Parexel International

ReiThera SRL.

RT-CoV-2

A Phase 1, Dose-Escalation Study to assess the Safety and Immunogenicity of a COVID-19 VaccineGRAd-COV2 in Healthy Adults and Elderly Subjects

Statistical Analysis Plan

Version: 4.0

Parexel Project Number: 252881

SPONSOR SIGNATURE PAGE

Signature below indicates that you have reviewed the document for clarity, completeness and consistency and approve it.

This document has been approved and signed electronically by the following:

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Parexel SIGNATURE PAGE

Signatures below confirm that the Statistical Analysis Plan was developed in accordance with SOP-GDO-WW-019 and that it is approved for release.

This document has been approved and signed electronically on the final page by the following:

	Signatory
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REVISION HISTORY

Version No.	Effective Date	Summary of Change(s)
Final 1.0	30 Oct 2020	New document.
Final 2.0	18 Jan 2021	Inclusion of imputation method for immunogenicity result below limit of detection. Further details on derivation of worst post-baseline laboratory value added for clarity. Typo corrections.
Final 3.0	27 Jan 2021	Administrative change in SAP language upon change of SOP used for protocol deviations. SOP-GDO-WW-012 changed to SOP-EPD-WW-012.
Final 4.0	21 Jun 2021	Imputation rule of BLQ value in anti-S-Ab concentration is updated to LLOQ/2 = 1.9. Clarification added on external data from frozen ELISpot samples collected on Week 2.

LIST OF ABBREVIATIONS

List and define all acronyms and abbreviations used in the document here. Abbreviations should be spelled out in full and the abbreviation indicated in parentheses at first appearance in the text. Abbreviations should appear in alphabetical order.

Abbreviation / Acronym	Definition / Expansion
AE	Adverse event
ATC	Anatomical therapeutic chemical
BLQ	Below the lower limit of quantification
BMI	Body Mass Index
BP	Blood pressure
Bpm	Beats per minute
CRF	Case Report Form
CS	Clinically significant
CTCAE	Common Terminology Criteria for Adverse Events
DB	Clinical database
DBHL	Clinical database hard lock
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DRM	Data Review Meeting
DRM	Data Review Meeting
DSMB	Data safety monitory board
ECG	Electrocardiogram
eDISH	Evaluation of Drug Induced Serious Hepatotoxicity
FA	Final analysis
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
IA	Interim analysis

Abbreviation / Acronym	Definition / Expansion
IAS	Immunogenicity analysis set
ICF	Informed consent form
IFNγ	Magnitude of interferon gamma
IMP	Investigational Medicinal Product
LSLV	Last subject last visit
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not available
NCI	National Cancer Institute (US)
NCS	Not clinically significant
NK	Not known
PBMC	Peripheral blood mononuclear cell
PT	Preferred term
SAE	Serious adverse event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical analysis system
SBP	Systolic blood pressure
SD	Standard deviation
SFC	Spot forming cell
SOC	System Organ Class
SOP	Standard operating procedure
TEAE	Treatment-emergent adverse event
TLFs	Tables, listings, and figures
WHO-DD	World Health Organisation - Drug Dictionary

1 INTRODUCTION

The analyses described in this SAP are based upon the following study documents:

- Study Protocol, Version 2.0 (September 8, 2020)
- electronic Case Report Form (eCRF), Version 4 (generated on August 4, 2020)
- DSMB Charter, Version 2.0

Per protocol, the collected data will be analyzed and reported in three steps according to stages achieved during the course of the study, ie:

Delivery 1: Interim Analysis 1

A report on safety and immunogenicity at 4 weeks for Adults-Cohort will be developed; this report will include:

a. an analysis of safety issue in the three adults-arms;
b. pivotal considerations for authorizing enrollment in the Elderlies-Cohort;
c. a preliminary analysis of immunogenicity in the three adults-arms aimed at identifying the most convenient dose to be used in the eventual phase 2/3 trial.

Delivery 2: Interim Analysis 2

A report on safety and immunogenicity at 4 weeks for Elderlies-Cohort will be developed; this report will include pivotal considerations on:

a. an analysis of safety issues in the three elderlies-arms
b. a preliminary analysis of immunogenicity in the three elderlies-arms aimed at identifying the most convenient doses to be used in the eventual phase 2/3 trial.
c. comparison of safety and immunogenicity between adults and elderlies (across the cohort comparison) to define the most convenient dose to be used either in general population or special groups at risk.

Delivery 3: Final Analysis

Within one year since LSLV final report will be produced including:

a. Final analysis on safety and immunogenicity at 24 weeks after vaccination in Adults-Cohort
b. Final analysis on safety and immunogenicity at 24 weeks after vaccination in Elderlies-Cohort

Analyses described hereafter will cover all these three stages of the study with applicable closures on selected analyses if a stage requires.

2 STUDY OBJECTIVES

The aim of the program is to prove first in human safety and immunogenicity of the new vaccine GRAd-COV2 considering both healthy adult population and special groups at risk such as the elderlies. The approach includes a phase 1A/1B dose escalation trial with six sequential arms in two cohorts.

2.1 Primary Objective(s)

- Provide preliminary information of vaccine safety and immunogenicity in healthy adults within 4 months since trial approval (Adults-Cohort)
- Confirm vaccine safety and immunogenicity in a special population (such as older adults) within 6 months (Elderlies-Cohort)
- Implement an eventual phase 2/3 trial tailored on current Italian epidemic situation at the beginning of 2021.
- Provide full data about safety and immunogenicity at 6 months after vaccination within one year since the approval of the program.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a first-in-human, open-label, dose escalation, phase 1A/1B clinical trial to assess the safety and immunogenicity of the candidate GRAd-COV2 vaccine in adult volunteers aged 18-55 years and elderly volunteers aged 65-85 years. The vaccine will be administered intramuscularly once in time.

The aim is to enroll 90 volunteers in two cohorts with three arms each (i.e. 15 volunteers per arm in six arms). Each study arm will assess a unique dose of the candidate GRAd-COV2 in a particular population, either adults or elderlies.

Adults-Cohort (Phase 1A)

Arm-1 will investigate safety and immunogenicity of a dose of 5xE10 vp in adults.

Arm-2 will investigate safety and immunogenicity of a dose of 1xE11 vp in adults.

Arm-3 will investigate safety and immunogenicity of a dose of 2xE11 vp in adults.

Elderlies-Cohort (Phase 1B)

Arm-4 will investigate safety and immunogenicity of a dose of 5xE10 vp in elderlies.

Arm-5 will investigate safety and immunogenicity of a dose of 1xE11 vp in elderlies.

Arm-6 will investigate safety and immunogenicity of a dose of 2xE11 vp in elderlies.

vp: Viral particles

To minimize the risk of severe adverse events in frail subjects, enrollment of Elderlies-Cohort will start after that 4-week safety results in Adults-Cohort will be available. Moreover, as it is a first in human trial, we have scheduled volunteers to be enrolled according to a strict frame of authorization rules. In particular, each arm includes two groups (A and B). Group As are made of 3 volunteers each to be enrolled throughout a week. The occurrence of SAE within 48h since the injection will be reported to data safety monitory board (DSMB) and the enrolment suspended until a direct effect of the vaccination is excluded. Group Bs are made of 12 volunteer each to be enrolled over a week. DSMB provide advice on sequential enrollment across groups and arms; this is:

- Enrolment of Arm-1 group B and Arm-2 group A is bound to a favorable 7-day safety profile in Arm-1 group A;
- Enrolment of Arm-2 group B and Arm-3 group-A is bound to a favorable 7-day safety profile in Arm-2 group A;
- Enrolment of Arm-3 group B is bound to favorable 7-day safety profile in Arm-3 group A;
- Enrolment of Arm-4 group A is bound to a favorable 4-week safety profile in the whole Arm- 1;
- Enrolment of Arm-4 group B and Arm-5 group A is bound to a favorable 7-day safety profile in Arm-4 group A;
- Enrolment of Arm-5 group B and Arm-6 group-A is bound to a favorable 7-day safety profile in Arm-5 group A;
- Enrolment of Arm-6 group B is bound to a favorable 7-day safety profile in Arm-5 group A.

3.2 Endpoints and Associated Variables

3.2.1 Safety Variables

- Adverse event (AE) assessments
- Clinical laboratory tests (hematology and clinical chemistry)
- Vital signs (supine blood pressure and pulse, body temperature, respiratory rate and SpO2)
- 12-lead electrocardiograms (ECG): Interpretation
- Physical examinations
- Concomitant medication assessments
- Subject Diary

3.2.2 Immunogenicity Variables

- Antibodies to SARS-CoV-2 S-protein (anti-S-Ab)
- Antibodies to SARS-CoV-2 N-protein(anti-N-Ab)
- SARS-CoV-2 micro-neutralization assay
- Magnitude of interferon gamma (IFNγ) ELISpot response (SFC/10^6 PBMC)
- T-cell response (number of positive spike pools-only applicable for fresh PBMC at week 2)

3.2.3 Exploratory Variables

4 STATISTICAL METHODS

4.1 Data Quality Assurance

All tables, figures and data listings to be included in the report will be independently checked for consistency, integrity and in accordance with standard Parexel procedures.

4.2 General Presentation Considerations

The overall outline of the scheduled assessment and other study procedures can be found in the Schedule of Assessment, Table 5. In the following, the data handling conventions should be interpreted within the framework of the Schedule of Assessment.

The date of Enrolment Visit (Time 0) is defined as Day 1. All other study days will be labeled relative to Day 1.

- For observation/event dates on or after Day 1, study day for an observation/event date is derived as:
  Observation/event date – visit Time 0 date + 1 day, which could be Day 1, Day 2, Day 3, etc.
- For observation/event dates prior to Day 1, study day for an event date is derived as:
  Observation/event date – visit Time 0, which could be Day -1, Day -2, etc., referring to 1 day, 2 days, etc., prior to Day 1, respectively.
- Day 0 is not defined.

Baseline is considered as the result of last non-missing evaluation before vaccine administration, unless specified otherwise.

End of Study is defined as the last available post-treatment assessment.

Continuous data will be summarized in terms of the mean, standard deviation (SD), median, minimum, maximum and number of observations, unless otherwise stated.

Count data will be summarized in terms of the median, lower and upper quartile, minimum, maximum and number of observations, unless otherwise stated. Geometric mean titer will be provided also for result in titer.

Categorical data will be summarized in terms of the number of subjects providing data at the relevant time point (n), frequency counts and percentages. Any planned collapsing of categories will be detailed in the SAP text and the data displays.

The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean, median, lower quartile and upper quartile will be reported to one more decimal place than the raw data recorded in the database. The SD will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistic. Confidence intervals will be presented to one more decimal place than the raw data.

Percentages will be presented to one decimal place. Percentages will not be presented for zero counts. Percentages will be calculated using n as the denominator. If sample sizes are small, the data displays will show the percentages, but any textual report will describe frequencies only.

Changes from baseline in categorical data will be summarized using shift tables where appropriate.

4.2.1 Treatment Naming, Grouping and Ordering

The six treatment arms defined in the protocol will be labelled and ordered (unless specified otherwise) in the outputs by means of age cohorts and dose administered as provided in table below.

Table 1 Treatment Group Labelling and Order

Order	Treatment Group [1]	Cohort	Dose	Treatment Group Label [2]
1	Arm-1	Adults	5*E10 vp	Adults, 5*E10 vp (Arm-1)
2	Arm-2		1*E11 vp	Adults, 1*E11 vp (Arm-2)
3	Arm-3		2*E11 vp	Adults, 2*E11 vp (Arm-3)
4	Arm-4	Elderlies	5*E10 vp	Elderlies, 5*E10 vp (Arm-4)
5	Arm-5		1*E11 vp	Elderlies, 1*E11 vp (Arm-5)
6	Arm-6		2*E11 vp	Elderlies, 2*E11 vp (Arm-6)

[1] As indicated in protocol. [2] As displayed in outputs.

Summaries will be grouped depending on data (e.g. demographics, AE, safety lab, etc.) as well as the type of delivery, i.e. interim analysis 1 and 2 and post database lock. Grouping method is indicated for each data type and each delivery in the following.

Table 2 Grouping: Subject Classification, Disposition, Demographics, Exposure, AEs and Categorical Safety

Delivery									
IA 1	Arm-1	Arm-2	Arm-3	Total					
IA 2	Arm-4	Arm-5	Arm-6	Total					
FA	Arm-1	Arm-2	Arm-3	Arm-4	Arm-5	Arm-6	Total		
FA [1]	Arm-1	Arm-2	Arm-3	Adult Total	Arm-4	Arm-5	Arm-6	Elderly Total	Total

IA: Interim analysis. FA: Final analysis.

[1] AE and Categorical Safety only

Table 3 Grouping: Safety (excluding AEs and Categorical Safety), Immunogenicity, Labs and Diary Data

Delivery						
IA 1	Arm-1	Arm-2	Arm-3			
IA 2	Arm-4	Arm-5	Arm-6			
FA	Arm-1	Arm-2	Arm-3	Arm-4	Arm-5	Arm-6

IA: Interim analysis. FA: Final analysis.
For interest of space table displays treatment arms instead of treatment labels, but outputs will display treatment labels.

4.2.2 Imputation of Missing and Incomplete Dates/Times

Every effort will be made to collect complete dates for all study assessments. The following date imputation rules will be applied for missing or partial dates:

- If month and year available, set start date to latest of (1st of the month, first dose date), if end date is complete and imputed start date > end date, set imputed start date to end date – 1

- If year available: Set start date to latest of (January 1st, first dose date), if end date is complete and imputed start date > end date, set imputed start date to end date – 1

- If start date is completely missing, set to first dose date, if end date is complete and imputed start date > end date, set imputed start date to end date – 1.

Completely missing time information will be imputed as 00:00. Partially missing time information with missing hour (xx:mm) or minute (hh:mm) part will be imputed as 00:mm or hh:00, respectively. Missing part will be shown as NK in the listings (where NK = Not Known).

If time is not collected and date of assessment is the same as the first dose date, then assessment will be assumed to be collected prior to treatment unless schedule of assessments and protocol indicate planned collection is always after dosing.

Any AEs with incomplete start and end dates/times will be treated as follows:

Adverse events with unknown start and/or end times (but where the date is known) will be imputed with a time of 00:00 h for the tabulations but will be shown as NK:NK in the listings (where NK = Not Known).

Adverse events with completely unknown start dates will be considered as treatment-emergent for the tabulations and will be shown as NK in the listings. Partially complete AE dates will not be imputed in the listings and the available day, month and year components will be used to determine if an adverse event is treatment-emergent taking the first dosing date and end of study date as references.

Imputing partial AE and prior/concomitant medication start dates:

a) If the year is unknown, the date will not be imputed and will be assigned a missing value.

b) If the month is unknown, then:

1. If the year matches the first dose date, then impute to the month and day of the first dose date.

2. Otherwise, assign 'January'.

c) If the day is unknown, then:

1. If the month and year match the first dose date, then impute to the day of the first dose date.

2. Otherwise, assign '01'.

Imputing partial AE and prior/concomitant medication stop dates:

a) If the year is unknown, the date will not be imputed and will be assigned a missing value.

b) If the month is unknown, then assign 'December'.

c) If the day is unknown, then impute to the last day of the month.

4.3 Software

All report outputs will be produced using SAS® version [9.4] or a later version in a secure and validated environment.

All report outputs will be provided to the Sponsor in RTF and PDF format.

4.4 Study Subjects

4.4.1 Disposition of Subjects

A clear accounting of the disposition of all subjects who enter the study will be provided, from screening to study completion.

An overview will be provided by treatment and overall on the number and percentage of subjects with ICF. The summary will include the number and percentage of subjects who entered, completed or discontinued (with reason for discontinuation) the study. Summary will be provided for each site and overall.

Summaries will include the number and percentage of subjects entering, completing and withdrawing from the study. Withdrawals from the study will also be summarized by major reason.

By-subject listings of eligibility and randomization details, visit dates, and withdrawal details (including reason for discontinuation and on study duration prior to discontinuation) will also be provided.

4.4.2 Protocol Violations

Protocol deviations are defined in a study specific Protocol Deviation Specification document.

Protocol deviations will be discussed during Data Review Meeting(s) (DRM) between PAREXEL and Sponsor, per the applicable SOPs (SOP-EPD-WW-012 and SOP-GDO-WW-017). All recorded PDs will be classified to minor/major/none and determined whether such deviations may warrant exclusion of a subject from the statistical analyses. Decisions made regarding the exclusion of subjects and/or subject data from analyses will be documented in the DRM report and approved by the Sponsor prior to database lock (DBL).

A summary of the number and percentage of subjects with major protocol deviation will be provided by treatment and protocol deviation for each site and overall.

By subject listing of protocol deviations will be provided for all screened subjects. This listing will be presented by treatment group and include subject identifier, visit (of occurrence), verbatim protocol deviation, protocol deviation code and classification (major/minor).

4.5 Analysis Sets

The safety summaries and analyses will be based on the Safety Analysis Set (SAF). The Safety Set is defined as all subject received any amount of study drug. Analyses using SAF will be performed by 'as treated' approach i.e. by actual treatment received.

The immunogenicity summaries and analyses will be based on the Immunogenicity Analysis Set (IAS). The immunogenicity analysis set is defined as all subject received any amount of study drug and have at least one non-missing post baseline immunogenicity evaluation. Subjects with any major protocol deviation thought to interfere with either the mechanism of action or immunogenicity test measurements will be excluded from IAS. Analyses using IAS will be performed by 'as treated' approach i.e. by actual treatment received.

A summary of the number and percentage of subjects assigned to each analysis set by treatment and overall will be provided for all subjects enrolled.

A by-subject listing of analysis set details will be provided for all subject enrolled. This listing will be presented by parts and dose group and include subject identifier, inclusion/exclusion flag for each analysis set and reason for exclusion, if applicable. This listing will be provided for all subjects dosed.

DRM will be held after the last data has been entered into the clinical database (DB) and prior to DB hard lock (DBHL). The primary purposes of the DRM are to:

- Confirm that the database is ready to be hard locked.
- Agree assignment of each enrolled subject to the analysis sets defined by the criteria described in Statistical Analysis Plan (SAP, Section 4.5), also known as analysis sets classification.

Results and decisions made during the meeting will be summarized in the DRM report which will be signed off by PAREXEL representatives and the Sponsor.

4.6 Demographics and Baseline Characteristics

Age will be calculated as the number of complete years between a subject's birth date and the date of informed consent.

Descriptive statistics (see Section 4.2**Error! Reference source not found.**) for demographics and other baseline characteristics will be presented for subjects assigned to SAF. All descriptive

summaries will be presented by treatment and overall. Unless otherwise specified, percentages (%) will be based on the number of patients assigned to SAF with data available.

Demographics will be summarized, including:

- Age (years)
- Sex
- Race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, Other/Specification)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Stated, Unknown)
- Other Baseline characteristics, as recorded in the Vital Signs - Height and Weight eCRF at Screening
    - Height (cm).
    - Weight (kg).
    - Body mass index (BMI) (kg/m2).

4.7 Medical History and Concomitant Illnesses

Medical history/current medical conditions will be presented for all subjects in SAF according to the Medical Dictionary for Regulatory Activities (MedDRA version 23.0 or higher) primary system organ class (SOC) and preferred term (PT), regardless of whether the status is reported as ongoing or not ongoing at visit Day 0. Medical history/current medical condition, start date of event and ongoing status (yes/no) of each medical history/current medical condition as pre-specified in the Medical history eCRF will be presented in by-subject data listing.

4.8 Prior and Concomitant Medications

Concomitant medications will be analyzed using the SAF. Concomitant medications will be coded using the World Health Organization-Drug Dictionary (WHO-DD B3) Version March 2020 and will be classified by Anatomical Therapeutic Chemical (ATC) categories.

Medication start and stop dates will be compared to the date of first dose of study medication to allow medications to be classified as either Prior only, both Prior and Concomitant, or Concomitant only. Medications starting after the completion/withdrawal date will be listed but will not be classified or summarized. Medications that start and stop prior to the date of first dose of study medication will

be classified as Prior only. If a medication starts before the date of first dose of study medication and stops on or after the date of first dose of study medication, then the medication will be classified as both Prior and Concomitant. Medications will be classified as Concomitant only if they have a start date on or after the date of first dose of study medication.

If medication start and/or stop dates are missing or partial, the dates will be compared as far as possible with the date of first dose of study medication. Medications will be assumed to be Concomitant only, unless there is clear evidence (through comparison of partial dates) to suggest that the medication started prior to the first dose of study medication. If there is clear evidence to suggest that the medication started prior to the first dose of study medication, the medication will be assumed to be both Prior and Concomitant, unless there is clear evidence to suggest that the medication stopped prior to the first dose of study medication. If there is clear evidence to suggest that the medication stopped prior to the first dose of study medication, the medication will be assumed to be Prior only.

Prior and concomitant medications as recorded in the Concomitant Medication eCRF will be presented in by-patient data listing. Listing will include ATC Level 1/Treatment Name/Name, dose, frequency and route, start/stop date and classification (prior, previous or both).

4.9 Treatment Exposure

Descriptive statistics for exposure will be presented for all subjects in SAF. Number and percentage of subjects received any amount of study drug will be summarized by treatment group and overall.

By-subject listing of drug exposure data as recorded on eCRF will be provided for SAF.

4.10 Immunogenicity

Immunogenicity analyses will be conducted on IAS as defined in Section 4.5. In case a subject contracts SARS-CoV-2 after vaccination (seropositivity for N-Ab and/or documented SARS-CoV-2 infection) or receives an approved COVID-19 vaccine , immunogenicity data collected from such subject will be excluded from immunogenicity analyses from that time point on.

Summaries will be provided by visit and treatment group for each immunogenicity endpoint as indicated in IA: Interim analysis. FA: Final analysis.

[1] AE and Categorical Safety only

Table 3 at Section 4.2. Immunogenicity evaluations will be assessed as shown in the Schedule of Assessments (Appendix 6.1).

Concentration below lower limit of quantification (BLQ) will be set to LLOQ/2 = 1.9 for summary calculations and graphical presentation. BLQ values will be listed as is in the source. Results of anti-S-Ab concentration will be summarized descriptively as indicated in Section 4.2.

Categorical results of anti-S-Ab, anti-N-Ab and PBMC ELISpot test will be tabulated by number and percentage of subject in each category.

Count data (micro neutralization test [titer]) will be summarized descriptively as indicated in Section 4.2.

Numeric assay result will be derived using the dilution factor from source data, ie 1:10 = 10, 1:20 = 20, etc. For result below limit of detection (LOD) which reported as <1:10 numeric result will be set to five (5) for summaries and graphical analyses.

Geometric mean titer for time point j will be calculated as

$$GMT_j = e^{\frac{\sum_{i=1}^{n_j} \ln(x_{ij})}{n_j}}$$

, where x_{ij} is the titer observed at time point j for subject i, and n_j is the number of observations at time point j.

To visualize the comparison between treatment groups the following graphs will be presented:

- Mean concentration – time profile of anti-S-Ab on semi-logarithmic scale.
- Individual concentration – time profiles (spaghetti plot) of anti-S-Ab on semi-logarithmic scale.
- By-visit combined plots (box + scatter) for micro neutralization and PBMC ELISpot results.

Inclusion of subjects for ELISpot analyses will depend on validity of the negative control results and be decided on case by case basis. Specifically, for the IFNγ ELISpot test on fresh PBMC at week 2, number of pools with positive results obtained in PBMC ELISpot test will assigned into two categories such as negative or positive. Result will be negative if number of positive pools is zero and positive if number of positive is greater than or equal to one.

ELISpot assay results on frozen PBMC samples obtained on Week 2, will be provided by the sponsor (outside of EDC system used in the study) and will be analyzed with other assay results recorded in EDC.

4.11 Safety Evaluation

All safety summaries and analyses will be based upon the SAF as defined in Section 4.5.

Safety summaries will be grouped by treatment as indicated in Table 2 and IA: Interim analysis. FA: Final analysis.

[1] AE and Categorical Safety only

Table 3 at Section 4.2.

Unscheduled tests will be listed only, unless it contributes as a baseline or as a worst post-baseline value.

4.11.1 Adverse Events

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0.

An AE is any untoward medical occurrence in a study subject administered an IMP which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP. Adverse events may include the onset of new illness and the exacerbation of pre-existing conditions.

Other untoward events occurring in the framework of a clinical study will be recorded as AEs, e.g. those occurring during treatment-free periods (including Screening or post-treatment Follow-up periods), in association with study-related procedures and assessments or under placebo.

Concomitant illnesses, which existed before entry into the clinical study, will not be considered AEs unless they worsen during the treatment period. Pre-existing conditions will be recorded as part of the subject's medical history.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins or worsens in severity in 28 days after study drug administration.

Adverse event with 'No Relationship' or 'Unlikely Related' causality will be evaluated as not related (relationship = No), otherwise (including missing causality) AE will be evaluated as related (relationship = Yes).

Adverse events with completely unknown start dates will be considered as treatment-emergent for the tabulations and will be shown as NK in the listings. Partially complete AE dates will not be imputed in the listings and the available day, month and year components will be used to determine if an adverse event is treatment-emergent taking the first dosing date and end of study date as references.

Solicited adverse reactions are foreseeable AEs following vaccination with GRAd-COV2 including the following AEs: injection site pain, erythema, warmth, swelling, pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea. Solicited adverse reaction is recorded as such on the corresponding AE's eCRF.

Adverse events of special interest (AESI) are any grade >=3 abnormalities on safety blood test according to Common Terminology Criteria for Adverse Events v.5.0 (CTCAE, see section 4.11.3).

An overview will be provided with the number and percentage of subjects as well as the total number of occurrences of the following event categories by treatment group:

- Any TEAE;
- Any TEAE related to study drug;
- Any treatment-emergent SAE (see section 4.11.2);
- Any treatment-emergent SAE related to study drug;
- Any TEAE leading to study discontinuation;
- Any AESI;
- Any solicited adverse reactions emerged in the first 7 days post dose.

Adverse event summaries by system organ class (SOC) and preferred term (PT) with number and percentage of subjects as well as the total number of occurrences will be provided by treatment group for the following:

- All TEAE;
- TEAEs by maximum severity;
- TEAEs by causality;
- All AESI;
- All solicited adverse reactions emerged in the first 7 days post dose.

Additional summary for all TEAE will be provided by PT and treatment group.

Adverse event summaries will be ordered in terms of decreasing frequency for SOC and PT within SOC in the Total group and then alphabetically for SOC and PT within SOC. Summaries will be ordered in terms of decreasing frequency for PT in the Total group in summaries where SOC is not included.

For each subject and each adverse event, the worst severity recorded will be attributed and used in the by-severity summaries. Similarly, the worst causality (most related to treatment) will be attributed and used in the by-causality summaries. If severity or causality is missing, a conservative approach for AE assessment (taking into account the worst case) will be followed.

By-subject listing of all adverse events (including non-treatment-emergent events) will be provided by treatment group. Adverse event listing will include center, subject identifier, age, sex, race, adverse event (SOC, PT, and verbatim term), date of onset, date of resolution, duration, solicitation flag, special interest flag. severity, seriousness, action taken, outcome and causality.

Adverse event listings will be provided for the following:

- All AEs
- All severe TEAE;
- All TEAE related to study drug;
- All AESI;
- All solicited adverse reactions emerged in the first 7 days post dose.

4.11.2 Deaths, Serious Adverse Events, and Other Significant Adverse Events

A serious AE (SAE) is defined as an AE which fulfills one or more of the following criteria: is a medically significant event (examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions regardless eventual need of hospitalization), is a congenital anomaly/birth defect, requires hospitalization or prolongation of existing hospitalization, causes persistent or significant disability or incapacity, is a life-threatening event or results in death. Serious adverse event is recorded as such on the corresponding AE's eCRF.

Separate AE listings will be provided for the following:

- Deaths;
- Serious AEs;

- TEAEs leading to study discontinuation.

Listings format will follow format described for adverse events in Section 4.11.1.

4.11.3 Clinical Laboratory Evaluation

Safety laboratory evaluations (hematology, biochemistry and urinalysis) will be assessed as shown in the Schedule of Assessments (Appendix 6.1). For list of safety laboratory test parameters refer to Blood test and other analysis for monitoring safety (section 8.3) in study protocol.

Urinalysis results will be listed only.

Hematology and biochemistry results will be graded according to NCI CTCAE (Version 5.0). Individual CTCAE grades will be provided with the by-subject listings.

For each hematology and biochemistry parameter:

- Results and change from baseline will be summarized descriptively by visit and treatment group;
- Number and percentage of subject in normal range categories will be tabulated by visit and treatment groups.
- Number and percentage of subject in each CTCAE severity grades will be tabulated by visit (and overall) and treatment groups.

Worst post-baseline record will be selected (flagged) by utilizing the rules as follow:

If there is any LOW or HIGH record, flag the most extreme record. Most extreme record will be derived by using the factor (f) for corresponding normal limits as appropriate (f × LLN or f × ULN). If there are multiple records with most extreme value, flag the earliest occurrence. If not out of normal range results is observed flag the first post-baseline observation.

By-subject listing of hematology and biochemistry with change from baseline will be provided by dose and time point for each treatment group separately.

All values outside the clinical reference ranges will be flagged in the data listings. The abnormal values will be flagged with ‘L’ for values below the lower limit of the clinical reference range and ‘H’ for values above the upper limit of the clinical reference range and included in the listings. The Investigator will assess whether the values outside the clinical reference range are clinically

significant (CS) and these will be reported as abnormal NCS or abnormal CS. Clinically significant laboratory values will be recorded by the Investigator as AEs.

4.11.4 Liver Safety

Based on the maximum post baseline value, the following criteria will be used to categorize the liver function tests, according to the Upper Limit of Normality (ULN):

- ALT >= 3X, >= 7 5X, >= 10X and >= 20X ULN
- AST >= 3X, >= 5X, >= 10X, and >= 20X ULN
- AST and/or ALT >= 3X ULN
- Total Bilirubin >= 2X ULN
- ALT and/or AST >= 3X ULN and Total Bilirubin >= 2X ULN (Hy's law)

For the ALT and/or AST >= 3X ULN and Total Bilirubin (TBL) >= 2X ULN criterion, a subject's ALT and Total Bilirubin laboratory draw date or AST and Total Bilirubin laboratory draw date must occur on the same date to be considered.

Frequency tables showing the number and percentage of subjects with potentially clinically significant values in liver function tests after first dose administration in the study will be provided.

A listing of the subjects meeting any of the criteria will be provided.

Helping visualization of possible Hy's law cases, an Evaluation of Drug Induced Serious Hepatotoxicity (eDISH) plot will be provided for post-baseline biochemistry results.

4.11.5 Vital Signs

Vital signs will be assessed as shown in the Schedule of Assessments (Appendix 6.1). The following vital signs measurements will be obtained:

- Systolic blood pressure (SBP) [mmHg].
- Diastolic blood pressure (DBP) [mmHg].
- Pulse rate (bpm).
- Blood oxygen saturation (SpO2) [%]
- Body temperature [°C].

Severity grading for vital signs will be derived according to the Table 4 below.

Table 4 Vital Signs Severity Grading

	Severity Grade		
Parameter	Mild	Moderate	Severe
SBP	139 < x =< 159	159 < x =< 179	x > 179
DBP	90 < x =< 99	99 < x =< 109	x > 109
Pulse rate	49 < x =< 55 or 100 < x =< 115	40 < x =< 49 or 115 < x =< 130	x =< 40 or x > 130
SpO2	93 < x =< 96	91 < x =< 93	x =< 91
Body temperature	35.0 < x =< 35.9 or 37.5 < x =< 38.0	38.0 < x =< 39.0	x =< 35 or x >39.0

If more than one assessment is performed for a time point, then for summaries the most severe grade will be used.

The following summaries will be provided for each vital sign parameter:

- Results and change from baseline will be summarized descriptively by timepoint and treatment group;
- Number and percentage of subjects will be tabulated by severity grade, visit and overall, for each treatment group;
- Shift from baseline severity grade will be presented by visit and overall, for each treatment group;

By-subject listing of vital signs results with change from baseline and severity grade will be provided by subject, vital sign parameter and timepoint for each treatment group.

4.11.6 Physical Examination

Physical examinations will be performed as shown in the SoA. By subject listing using SAF will be provided for overall results and body system with abnormal observation. Listing will be provided by subject and timepoint for each treatment group.

4.11.7 Safety Monitoring (Data and Safety Monitoring Board [DSMB])

The main roles of the DSMB for this study will be:

- decision-making on dose escalation, escalation intervals and cohort extension (if applicable).
- Monitoring all safety data recorded while the study is on-going.
- Proposing changes in the design and operations of the trial during the course of the study. For documented safety reasons, the DSMB can suggest and discuss with the Sponsor the interruption of the study. In case this decision will be agreed, the Sponsor should ensure the interruption in a reasonable timeframe, complying with ICH-GCP and all applicable Regulations.

The justification for the establishment of the DSMB and the grounds for dose escalation, as well as the DSMB procedures is provided in the DSMB charter.

Seven DSMB meetings are planned prospectively, in order to:

A. DSMB authorizes ARM1 group B and ARM2 Group A
B. DSMB authorizes ARM2 group B and ARM3 Group A
C. DSMB authorizes ARM3 group B
D. DSMB revises 4-week safety of whole Arm-1. DSMB authorize enrollment in ARM4 Group A.
E. DSMB authorizes ARM4 Group B and ARM5 Group A
F. DSMB authorize ARM5 Group B and ARM6 Group A
G. DSMB authorize ARM6 Group B

Tables, listings, and figures (TLFs) provided for the DSMB are created by the Parexel statistical team All TLFs (planned and ad-hoc) created for the DSMB are developed following the guidelines defined in this SAP, as appropriate. Specifications for these outputs are included in a separate TLF shells dedicated to solely supports the review conducted by the DSMB.

4.12 Other Analyses

4.12.1 Subject Diary Data

Diary data summaries will be based upon the SAF as defined in Section 4.5. Summaries will be grouped by treatment as indicated in IA: Interim analysis. FA: Final analysis.

[1] AE and Categorical Safety only

Table 3 at Section 4.2.

Subject are provided with diary cards as indicated in Schedule of Assessments (Appendix 6.1) and instructed to record systemic and injection site adverse event in diary for four weeks post vaccination. Daily source data are entered in the respective Diary eCRFs.

Summary on severity will be provided over all systemic adverse events (Nausea, Vomiting, Diarrhea, Abdominal Pain, Headache, Muscular Pain, Fatigue and Chills), injection site pain and ulcer by treatment groups, days and weeks. By time point severity over all systemic adverse events will be calculated by counting the number of subjects in each severity categories in any AE term.

By-subject listings of diary data will include records with any severity as well as swelling, redness, ulceration and body temperature daily assessments.

4.13 Determination of Sample Size

No formal testing of hypotheses has been planned for this study. Therefore, no formal sample size calculations were performed. 45 subjects are considered to be adequate to analyze the study objectives.

4.14 Interim Analyses

There will be two interim analyses (IAs) before general database lock and provision of final study results.

Each interim analyses will be preceded by a DRM focusing on the subjects to be included in the actual IA, i.e. for the first interim analysis (Deliverable 1) the target subject pool will be those who were enrolled for Arm 1 – 3 (Adults), for the second interim analysis (Deliverable 2) the target pool will be those who were enrolled for Arm 4 – 6 (Elderlies).

The first interim analysis will use data collected in Arm 1 – 3 up to the last subject in Arm-3 completed its Week 4 visit (Adults, highest dose). The second interim analysis will use data collected in Arm 4 – 6 up to last subject in Arm-6 completed its Week 4 visit (Elderlies, highest dose).

All outputs specified in the study's TLF Specification will be provided for both IAs. Grouping of IA summaries will follow guidelines specified in Section 4.2.1.

Selected immunogenicity endpoints such as ELISpot and nAb data will be analyzed at single timepoints during interim analyses. Results of ELISpot and nAb will be reported only for Week 2 and Week 4, respectively.

4.15 Changes in the Conduct of the Study or Planned Analysis

In reflection to DSMB recommendations on the meeting held 3rd October 2020, additional analyses on liver enzyme data will be performed with categorized liver test results and standard eDISH plot.

5 REFERENCES

Food and Drug Administration. (2014). Drug-induced liver injury: premarketing clinical evaluation.

Meeting Minutes (Draft). Study DSMB meeting held on 3rd October 2020.

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6 APPENDICES

6.1 Schedule of Assessments

Table 5 Schedule of Assessments

	Time of the visit since vaccination								
	Screening	T0	D2	W1	W2	W4	W8	W12	W24
Window (days)	≤ 28	0	0	±1	±1	±2	±4	±7	±14
Informed consent	X								
Eligibility	X								
Vaccination		X							
AE reporting	X	X	X	X	X	X	X	X	X
Diary card		A		B	B	B§			
Checking diary card completeness			X	X	X	X			
Medical history									
Adverse Events	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X
Body Temperature	X	X	X	X	X	X	X	X	X
SpO2 %	X	X	X	X	X	X	X	X	X
Pulse	X	X	X	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X	X	X	X
ECG	X								
Urine analysis	X								
Urine B-HCG	X	X							
Blood counts	X	X	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X	X	X
Coagulation	X								
HIV-Ab	X								
HBsAg and HCV	X								
HLA Typing				X					
SARS-CoV-2 Serology*	X								
CLIA anti-S (IgG)		X		X	X	X	X	X	X
CLIA anti-N (IgG)		X		X	X	X	X	X	X

	Time of the visit since vaccination								
	Screening	T0	D2	W1	W2	W4	W8	W12	W24
Micro neutralization						X			X
ELISpot SARS-CoV-2		X			X	X	X	X	X

Scr=screening to be done no more than 21 days before vaccination; T0= day of the vaccination; D2= day 2 after vaccination; W1= one week after vaccination; W2= two weeks after vaccination W4= four weeks after vaccination; W8= eight weeks after vaccination; W12= twelve weeks after vaccination; W24= twenty-four weeks after vaccination. A) Diary given to volunteers; B) Diary received form volunteers. §At week 4, diary cards for W3 and W4 will be collected.

* This is any serological assay to detect anti-SARS-CoV-2 total IgG which is validated for clinical use in Italy (i.e. validated for diagnosis of SARS-CoV-2 infection). †no CRP testing at screening.

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Reason for signing: Approved	Name: Istvan Nemeth Role: Biostatistics Date of signature: 23-Jun-2021 15:32:54 GMT+0000

Reason for signing: Approved	Name: Stefania Capone Role: Sponsor Date of signature: 24-Jun-2021 09:25:42 GMT+0000

Reason for signing: Approved	Name: Roberto Camerini Role: Sponsor Date of signature: 28-Jun-2021 08:32:34 GMT+0000

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