaccinations

Visit Type (Study Day)	Booster Administration	Booster Follow-up 1	Study Calls	Booster Follow-up 2	Study Calls	Booster Follow-up 3[l]	Study Calls	Unscheduled Visit	Early Termination	Final Visit
	Clinic	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	Clinic[a]	Clinic[a]
Study Day	208	215	222, 229	236	264, 292, 320, 348, 376	388	416,444, 472 500, 528, 556	n/a	n/a	570
Procedure/Visit Window (in days)	+60	0	±3	±3	±3	±3	±3	n/a	n/a	+7
Physical examination[b]	X	X		X		X		X	X	X
Vital signs, pulse oximetry, and body temperature[c]	X	X		X		X		X	X	X
Pregnancy test[d]	X									
Blood sampling for safety[e]	X	X								
Blood sampling for SARS-CoV-2 antibodies[f]	X			X		X		X	X	X
Blood sampling for CMI (CMI subset only)[g]	X	X		X		X			X	X
Saliva sample for SARS-CoV-2[h]	X	X		X		X		X	X	X
Study vaccine administration[i]	X									
Post vaccination observation[j]	X									
Adverse Events[k]	X	X	X	X	X	X	X	X	X	X
Concomitant meds collection	X	X	X	X	X	X	X	X	X	X

Visit Type (Study Day)	Booster Administration	Booster Follow-up 1	Study Calls	Booster Follow-up 2	Study Calls	Booster Follow-up 3[i]	Study Calls	Unscheduled Visit	Early Termination	Final Visit
	Clinic	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	TC	Clinic[a]	Clinic[a]	Clinic[a]
Recording of symptoms of COVID-19, and exposure to COVID-19 cases	X	X	X	X	X	X	X	X	X	X

[a] Visits will be performed in person unless the participant is otherwise directed to remain at home or be seen at a hospital. **The target dates for the Day 215- Day 570 visits are based on the actual date of the Day 208 visit.**

[b] Complete physical examination will be performed at Screening and final visit (Booster Follow-up Visit 3 or Early Termination); symptom-directed examination (if any symptoms) will be performed at other time points as indicated to assess changes from Screening.

[c] Blood pressure, heart rate, respiratory rate, body temperature, and pulse oximetry will be measured. On days of study vaccine administration, these will be measured before and after vaccine administration.

[d] Pregnancy testing will be performed on women who are not surgically sterile or postmenopausal. Pregnancy testing by urine dipstick is acceptable. Pregnancy testing will be performed and evaluated prior to study vaccine administration.

[e] Analytes for safety are listed in Appendix 2: Clinical Laboratory Tests. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. Throughout the study, additional laboratory analyses may be performed for enrolled participants if relevant to understanding participant safety. The decision to conduct these analyses will be based on discussion between the Medical Monitor and the Investigator and subject to Medical Monitor approval.

[f] Immunogenicity samples will include anti-SARS-CoV-2 neutralizing and binding antibodies and serum samples for exploratory antibody testing. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. See Section 6.3 for further detail. On unscheduled visits that are performed for assessment of non-COVID-19-related AEs, a blood sample for immunogenicity is not required.

[g] At each time point, blood will be drawn in the participants assigned to the CMI subset. On days when study vaccines are administered, blood must be drawn prior to vaccine administration. Participants assigned to the CMI subset and who re-enroll into the study will not undergo blood sampling for CMI after re-enrollment. The CMI assays are described in Section 7.6.5

[h] Nasal swabs and nasal turbinate swabs may replace saliva testing with alternate means of confirmatory testing of SARS-CoV-2. Participant will receive a Home Test Kit if the participant reports possible COVID-19 symptoms or risk of exposure to SARS-CoV-2.

[i] Study vaccine will be administered by intramuscular injection into the lateral aspect of the deltoid muscle of the non-dominant arm. Study vaccine administration will not be performed at these visits if the participant has not met reasons for study vaccine withdrawal. See Section 4.2.2 for further detail.

[j] Vaccinated participants will be observed at the site for at least 30 minutes following vaccine administration or until clinically stable.

13.2. Appendix 2: Imputation Rules

13.3. Missing Dates of Prior/Concomitant Medications and Non-Study Vaccinations

Imputation rules for missing or partial start/stop dates of medication are defined below:

- Missing or partial medication start date:
    - If only Day is missing, use the first day of the month, unless:
        - The medication end date is on/after the date of first injection or is missing/partial AND the start month and year of the medication coincide with the start month and year of the first injection. In this case, use the date of first injection.
    - If Day and Month are both missing, use the first day of the year, unless:
        - The medication end date is on/after the date of first injection or is missing/partial AND the start year of the medication coincide with the start year of the first injection. In this case, use the date of first injection.
    - If Day, Month, and Year are all missing, the date will not be imputed, but the medication will be treated as though it began prior to the first injection for purposes of determining if status as prior or concomitant.
- Missing or partial medication stop date:
    - If only Day is missing, use the earliest date of (last day of the month, study completion, discontinuation from the study, or death).
    - If Day and Month are both missing, use the earliest date of (last day of the year, study completion, discontinuation from the study, or death).
    - If Day, Month, and Year are all missing, the date will not be imputed, but the medication will be flagged as a continuing medication.

In summary, the prior, concomitant or post categorization of medications and non-study vaccinations is described in **Table 13-2**below.

Table 13-2: Prior, Concomitant, and Post Categorization of Medications and Non-study Vaccinations

	Medication Stop Date				
Medication Start Date	**< First Injection Date of IP**	**≥ First Injection Date and ≤ 28 Days After Day 28 Injection**	**> 28 days after Second injection to Third (Booster) injection or Early Termination for subjects without Third Injection [2]**	**≥ Third injection date and ≤ 28 days after Third injection**	**> 28 days after Third Injection [2]**
< First injection date of IP [1]	P	P	P	P	P
≥ First injection date and ≤ 28 days after Second injection	-	C	C	C	C
> 28 days after Second injection to Third (Booster) injection or Early Termination for subjects without Third injection	-	-	A	A	A
≥ Third injection date and ≤ 28 days after Third injection	-	-	-	C	C
> 28 days after Third Injection	-	-	-	-	A

A: Post; C: Concomitant; P: Prior

[1] includes medications with completely missing start date

[2] includes medications with completely missing end date

13.4. Missing Dates of Adverse Events

(UK, UKN, and UNKN indicate unknown or missing day, month, and year, respectively):

Missing Onset Dates:

- First, remove any non-missing date component following any missing date component (e.g., set day to UK if month equals UKN or set month and day to UK and UKN, respectively, if year equals UNKN)
- UK-MMM-YYYY: Assume 01-MMM-YYYY but, if month and year are the same as the first study vaccination month and year, then assume the date of first study vaccination
- UK-UKN-YYYY: Assume 01-JAN-YYYY, but if year is the same as the first study vaccination year, then assume the date and month of first study vaccination
- UK-UKN-UNKN: Assume date of first study vaccination

Should the imputed start date fall after a complete stop date provided, use the stop date instead of the date that would otherwise be imputed.

Missing End Dates:

- First, remove any non-missing date component following any missing date component (e.g., set day to UK if month equals UKN or set month and day to UK and UKN, respectively, if year equals UNKN)
- UK-MMM-YYYY: Assume the last day of the month
- UK-UKN-YYYY: Assume 31-DEC-YYYY
- UK-UKN-UNKN: Do not impute and assume ongoing

Should the imputed stop date fall before a complete start date provided, use the start date instead of the date that would otherwise be imputed.

13.5. Appendix 3: Estimands and Intercurrent Events

Possible intercurrent events are presented in the table below.

Table 13-3 Intercurrent Event Types

Label	Intercurrent Event Type
IcEv1 (Death)	Death due to any cause (including pneumonia) other than COVID-19.
IcEv2 (Missed dose)	Does not receive the second vaccination at Day 28 where second vaccination assigned was ARCT-021 or does not receive booster vaccination at Day 208 where booster vaccination assigned was active ARCT booster. It is not necessary to exclude placebo missed second doses as there is no impact.
IcEv3 (Immune-modifying)	Use of immune-modifying drugs or non-study vaccines which interfere with immunogenicity. Immune-modifying drugs will be determined from a medical review of medications taken during the study.
IcEv4 (Early infection)	Priming vaccination period: Antigens or antibodies indicating exposure to SARS-CoV-2 prior to vaccination or early infection before Day 7. (This IcEv is particularly relevant to assessing immunogenicity.) Early infection is defined as follows: o Positive PCR saliva test at baseline OR o Positive PCR test at any time up to 7 days post-baseline OR o Positive SARS-CoV-2 baseline serostatus Booster vaccination period: Antigens or antibodies indicating exposure to SARS-CoV-2 prior to vaccination or early infection before Day 7 post booster vaccination. (This IcEv is particularly relevant to assessing immunogenicity.) Early infection in the booster period is defined as follows: o Positive PCR saliva test on Day 208 OR o Positive PCR test at any time up to 7 days post-booster vaccination
IcEv5 (Study infection)	Develops COVID-19 or asymptomatic SARS-CoV-2 infection on or after Day 7. (This IcEv is particularly relevant to immunogenicity and CESI events.). Study infection is defined as COVID-19 infection recorded on the adverse event CRF page with start date on or after Day 7. The following AE preferred terms will be considered: Asymptomatic COVID-19, COVID-19, Suspected COVID-19. Positive MSD nucleocapsid antibody value of 5000 AU/mL or higher at any point after baseline (with start date on or after Day 7) will also be considered as on-study infection.

Attributes for the primary safety estimands with strategies for IcEvs are presented in Table 13-4 below.

Table 13-4: Primary Safety Estimands With Rationale and Strategies to Address Intercurrent Events

Estimand Label	Estimand 1 (Safety)
Estimand Description	Count and percentage of vaccinated healthy adults who would develop MAAEs, NOCDs, SAEs, AEs leading to discontinuation/withdrawal, solicited AEs, and unsolicited AEs: These will be evaluated after each dose of study vaccine by priming vaccine study group and by booster vaccine received (ARCT 021, ARCT 154, ARCT 165, or placebo). A treatment policy strategy is used for assessing safety irrespective of an early infection within 7 days after the first vaccination or an infection during the study or missed subsequent vaccination. Infections and death are included in the endpoint (composite strategy).
Target Population	Participants receiving at least 1 dose of study vaccine (as defined by eligibility criteria).
Endpoint	Occurrence of MAAEs, NOCDs, AEs leading to discontinuation/withdrawal, SAEs, solicited, or unsolicited AEs: • Solicited AEs within 7 days of each vaccination (by Toxicity Grade, Appendix 5): Day 7, Day 35, Day 215 • Unsolicited AEs within 28 days of each vaccination (by mild/moderate/severe severity): Day 28, Day 56, Day 236 • MAAEs, NOCDs, SAEs, AEs leading to discontinuation/withdrawal from vaccination 1 to prior to vaccination 2, vaccination 2 to prior to booster, overall prior to booster vaccination, and post-booster vaccination.
Treatment Conditions	Placebo or 5.0 µg or 7.5 µg ARCT-021 administered on Day 0, Day 28; Placebo, ARCT-021, ARCT-154, or ARCT-165 administered on Day 208.
Population-Level Summary	Percentage of vaccinated participants (who would develop each type of AE).
Intercurrent Event Strategy	
IcEv1 (Death)	Composite strategy
IcEv2 (Missed second)	Treatment policy strategy
IcEv3 (Immune-modifying)	Treatment policy strategy
IcEv4 (Early infection)	Treatment policy strategy (based on study phase)
IcEv5 (Study infection)	Composite strategy
Rationale for Strategies	Deaths and symptomatic infections during study would contribute as part of the endpoint (i.e., included as MAAEs, SAEs, NOCDs, AEs leading to discontinuation/withdrawal, solicited AEs or unsolicited AEs) as per normal practice. A treatment policy strategy is used for assessing safety irrespective of early infection and missed additional (second, booster) study vaccinations.

Table 13-5 Primary Immunogenicity Estimands With Rationale and Strategies to Address Intercurrent Events

Estimand Label	Estimand 2 (Geometric Mean Titers/Concentrations)
Estimand Description	The GMT of NAbs (primary) and BAbs (secondary) against SARS-CoV-2 up to Early Termination after first vaccination with ARCT-021 of each group (5.0, 7.5 µg, or placebo) and with/without booster dose in healthy adults. Interest will lie in comparing the GMT of each dosing regimen to placebo based on the Geometric Mean Ratio (GMR). The hypothetical strategy is used to estimate antibody levels had all scheduled vaccinations been received, and without subsequent SARS-CoV-2 infection or influence from immune-modifying drugs or non-study vaccines. The principal stratum strategy excludes those with active or early SARS-CoV-2 infection within 7 days after the first study vaccine administration.
Analysis Population	Vaccinated healthy adults (as defined by eligibility criteria) without active or early SARS-CoV-2 infection (infection within 7 days after the first vaccination).
Endpoint	Total NAbs (primary) and BAbs (secondary) specific for the SARS-CoV-2 measured pre- and post-priming vaccine administration (on Day 0, 14, 28, 42, 56, and 208) and following booster vaccine administration (Day 236, Early Termination).
Treatment Conditions	Placebo or 5.0 µg or 7.5 µg ARCT-021 administered on Day 0, Day 28; placebo, ARCT-021, ARCT-154, or ARCT-165 administered on Day 208.
Population-Level Summary	The GMT and GMR of comparisons of interest at each time point. GMR will compare responses in placebo-, ARCT-021-, ARCT-154-, and ARCT-165-vaccinated groups at relevant timepoints. For additional considerations on population-level summary see the SAP.
Intercurrent Event Strategy	
IcEv1 (Death)	Hypothetical strategy
IcEv2 (Missed dose)	Hypothetical strategy
IcEv3 (Immune-modifying)	Hypothetical strategy
IcEv4 (Early infection)	Principal stratum strategy (based on study phase)
IcEv5 (Study infection)	Hypothetical strategy
Rationale for Strategies	Hypothetical strategy is used to understand antibody levels achieved through receiving scheduled vaccination, without subsequent SARS-CoV-2 infection and without any influence from immune-modifying drugs or non-study vaccines. In estimation, the hypothetical uses a statistical model on the mITT (excluding data points following intercurrent events and not complete removal of all data for the participant).

Estimand Label	Estimand 3 (Rise/Seroconversion)
Estimand Description	Percentage of participants with at least 2- and 4-fold increase in antibody responses from baseline (seroconversion) in NAbs (primary) and BAbs (secondary) at each time point from Day 14 to Early Termination. The hypothetical strategy is used to estimate antibody levels had all scheduled vaccinations been received, and without subsequent SARS-CoV-2 infection or influence from immune-modifying drugs or non-study vaccines. The principal stratum strategy excludes those with active or prior SARS-CoV-2 infection at first vaccination.
Target Population	Vaccinated healthy adults (as defined by eligibility criteria) without active or early SARS-CoV-2 infection within 7 days after first vaccination.
Endpoint	GMFR and seroconversion against SARSCoV2 NAbs (primary) and BAbs (secondary) measured pre- and post-priming vaccine administration (on Day 0, 14, 28, 42, 56, and 208) and following booster vaccine administration (Day 236, Early Termination).
Treatment Conditions	Placebo or 5.0 µg or 7.5 µg ARCT-021 administered on Day 0, Day 28; placebo, ARCT-021, ARCT-154, or ARCT-165 administered on Day 208.
Population-Level Summary	The GMFR and ≥2- and 4-fold seroconversion (SC) at each time point.
Intercurrent Event Strategy	
IcEv1 (Death)	Hypothetical strategy
IcEv2 (Missed dose)	Hypothetical strategy
IcEv3 (Immune-modifying)	Hypothetical strategy
IcEv4 (Early infection)	Principal stratum strategy
IcEv5 (Study infection)	Hypothetical strategy
Rationale for Strategies	Hypothetical strategy is used to understand seroconversion achieved through receiving scheduled vaccination, without subsequent SARS-CoV-2 infection and without any influence from immune-modifying drugs or non-study vaccines. In estimation, the hypothetical uses a statistical model on the mITT (excluding data points following intercurrent events and not complete removal of all data for the participant).

13.6. Overview of Statistical Methods: Estimation of Estimands and Supportive Analyses

A summary of the statistical methods and sensitivity analyses is presented in Table 13-6. Placebo participants will be pooled across dose groups summaries.

Table 13-6 Summary of Statistical Methods and Supportive Analyses

Estimand Label	Estimand Description	Main Estimation			Supportive Analysis
		Analysis Set	Imputation/ Data/ Censoring Rules	Analysis Model/Method	
Estimand 2	The GMT of total NAbs (primary) and GMC of BAbs (secondary) titers against SARS-CoV-2 from Day 14 up to Early Termination after initial priming vaccination with ARCT-021 and booster vaccinations (ARCT-021, ARCT-154, and ARCT-165) (exploratory) of each dose in healthy adults. Interest will lie in comparing the GMC of each dosing regimen to placebo (GMR). The hypothetical strategy is used to estimate antibody levels had both scheduled vaccinations been received, and without any influence from immune-modifying drugs or non-study vaccines, or subsequent SARS-CoV-2 infection.	mITT	Values below the limit of quantification (LOQ) or limit of detection (LOD) will be replaced by LOQ/2 and LOD/2, respectively.	Total NAbs and BAbs levels will be summarized with descriptive statistics, including boxplots (on log scale versus time) by time point: Days 14, 28, 42, 56, 208, 236, Early Termination. GMTs with corresponding 95% CI will be provided at each time point. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale. GMFRs with corresponding 95% CI will be provided at each post-baseline timepoint over baseline. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale.	Supportive: For the priming vaccination period ANCOVA will be fitted to the natural log transformed antibody titers and antibody concentrations measured against SARS-CoV-2 with terms for treatment group, age group and natural log baseline titer by each post-vaccination immunogenicity visit. The geometric least squares mean (GLSM) and corresponding 2-sided 95% CI for each treatment group will be provided by visit. Geometric mean ratio (GMR), estimated by the ratio of GLSM, and the corresponding 95% CI will be provided to assess the difference between ARCT-021 vs. placebo groups at each visit. Descriptive statistics, GMT/GMFRs based on t-distribution will be presented by the following analysis subgroups: age group, gender and country. ANCOVA models will be presented by gender and country.

Table 13-6 Summary of Statistical Methods and Supportive Analyses

Estimand Label	Estimand Description	Main Estimation			Supportive Analysis
		Analysis Set	Imputation/ Data/ Censoring Rules	Analysis Model/Method	
Estimand 3	Percentage of participants with at least 2- and 4-fold increase in antibody responses from baseline (seroconversion) in NAbs (primary) and BAbs (secondary) at each time point from Day 14 to Early Termination after initial priming vaccination with ARCT-021 (for each dose and placebo), and booster vaccinations (ARCT-021, ARCT-154, and ARCT-165) (exploratory) in healthy adults. Same hypothetical/principal stratum strategies and vaccine groups as for Estimand 2.	mITT	Titer values below the LOQ or LOD will be replaced by LOQ/2 and LOD/2, respectively, prior to calculation of fold changes and seroconversion.	The number and percentage of participants with ≥ 2- and 4- fold increase from baseline will be provided with 2-sided 95% CI using the Clopper-Pearson method at each post-baseline timepoint: Days 14, 28, 42, 56, 208, 236, Early Termination. The number and percentage of participants with seropositivity at a participant level (defined as a detectable level above LLOQ) will be provided with 2-sided 95% CI using the Clopper-Pearson method at each post-baseline timepoint.	Supportive: Similar tabulations will be done for the following analysis subgroups: age group, gender and country. For the priming vaccination period: the common difference of seropositivity and seroconversion rates and 95% CIs between each vaccine dose group and the placebo will be estimated using the Miettinen-Nurminen method with age group as stratification factor for each time point.

13.7. Appendix 4: Clinical Laboratory Tests

Table 13-7: Clinical Laboratory Tests

<table>
<tr><th colspan="2">Safety Laboratory Assessments</th><th>Screening Tests for Eligibility[a]</th><th>Immunogenicity Assessments</th></tr>
<tr>
<td>Clinical Chemistry Panel
• Sodium
• Potassium
• Chloride
• Bicarbonate
• Total protein
• Albumin
• Calcium
• Magnesium
• Phosphorus
• Glucose (random)
• BUN
• Creatinine
• Cholesterol
• Uric Acid
• Total bilirubin
• Direct (conjugated) bilirubin
• Indirect (unconjugated) bilirubin
• ALT
• AST
• Alkaline phosphatase
• Creatinine kinase
• GGT</td>
<td>Hematology
• Red blood cells
• Hemoglobin
• Hematocrit
• MCV, MCH, MCHC
• Platelets
• White blood cells
• WBC Differential (% and absolute)
• Neutrophils
• Eosinophils
• Basophils
• Lymphocytes
• Monocytes</td>
<td>• Hepatitis B surface antigen
• Hepatitis C antibody
• HIV antibody
• FSH (women only, as clinically warranted)
• Serum/urine βhCG
• Urine drug/alcohol screen
• HbA1c (if warranted for individuals with diabetes mellitus, Type 2)
Virology
• SARS-CoV-2 detection by RT-PCR</td>
<td>• SARS-CoV-2 neutralizing antibody titer by pseudoviral microneutralization assay [all participants] and optional PRNT assay [all/some participants]
• [All participants] Anti-S, N-, and RBD protein IgG by MSD multiplex assay
• [CMI subset only] Cytokine-producing SARS-CoV-2 spike protein-specific T-cells as measured by flow cytometry
• [CMI subset only] ICS assay (Tumor necrosis factor (TNF)-α, Interleukin-2 (IL-2), Interferon-γ (IFN- γ), IL-4, and IL-13)
• Additional exploratory immunogenicity tests will be specified in the SAP, if performed.</td>
</tr>
</table>

[a] Can be performed at a local laboratory if the tests are available. Repeat safety laboratory testing (except toxicity screen and pregnancy test) at Screening will not be permitted unless the result is uninterpretable, or Screening assessments are repeated due to lapse of the 14-day screening visit window.

13.8. Appendix 5: FDA CBER Toxicity Grading Scales

Table 13-8: Toxicity Grading for Solicited Adverse Events

Solicited Local AEs	Grade 0+	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially Life Threatening)
Injection Site Erythema *	< 2.5 cm	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Injection Site Induration/Swelling **	< 2.5 cm	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Injection Site Pain	None	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Injection Site Tenderness	None	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Solicited Systemic AEs					
Fever (°C) *** (°F) ***	<38.0 <100.4	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	>40 >104
Nausea/vomiting	None	No interference with activity or 1 – 2episodes/24 hours	Some interference with activity or > 2 episodes/24hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhoea	None reported or 1 loose stool/24 hours	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800 gms/ 24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	None	No interference with activity	Repeated use of non- narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia, chills, dizziness	None	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

\+ Grade 0 is defined as subject assessed with either no symptom or reported symptom less than Grade 1.
* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
*** Oral temperature; no recent hot or cold beverages or smoking.
Source: DHHS 2007

Table 13-9: Toxicity Grading for Safety Laboratory Assessments

Laboratory Parameter*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
Creatinine kinase– mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000

Laboratory Parameter*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)**
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a Grade 3 parameter (125-129 mEq/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
*** "ULN" is the upper limit of the normal range.
Source: DHHS 2007.

Toxicity Grading for Vital Signs

Vital Signs*	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially Life Threatening)
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.