# CLINICAL STUDY PROTOCOL

## Randomized, placebo-controlled, double-blind, multicenter, seamless adaptive phase II-III clinical trial to select the dose and evaluate safety and efficacy of MAD0004J08 monoclonal antibody in adult patients with recently diagnosed asymptomatic to moderately severe COVID-19

Protocol Number: A0001B

EudraCT Number:2020-005532-29

Sponsor: Toscana Life Sciences - Sviluppo (TLS-S), Siena, Italy

Version Number: 8.0 (final)

Date: 24 March 2021

# STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP E6) and applicable laws and regulations in force. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the competent Ethics Committee (EC), except where necessary to eliminate an immediate hazard to the trial participants. All personnel involved in the conduct of this study have completed ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the EC for review and approval. Approval of both the protocol and the consent form must be taken before any participant is enrolled. Any amendment to the protocol will require review and approval by the EC before the changes are implemented to the study. All changes to the consent form will be EC-approved; a determination will be made regarding whether a new consent needs to be taken from participants who provided consent using a previously approved consent form.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADA | Anti-Drug Antibody |
| ADE | Antibody-dependent Disease Enhancement |
| ADNK | Antibody-Dependent NK cell Activation |
| ADNP | Antibody-Dependent Neutrophil Phagocytosis |
| ADR | Adverse Drug Reaction |
| AE | Adverse Event |
| AESI | Adverse Events of Special Interest |
| AIDS | Acquired Immunodeficiency Syndrome |
| AIFA | Agenzia Italiana del Farmaco |
| ALT | Alanine amino Transferase |
| ANC | Absolute Neutrophil Count |
| ANOVA | Analysis of Variance |
| aPTT | Partial Thromboplastin Time |
| AR | Adverse Reaction |
| ASL | Azienda Sanitaria Locale |
| AST | Aspartate amino Transferase |
| ATS | Azienda Territoriale Sanitaria |
| ATC | Anatomic Therapeutic Chemical |
| AUC | Area Under Curve |
| BMI | Body Mass Index |
| BT | Body Temperature |
| CcCl | Creatinine Clearance |
| CI | Confidence Interval |
| CONSORT | Consolidated Standards of Reporting Trials |
| COVID-19 | Corona Virus Disease 2019 |

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|---|---|
| CPK | Creatine Phosphokinase |
| CRF | Case Report Form |
| CRO | Contract Research Organization |
| CRP | C-Reactive Protein |
| CSR | Clinical Study Report |
| DBP | Diastolic Blood Pressure |
| DMC | Data Monitoring Committee |
| EC | Ethics Committee |
| $EC_{50}$ | Half Maximal Effective Concentration |
| ECG | Electrocardiogram |
| ECMO | Extra Corporeal Membrane Oxygenation |
| eCRF | Electronic Case Report Form |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EMA | European Medicines Agency |
| EUA | Emergency Use Authorization |
| FAS | Full Analysis Set |
| FcγR | FC gamma Receptor |
| FDA | Food and Drug Administration |
| $FiO_2$ | Fractional Inspired Oxygen |
| GCP | Good Clinical Practice |
| Hep-2 | Human Epithelial Type-2 |
| HIV | Human Immunodeficiency Virus |
| HR | Heart Rate |
| IB | Investigator's Brochure |
| $IC_{50}$ | Half Maximal Inhibitory Concentration |
| ICF | Informed Consent Form |

| | |
|---|---|
| ICU | Intensive Care Unit |
| ICH | International Council for Harmonisation |
| IFN | Interferon |
| IgG | Immunoglobulin G |
| IM | Intramuscular |
| IMP | Investigational Medicinal Product |
| INR | Prothrombin time |
| IWRS | Interactive Web Response System |
| IV | Intravenous |
| LDH | Lactate Dehydrogenase |
| MLE | Maximum Likelihood Estimator |
| KD | Affinity |
| mAb | monoclonal Antibody |
| MD | Medical Doctor |
| MedDra | Medical Dictionary for Regulatory Activities |
| MMRM | Mixed Model for Repeated Measures |
| MP | Monitoring Plan |
| MUT | Mutated |
| NIH | National Institutes of Health |
| NK | Natural Killer |
| NoB | Neutralization of Binding Activity |
| OsSC | Osservatorio Nazionale sulla Sperimentazione Clinica dei Medicinali |
| $PaO_2$ | Arterial Oxygen Partial Pressure |
| PPAS | Per Protocol Analysis Set |
| PPE | Personal Protection Equipment |
| RBD | Receptor Binding Domain |

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|---|---|
| RR | Respiratory Rate |
| RSV | Respiratory Syncytial Virus |
| RT-PCR | Reverse Transcriptase – Polymerase Chain Reaction |
| SA set | Safety Analysis Set |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SAR | Serious Adverse Reaction |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome – Coronavirus – 2 |
| SBP | Systolic Blood Pressure |
| SC | Steering Committee |
| SD | Standard Deviation |
| SDV | Source Data Verification |
| SoA | Schedule of Activities |
| SoC | Standard of Care |
| SOP | Standard Operating Procedure |
| SpO2 | Saturation of Peripheral Oxygen |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TEADA | Treatment Emergent Anti-Drug Antibodies |
| TEAE | Treatment Emergent Adverse Event |
| TLS-S | Toscana Life Sciences - Sviluppo |
| UAR | Unexpected Adverse Reaction |
| ULN | Upper Limit of Normal |
| URT | Upper Respiratory Tract |
| US | United States |
| WHO | World Health Organization |
| WOBC | Women of Childbearing Potential |

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|---|---|
| WT | Wild Type |
| yoa | years of age |

# 1 PROTOCOL SUMMARY

## 1.1 Synopsis

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| **Title of study:** | Randomized, placebo-controlled, double-blind, multicenter, seamless adaptive phase II-III clinical trial to select the dose and evaluate safety and efficacy of MAD0004J08 monoclonal antibody in adult patients with recently diagnosed asymptomatic to moderately severe COVID-19 |
| **Protocol number:** | A0001B |
| **Sponsor:** | Toscana Life Sciences - Sviluppo (TLS-S), Siena, Italy |
| **Phase of development:** | II-III |
| **Rationale:** | MAD0004J08 is a potent neutralizing IgG1 monoclonal antibody (mAb) targeting the spike protein of SARS-CoV-2. MAD0004J08 blocks viral attachment and entry into human cells and neutralizes the virus. Because of its high affinity and potency, MAD0004J08 may accelerate clearance of the virus and prevent clinical deterioration of COVID-19 patients, especially when administered shortly after infection, and prevent SARS-CoV-2 infection in uninfected subjects. Because of its high potency, MAD0004J08 is expected to be effective at low doses (mg range) and thus will be administered by intramuscular (IM) injection, as opposed to the intravenous bolus required by high dose mAbs. The IM route of administration would facilitate treatment, which would be especially important in the context of the ongoing COVID-19 pandemic.<br><br>The goals of this Phase II-III seamless adaptive clinical trial are:<br><br>Stage-1 (Phase II)<br><br>1) Select one dose level for progression to Stage-2<br><br>Stage-1 + Stage-2 (Phase III)<br><br>2) Provide confirmatory evidence of safety and efficacy for regulatory approval.<br><br>If strong evidence is obtained, early submission to regulatory authorities for conditional / emergency use approval will be considered. |
| **Study description:** | This clinical trial is designed as a randomized, stratified, placebo-controlled double-blind, multicenter, seamless adaptive study.<br><br>The target study population is adult patients ≥ 18 years of age with recently diagnosed (≤ 3 days from 1st positive swab taken) asymptomatic to moderately severe COVID-19 at baseline. Patients with comorbidities will be allowed in the study assuming all inclusion and exclusion criteria are met. Participants will not require hospitalization at baseline.<br><br>The trial is designed in two stages: |

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| | In Stage-1 participants meeting inclusion/exclusion criteria will be randomized (1:1:1 ratio) to one of the following three study treatments on top of standard of care (SoC) treatment:<br>– MAD0004J08 400 mg, single dose<br>– MAD0004J08 100 mg, single dose<br>– Placebo, single dose<br><br>When a predefined number of events (see below for definition) is reached, the data will be analyzed following a pre-planned interim analysis plan. Based on the results of the interim analysis, and pre-defined criteria, the Data Monitoring Committee (DMC) will recommend whether the study should advance to Stage-2, and if so, will recommend selection of one of the two MAD0004J08 treatments for Stage-2. Alternatively, the DMC will recommend stopping the study either for futility or for efficacy following pre-defined criteria. Final decisions will be made by an unblinded sub-group of the Steering Committee (SC), including senior Sponsor representatives, based on summary results.<br><br>If the study continues, Stage-2 will start and participants meeting inclusion/exclusion criteria will be randomized (1:1 ratio) to one of two treatments on top of SoC treatment:<br>– MAD0004J08, dose level selected in Stage-1, single dose<br>– Placebo, single dose<br><br>Twelve (12) study visits and 2 telephone calls are scheduled for each participant over approximately 168 days (see the Schedule of Activities (SoA) in Section 1.3). Additional ad-hoc visit(s) may be necessary to confirm eradication of SARS-CoV-2 from the upper respiratory tract (URT) following the 1st negative swab.<br><br>Due to the need to minimize time between diagnosis and intervention, screening procedures, baseline procedures, randomization and administration of study treatment will typically occur on the same day (Day 1, Visit 1) at the study center. However, if the ≤3-day limit between swab taken and intervention is maintained, Visit1 can occur over two days (Days 0 and 1).<br><br>At Visit 1 (baseline) all participants will undergo testing for serum IgA and IgG vs. the spike (S) protein, and IgG vs. nucleocapsid (N) protein: participants testing negative to all three antibodies at baseline are referred to as seronegative; participants testing positive to any of the three antibodies at baseline are referred to as seropositive.<br><br>Visits from Day 3 to Day 21 (Visits 2 to 9) will be conducted by study staff at the participant's home, unless the participant is hospitalized. Visits from Day 28 to Day 168 (Visits 10 to 12) will be conducted at the study center.<br><br>Participants requiring hospitalization during the study period are to be hospitalized at the study center where Visit 1 was conducted. Visits and procedures during hospitalization will be conducted as per protocol to the extent the participant's condition allows it. Once a participant is discharged from the hospital, subsequent home and center visits will resume as per protocol. |

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| | Nasopharyngeal swabs will be carried out at each scheduled visit. Additional swabs may be taken ad hoc to confirm eradication after the 1st negative swab. Swabs will be tested for SARS-CoV-2 viral load by reverse transcriptase polymerase chain reaction (RT-PCR).<br><br>This is an event-driven study. The event for an individual participant is the 1st nasopharyngeal swab testing negative for SARS-Cov-2 by RT-PCR followed by an additional negative nasopharyngeal swab taken at least 24 hours thereafter.<br><br>Safety and efficacy endpoints will be analyzed as appropriate in two target populations and three time-windows:<br><br>Primary populations: 1) all randomized participants (ALL), and 2) seronegative randomized participants (SEROneg).<br><br>Time windows (defined at an individual participant level): 1) baseline (Visit 1) to end of Stage-1 or dropout (interim analysis), 2) baseline (Visit 1) to end of Stage-2 or dropout (primary analysis), 3) baseline (Visit 1) to end of study (Visit 12) or dropout (final analysis).<br><br>The interim and primary time windows are variable, whereas the final time window is fixed unless the participant drops out from the study.<br><br>The DMC composed of experts independent of the Sponsor will monitor the safety and wellbeing of participants throughout the study and make recommendations on study progress at the end of Stage-1 (see above).<br><br>The SC, which will include Sponsor personnel, will ensure transparent management of the study according to the protocol and make final decisions on study progress taking into account DMC recommendations (see above). |
| **Objectives:** | Safety<br><br>Primary: to assess the safety and tolerability of MAD0004J08 as determined by severe and serious adverse events.<br><br>Secondary: to assess the overall safety and tolerability, local reactogenicity, and production of anti-drug antibodies of MAD0004J08.<br><br>Efficacy<br><br>Primary: to demonstrate that MAD0004J08 shortens the time to clearance of SARS-CoV-2 from the URT.<br><br>Key secondary: to demonstrate that MAD0004J08 reduces the proportion of participants who experience one of the clinical outcomes that are part of the composite endpoint (see below).<br><br>Secondary: to assess the impact of MAD0004J08 on SARS-CoV-2 virus in the URT and on the clinical course of COVID-19. |
| **Endpoints** | All endpoints will be tested in the ALL and SEROneg primary populations.<br><br><u>Safety</u> |

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| | Primary: proportion of participants with severe (Grade 3) unsolicited AEs and/or serious unsolicited AEs (SAEs).<br><br>Secondary: proportion of participants with unsolicited AEs, including clinically relevant laboratory and ECG abnormalities, and with solicited local AEs at the injection site; proportion of tested participants who develop ADA (ADA testing limited to the first 60 randomized participants).<br><br><u>Efficacy</u><br><br>Primary: time to SARS-CoV-2 clearance in the URT. The primary endpoint is censored at Day 28.<br><br>Key secondary: proportion of patients experiencing at least one of the following events: peripheral capillary oxygen saturation ($SpO_2$) < 94%, newly established or increased (*) dose home oxygen therapy, hospitalization, death (clinical composite endpoint).<br><br>Secondary: viral clearance and viral load in the URT and $SpO_2$% by visit, lowest $SpO_2$, proportion of participants with SpO2 % < 94%, COVID-19 clinical symptoms, newly established or increased (*) dose home oxygen therapy, proportion of patients requiring hospitalization, hospital oxygen therapy, admission to intensive care unit (ICU) and deaths; duration of hospital and ICU stay and of oxygen therapy.<br><br>(*) Patients enrolled in the trial must not require home oxygen therapy due to COVID-19 at baseline (see below); increased home oxygen therapy only applies to patients with underlying conditions other than COVID-19 requiring such therapy, (e.g., COPD). |
| **Study population:** | The estimated target sample size of the study is 806 randomized participants, of whom 403 (~50%) seronegative. Should the proportion of seronegative participants be lower than 50%, recruitment will continue until the predefined number of events is reached in the SEROneg population.<br><br>The interim analysis will occur when 334 primary endpoint events (see definition above) have been accrued in the ALL population and 170 events have been accrued in the SEROneg population. The primary analysis will be conducted when 546 and 277 events have occurred in the two populations, respectively. Based on several assumptions, it is expected that the sample size of Stage-1 (all patients randomized before the interim analysis) will be approximately 50% of the total sample (i.e., ~402).<br><br>**Inclusion criteria**<br><br>1. Signed written informed consent taken before any study procedure from any patient capable of giving consent, or, when the patient is incapable of doing so, by his or her legal/authorized representative.<br><br>2. Age ≥18 years.<br><br>3. First nasopharyngeal swab testing positive for SARS-CoV-2 by RT-PCR taken no more than 3 days before randomization (Visit 1). Results of "rapid" semiquantitative tests are not acceptable. |

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| | 4. Asymptomatic to moderately symptomatic outpatients with no need for immediate hospitalization: grade 1, or grade 2 or grade 3 of Clinical Severity Scale.<br>5. No childbearing potential (post-menopause, surgically-induced, or pharmacologically-induced sterility) or, if of childbearing potential, negative urinary pregnancy test (women) and commitment to use at least 2 forms of contraception for at least 168 days from administration of study drug (men and women).<br>**Exclusion criteria**<br>1. Severe or critical COVID-19: grade 4 or grade 5 of clinical severity scale.<br>2. Current hospitalization and/or hospitalization or emergency room visit in the past 14 days.<br>3. Need for immediate hospitalization for any reason in the investigator's opinion.<br>4. Severe liver disease as determined by values of ALT and/or AST >5x upper limit of normal (ULN) and/or history of liver cirrhosis.<br>5. Severe renal disease as determined by estimated creatinine clearance (CcCl) <30 mL/min or serum creatinine >2 mg/dL (>176.8 μmol/L) or ongoing renal dialysis.<br>6. Absolute neutrophil count (ANC) < 1000/μL.<br>7. Demyelinating and connective tissue disease.<br>8. Active tuberculosis or suspected active bacterial, fungal, viral, or other infection (besides COVID-19).<br>9. Any condition that in the Investigator's opinion may be negatively affected by the study treatments and/or study procedures.<br>10. Any condition, including psychiatric disorders, alcohol, or substance abuse, which in the Investigator's opinion may interfere with completion of the study procedures.<br>11. Any condition with life expectancy <6 months in the Investigator's opinion.<br>12. Ongoing or planned pregnancy.<br>13. Ongoing breast feeding.<br>14. History of life-threatening event in the 1 month before Visit 1.<br>15. History of surgery in the 1 month before Visit 1.<br>16. History of treatment with blood components in the 6 months before Visit 1.<br>17. History of cancer treated with chemotherapy in the 6 months before Visit 1.<br>18. History of solid organ transplant at any time before Visit 1.<br>19. History of severe and/or serious allergic reaction to monoclonal antibodies or any component of MAD0004J08, including anaphylaxis at any time before Visit 1.<br>20. Treatment with an investigational drug or vaccine within 5 half-lives or 30 days (whichever is longer) of randomization. |

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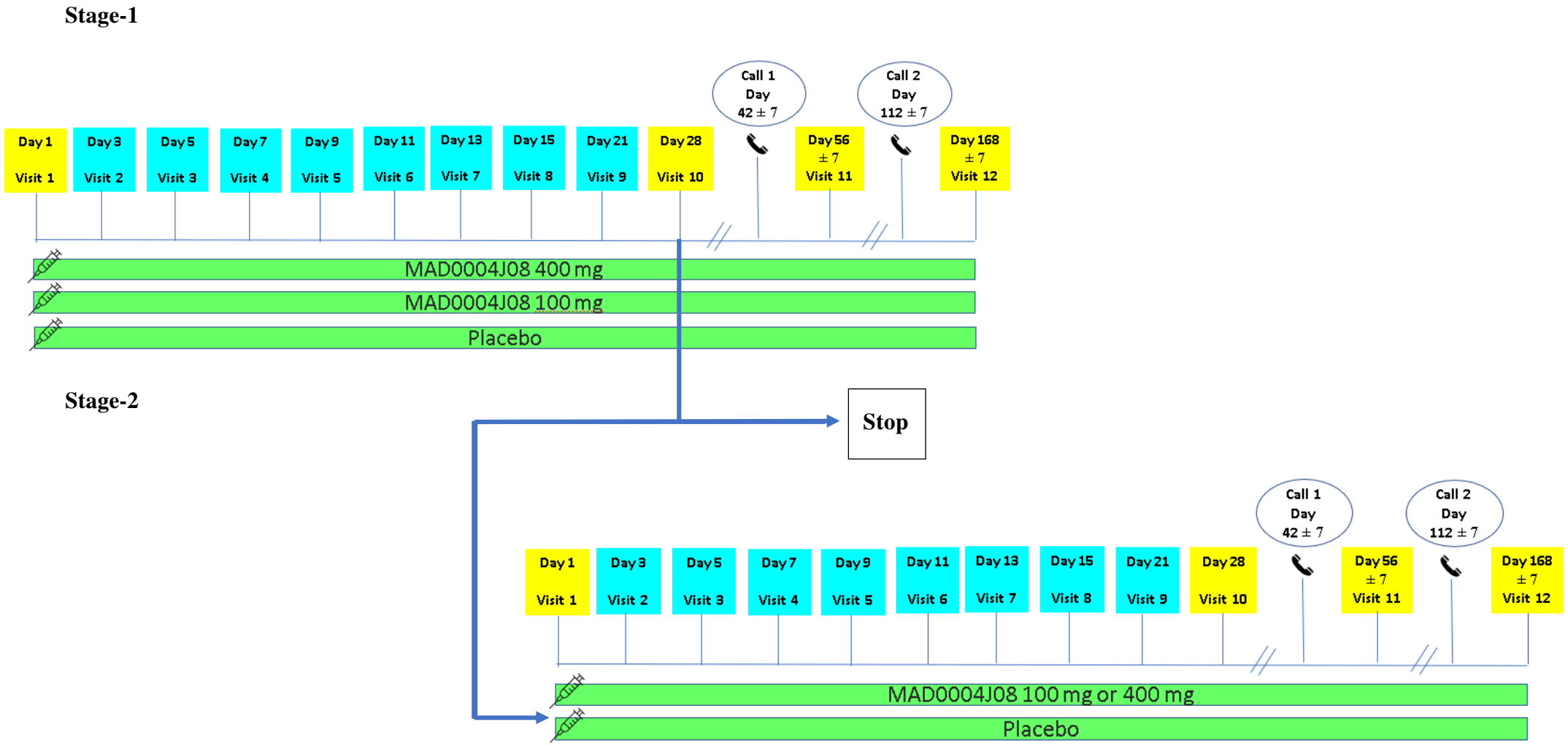
| | 21. Treatment at any time with monoclonal antibodies bamlanivimab, bamlanivimab + etesevimab combination, and casiribimab + imdevimab combination.<br><br>Receipt of an approved vaccine vs. COVID-19 is NOT an exclusion criterion, i.e., is compatible with enrolment in the study if all inclusion and exclusion criteria are met. |
| **Study intervention:** | **Investigational Medicinal Products (IMP, also referred to as study drugs)**<br>– MAD0004J08 (human monoclonal Antibody (mAb)), 100 mg in a 2.5 mL 2R vial<br>o Drug Substance: MAD0004J08 42.5 ± 2.5 mg / mL = 100 ±10 mg/vial<br>o Buffers: sodium phosphate monobasic monohydrate (1,89 g/L), sodium phosphate dibasic anhydride (0.92 g/L), 0.9% NaCl saline solution (to 2.5 mL)<br>– Placebo: 0.9% NaCl saline solution in a 2.5 mL 2R vial.<br><br>Participants will be randomized to one of three treatments (1:1:1 ratio) in Stage-1 and to one of two treatments (1:1 ratio) in Stage-2 as described above.<br><br>Each participant will receive a single dose of study treatment administered as two IM injections of 5.0 mL each.<br><br>Along with the study intervention, patients in all three treatment arms will receive SoC as deemed appropriate by the treating physician and the Investigator. |
| **Version and date** | Version 8.0, 24 March 2021 |

## 1.2 Study schema

*Figure 1-1 Study schema*



## 1.3 Schedule of Activities (SoA)

***Table 1-1*** **Schedule of Activities**

| Day | 1 (or 0-1 ◆ ) Screening, Bsl, Randomization | 3, 5 | 7 | 9, 11, 13, 15, 21 | 28 | 42 ± 7 | 56 ± 7 | 112 ± 7 | 168 ± 7 |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Visit 1** | **Visits 2-3** | **Visit 4** | **Visits 5-6-7-8-9** | **Visit 10** | 📞 | **Visit 11** | 📞 | **Visit 12** |
| **Location** | **Study Center** | **Home ♯** | **Home ♯** | **Home ♯** | **Study Center** | | **Study Center** | | **Study Center** |
| **1. Informed consent (a)** | X | | | | | | | | |
| **2. Demographics and medical history** | X | | | | | | | | |
| **3. Physical examination** | X (k) | | | | X (l) | | X (l) | | X(l) |
| **4. Vital signs (BT, HR, RR, DBP, SBP)** | X | X | X | X | X | | X | | X |
| **5. ECG** | X | | | | X | | X | | X |
| **6. SpO2** | X | X | X | X | X | | X | | X |
| **7. Chest imaging (ultrasound or X-rays)** | X | | | | | | | | |
| **8. $PaO_2/FiO_2$** | X | | | | | | | | |
| **9. COVID-19 Clinical Severity Scale** | X | | | | | | | | |
| **10. Solicited local AEs** | X | X | X | X | X | | | | |
| **11. Unsolicited AEs (b)** | X | X | X | X | X | X | X | X | X |
| **12. Previous (c) and concomitant medications** | X | X | X | X | X | X | X | X | X |
| **13. Blood samples for routine safety tests (d)** | X* | | X | | X | | X | | X |
| **14. Urine sample for pregnancy test (d, e)** | X | | | | X | | X | | X |
| **15. Inclusion and exclusion criteria** | X | | | | | | | | |
| **16. Blood sample for anti-SARS-CoV-2 Abs (f)** | X | | | | | | | | |
| **17. Blood samples for ADA and MAD0004J08 concentration (f, g)** | X | | X | | X | | X | | X |
| **18. Blood sample for exploratory research (f, h)** | X | | X | | X | | | | |
| **19. Nasopharyngeal swab (f, i, j)** | X | X | X | X | X | | X | | X |
| **20. Randomization and IMP administration** | X | | | | | | | | |
| **21. COVID-19 Symptom and oxygen intake quest.** | X | X | X | X | X | | X | | X |
| **22. Hospitalization events** | ----------Daily recording in case of hospitalization---------- | | | | | | | | |

♦ Screening and baseline procedures, randomization and administration of IMP will typically occur on the same day (Day 1). However, if the ≤3-day limit is maintained between swab taken and study drug given, Visit 1 can occur over two days (Days 0 and 1).
✆ Phone call.
♯ The visit will occur at the study center hospital if patient requires hospitalization.
(a) Informed consent must be explained to, reviewed, and signed by the participant before any study procedure, including screening procedures.
(b) Participants will be encouraged to call the study center at any time between visits to report AEs.
(c) "Previous" refers to discontinued medications including vaccines and non-prescription medications, taken in the past year for Visit 1 and since the last visit for all other visits.
(d) Results must be available before randomization and dosing.
(e) Limited to women of childbearing potential.
(f) To be conducted at Visit 1 only if the participant meets all inclusion / exclusion criteria and is enrolled.
(g) Limited to the first 60 randomized participants who receive IMP: a sample will be taken at each scheduled time point.
(h) To be taken only if the participant agrees to sampling for exploratory research in a dedicated informed consent form. If the participant agrees, a 7 mL sample and a 9 mL sample will be taken at each scheduled time point.
(i) Additional nasopharyngeal swabs may be taken at an ad-hoc visit at least 24 hours after the 1$^{st}$ negative swab to confirm eradication of SARS-CoV-2 from the URT.
(j) If a nasopharyngeal swab cannot be taken in a hospitalized participant at a scheduled visit, the swab will be taken at an ad hoc visit as soon as feasible in the investigator's judgement.
(k) Full physical examination, including calculation of Body Mass Index (BMI) but excluding pelvic, rectal and breast examinations unless clinically indicated.
(l) Symptom/history-driven partial physical examination.

Legend: Abs: antibodies, -ADA = anti-drug antibodies, AE = adverse event, bsl = baseline, BT = body temperature, DBP = diastolic blood pressure, ECG = electrocardiogram, HR = heart rate, RR = respiration rate, SBP = systolic blood pressure, $SpO_2$ = Peripheral Capillary Oxygen Saturation.

# 2 GENERAL INFORMATION

Protocol title: Randomized, placebo-controlled, double-blind, multicenter seamless adaptive phase II-III clinical trial to select the dose and evaluate safety and efficacy of MAD0004J08 monoclonal antibody in adult patients with recently diagnosed asymptomatic to moderately severe COVID-19.

Name and address of Sponsor: Toscana Life Sciences - Sviluppo (TLS-S), Siena, Italy

Name, title and contact details of Sponsor's representative: Andrea Frosini, TT & IPR manager, a.frosini@tlssviluppo.com.

Name, title and contact details of Principal Investigator or Coordinating Investigator: Simone Lanini, M.D., Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome, Italy, e-mail:simone.lanini@inmi.it

# 3 INTRODUCTION

## 3.1 Background and study rationale

COVID-19 can be unpredictable in its severity. The clinical presentation of SARS-CoV-2 infection ranges from asymptomatic to life threatening with multi-organ failure [1].

All subjects infected with SARS-CoV-2, including those with no or mild symptoms, are quarantined until they are considered unlikely to be infectious, typically when nasal or nasopharyngeal swabs turn negative for SARS-CoV-2, which may require several weeks, even in asymptomatic carriers [2, 3].

Such quarantine may have a major impact on income and employment, family and social life and quality of life in general for individuals and on medical, social, and economic functioning of local communities.

An intervention that shortens time to eradication of SARS-CoV-2 would have a positive impact on the patients, the health care system, and the communities. These benefits would be even greater if the intervention also has an effect on preventing or shortening hospitalizations.

Human monoclonal antibodies (mAbs) have shown potential in the fight against infectious diseases [4]. Examples are mAbs against respiratory syncytial virus (RSV), which have shown therapeutic effect in infants with one 50 mg intramuscular injection [5], human immunodeficiency virus (HIV), where some mAbs have demonstrated broad neutralization to several clinical strains and high virologic suppression [6, 7], and Ebola virus, where mAb therapy was the only effective tool to tackle the dramatic disease caused by this virus [8].

Since the start of the COVID-19 pandemic, several human mAbs capable of neutralizing the SARS-CoV-2 virus *in vitro* and preventing/treating the infection *in vivo* in animal models have been identified and some are currently in clinical development [9, 10].

Interim results of ongoing clinical studies testing Eli Lilly's mAb bamlanivimab (LY-CoV555) and mAb combination bamlanivimab + etesevimab and Regeneron's mAb combination casiribimab + imdevimab (REGN-COV2) were recently published, suggesting that a single high dose given as an intravenous (IV) infusion may reduce viral load, and/or medically attended visits and/or hospitalizations in non-hospitalized patients with mild to moderate COVID-19 [11, 12, 53]. In November 2020, the US Food and Drug Administration (FDA) granted bamlanivimab and casiribimab + imdevimab emergency use authorization (EUA) in COVID-19 patients 12 years of age or older weighing at least 40 kg and who are at high risk of progression to severe disease and/or hospitalization [13, 14]. For both products, the lowest dose among those tested in the ongoing trials was approved for EUA, namely 700 mg for bamlanivimab and 2400 mg for casirivimab + imdevimab (1200 mg each).

In February 2021, the Italian regulatory agency AIFA made available bamlanivimab and casiribimab + imdevimab before registration for early treatment of outpatients with mild to moderate COVID-19 at risk of disease progression and hospitalization [54]. The risk factors indicated by AIFA are similar to those indicated by the FDA. AIFA emphasizes that access to these unapproved drugs is granted because of the exceptional circumstances despite the "immaturity of data and the consequent uncertainty on the entity of the benefit offered by such dugs" [56].

At the end of February 2021, the European Medicines Agency (EMA) completed the review of the available documentation for casiribimab + imdevimab (REGN-COV2) under Article 5(3) of Regulation 726/2004 and concluded that this combination can be used prior to marketing authorization for the treatment of confirmed COVID-19 in patients who do not require supplemental oxygen and who are at high risk of progressing to severe COVID-19. EMA's review under Article 5(3) for the bamlanivimab + etesevimab combination is ongoing [55, 56].

## 3.2 Anti-SARS-CoV-2 MAD0004J08 monoclonal antibody

MAD0004J08 is a novel anti-SARS-CoV-2 monoclonal antibody recently developed by Toscana Life Sciences (TLS), Siena, Italy.

This monoclonal antibody has shown a very high neutralization potency *in vitro* and completely neutralizes the SARS-CoV-2 virus ($IC_{100}$) at a concentration as low as 0.003 µg/mL. The high potency of MAD0004J08 makes it suitable for IM administration.

Furthermore, the constant portion (Fc) of MAD0004J08 was engineered to extend half-life and to abrogate the Fc binding to cellular receptors, which has been associated to antibody dependent enhancement (ADE) disease [15].

Nonclinical studies for MAD0004J08 *in vitro* characterization, autoreactivity evaluation, and Fc engineering to abrogate Fc binding to cellular receptors and also to extend half-life are summarized hereafter. In addition, results of an *in vivo* study to assess MAD0004J08 prophylactic activity in an animal model of SARS-CoV-2 infection are presented.

A tissue cross-reactivity study of MAD0004J08 is currently ongoing with the aim to assess its potential cross-reactivity with a selected panel of human tissues.

### 3.2.1 *In vitro* analysis for MAD0004J08 characterization

Several *in vitro* assays were performed on MAD004J08 to profile its binding characteristics and evaluate its functional activity against the live SARS-CoV-2 virus.

- Initially, an enzyme-linked immunosorbent assay (ELISA) was performed against the trimeric pre-fusion stabilized SARS-CoV-2 spike protein (S-protein) and the two different subunits of the spike monomer named S1 and S2 domains.
- MAD0004J08 is able to tightly bind the trimeric S-protein with a half maximal effective concentration ($EC_{50}$) of 5.8 and an affinity (KD) of $0.03E^{-11}$ M.
- Binding was also detected when the antibody was tested against the S1 domain with an $EC_{50}$ of 4.8 ng/mL. Vice versa, no signal was detected for binding to the S2 domain.

Following the binding profiling of the antibody, MAD0004J08 was assessed for its neutralization of binding activity (NoB), aimed at evaluating the abrogation of S-protein/receptor binding, and neutralization potency against both the wild type (WT: SARS-CoV-2/INMI1-Isolate/2020/Italy: MT066156) and the widespread SARS-CoV-2 D614G mutated strain (SARS-CoV-2/human/ITA/INMI4/2020, clade GR, D614G (S): MT527178) [16]. MAD0004J08 neutralized the S-protein/receptor binding with an $EC_{50}$ of 78.6 ng/mL and was very efficient in neutralizing both viral strains with an $IC_{100}$ of 3.9 and 7.8 ng/mL for the WT and D614G strain, respectively.

### 3.2.2 Autoreactivity evaluation of MAD0004J08 to human epithelial cells

Recent findings showed that COVID-19 patients can develop autoantibodies. In particular it was reported that around 10% of patients with life-threating COVID-19 pneumonia develop autoantibodies against type I interferons, tipping the balance of the infection in favor of the virus [17]. These patients may develop anti-phospholipid autoantibodies and phospholipid-binding proteins resulting in a potentially life-threatening thrombophilia. In one study over 50% of the serum samples from COVID-19 patients tested positive for antiphospholipid autoantibodies [18].

To eliminate any possible risk of autoreactivity to human antigens, MAD0004J08 wild type (WT) and mutated (MUT) versions were tested through an indirect immunofluorescent assay against human epithelial type 2 (HEp-2) cells which expose clinically relevant proteins to detect autoantibody activities. While the positive control showed a low but detectable signal at 1:100 dilution, MAD0004J08 did not show any signal in both its WT and MUT version at a concentration of 100 µg/mL.

### 3.2.3 MAD0004J08 Fc engineering to extend half-life and minimize risk of antibody-dependent disease enhancement

Five different point mutations were introduced in the MAD004J08 antibody constant region.

Two-point mutations (M428L/N434S) were introduced to enhance antibody half-life [19].

Three-point mutations (L234A/L235A/ P329G) were introduced to reduce antibody dependent functions such as binding to FCγRs and cell-based activities [20]. Binding to FCγRs has been associated to ADE, previously shown to be a potential clinical risk following coronaviruses infection [21].

To confirm the lack of FCγR binding as well as the extended half-life, a beads-based Luminex assay was performed. Briefly, the beads were coated with SARS-CoV-2 S-protein receptor binding domain (RBD). MAD0004J08 was tested at 8-point dilutions and the binding detected with FCγR2A and FcRn (Neonatal Fc receptor) at pH 6.2 and 7.4. The FCγR2A was selected as it is predominantly expressed on the surface of phagocytic cells (such as monocytes, macrophages and neutrophils) and is associated with phagocytosis of immune complexes and antibody opsonized targets [22]. On the other hand, FcRn, which is highly expressed on endothelial cells and circulating monocytes, was selected as it is responsible for the recycling and serum half-life of IgG in the circulation [23].

The results demonstrate that the binding to the FCγR2A was completely abrogated for the mutated version of MAD0004J08 (MAD000J08-MUT) compared to the respective WT version (MAD000J08-WT) and control antibody (CR3022). Furthermore, FC-engineered antibodies showed an increased binding activity to the FcRn at both pH 6.2 and 7.4 compared to their WT counterpart.

Finally, to evaluate the lack of FC-mediated cellular activities by our three candidate mAbs, the antibody-dependent neutrophil phagocytosis (ADNP) and antibody-dependent NK cell activation (ADNK) were evaluated [24-26]. For the ADNP assay, primary human neutrophils were used to detect the antibody binding to SARS-CoV-2 S-protein RBD coated beads, while ADNK activity was evaluated by using primary human NK cells and detecting the release of the proinflammatory cytokine interferon (IFN)-γ. Complete abrogation of both ADNP and ADNK was observed for all

three FC-engineered candidate mAbs compared to their WT versions and control antibody (CR3022) confirming the lack of FC-mediated cellular activities.

### 3.2.4 *In vivo* study in a golden Syrian hamster SARS-CoV-2 infection model

The golden Syrian hamster model has been widely used to assess monoclonal antibody prophylactic and therapeutic activities against SARS-CoV-2 infection. This model has shown to manifest severe forms of SARS-CoV-2 infection closely mimicking the clinical disease observed in humans [27-30].

A prophylactic study in golden Syrian hamster was designed to evaluate the efficacy of MAD0004J08 in preventing SARS-CoV-2 infection. Thirty hamsters were divided into 5 arms (6 animals each). The monoclonal antibody was administered at 3 different concentrations (4 – 1 – 0.25 mg/kg) via intraperitoneal injection. Control groups receiving placebo (saline solution) and IgG1 anti-influenza antibody isotope (4 mg/kg) were included in the study. MAD0004J08 4 mg/kg group and the 1 and 0.25 mg/kg groups were tested in two independent experiments. The IgG1 isotype control group was tested in parallel with the MAD0004J08 4 mg/kg group while the placebo is an average of the two experiments.

Twenty-four hours post-administration of the antibody or saline solution, animals were challenged with 100 µL of SARS-CoV-2 solution ($5x10^5$ PFU) via intranasal distillation. Three hamsters per group were sacrificed at three days post-infection while the remaining animals were culled at day 8. Body weight changes were evaluated daily throughout the study. MAD000J08 significantly reduced weight loss at all concentrations tested in a dose-response fashion compared to both the placebo and the IgG1 isotype control groups. When MAD0004J08 was administered at 4 mg/kg, complete protection from SARS-CoV-2 infection was observed and only a minimal weight loss was measured one day post viral challenge. All animals quickly recovered at day 3, reaching their initial weight. From day 4 on, hamsters gained weight increasing up to 5% from their initial body weight. A slightly greater body weight loss was observed 1day post infection in hamsters that received MAD0004J08 at 1 and 0.25 mg/kg. However, hamsters in these groups completely recovered their initial body weight at day 6 and 8 for the 1 and 0.25 mg/kg doses, respectively. Hamsters in the control groups did not recover their initial body weight and at day 8 still show around 5% weight loss.

In conclusion, MAD0004J08 was able to prevent SARS-CoV-2 infection in a golden Syrian hamster model even at concentrations as low as 0.25 mg/kg (around 50 µg/animal).

## 3.3 Risk/Benefit Assessment

Human mAbs are one of the greatest scientific and medical breakthroughs of the last decades. MoAbs are now effectively used to treat diseases in many therapeutic areas including malignancies, immune-mediated diseases (e.g., rheumatoid arthritis, inflammatory bowel disease), respiratory, and infectious diseases [31].

Human mAbs have been used in millions of patients with a generally satisfactory safety profile [31, 32]. Clinically meaningful risks associated with mAb therapy with direct antiviral activity include anaphylaxis and other allergic reactions, development of anti-drug antibodies (ADA), and potential induction of antibody-dependent enhancement of disease (ADE). Anaphylaxis is a serious allergic reaction that may rapidly manifest after administration of exogenous compounds. Anaphylaxis is rare after mAb administration, especially with single dose treatments, and prompt intervention is typically

associated with resolution. Production of ADA may be associated with loss of efficacy. As ADA tend to develop after multiple drug administration, the risk of ADA in this study is considered low. ADE has been associated with the ability of low-level non-neutralizing antibodies to bind viral antigens and dysregulate immune response trough Fc-dependent pathways [33]. As MAD0004J08 is highly neutralizing even at a very low dose and lacks Fc portions that appear to be key for ADE (see Section 3.2.3 above), the risk of ADE with MAD0004J08 is also considered low.

A single dose of MAD0004J08 will be administered as two intramuscular (IM) injections of 5 mL each given in rapid sequence. Large volume IM injections are approved by the FDA for several drugs, for short term as well as chronic use, including fulvestrant (Falsodex), approved for the treatment of some forms of breast cancer as two 5 mL injections (same as in this trial). IM injections of 5 mL are safe and well tolerated in the vast majority of patients. More information including injection techniques and references are provided in Section 7.1.2.

By the time this trial starts, interim safety results from a Phase I trial testing MAD0004J08 will be available. The same doses (100 mg and 400 mg) and the same modality of administration (two 5 mL injections) will be used in the Phase I trial and in the present Phase II-III trial.

Overall, the risk of clinically meaningful adverse events following administration of MAD0004J08 is considered low.

The key expected benefits are consequences of the expected reduction of time to virus clearance in the upper respiratory tract and viral load when MAD0004J08 is given to patients with asymptomatic to moderate COVID-19 shortly after diagnosis:

- Shorter duration of quarantine, with positive impact on income, family life and psychological wellbeing.
- Reduced likelihood of infecting household members and health care providers during quarantine.
- Recent evidence suggests that reduction of viral load in the upper respiratory tract is associated with improved clinical outcome and reduced need for medically attended visits and/or hospitalization [11].

Another important benefit is the IM administration, which is simpler than the IV infusion required for the mAbs vs. SARS-CoV-2 that have received EUA thus far. This is likely to be a decisive advantage in the context of a pandemic, where skilled personnel trained in IV infusion may become the rate-limiting step for such intervention.

Overall, the benefit-risk ratio of MAD0004J08 in this Phase II-III trial is considered favorable.

More detailed information about the known and expected risks and reasonably expected adverse events of MAD0004J08 may be found in the Investigator's Brochure (IB).

# 4 OBJECTIVES AND ENDPOINTS

The goals of this Phase II-III seamless adaptive clinical trial in adult patients with recently diagnosed asymptomatic to moderately severe COVID-19 at baseline are:

Stage-1 (Phase II)

1) Select one of two dose levels of MAD0004J08 for progression to Stage-2

Stage-1 + Stage-2 (Phase III)

2) Provide confirmatory evidence of safety and efficacy for regulatory approval

If strong evidence is obtained, early submission to regulatory authorities for conditional / emergency use approval will be considered.

Study objectives and related endpoints are reported in Table 4-1 below.

***Table 4-1 Objectives and related endpoints***

| | |
|---|---|
| Safety and efficacy endpoints will be analyzed as appropriate in two primary target populations and three time-windows:<br>Primary populations: 1) all randomized participants (ALL), 2) seronegative randomized participants (SEROneg).<br>All endpoints will be tested in the ALL and SEROneg primary populations.<br>Time windows (defined at an individual participant level): 1) baseline (Visit 1) to end of Stage-1 or dropout (interim analysis), 2) baseline (Visit 1) to end of Stage-2 or dropout (primary analysis), 3) baseline (Visit 1) to end of study (Visit 12) or dropout (final analysis). | |
| **Safety** | |
| **Safety objectives** | **Safety endpoints** |
| **Primary safety objective**<br>To assess the safety and tolerability of MAD0004J08 as determined by severe and serious adverse events | **Primary safety endpoint**<br>• Proportion of participants with severe (Grade 3) unsolicited AEs and/or serious unsolicited AEs (SAEs). |
| **Secondary safety objectives** | **Secondary safety endpoints** |
| To assess the overall safety and tolerability of MAD0004J08 | • Proportion of participants with unsolicited AEs, including clinically relevant laboratory and ECG abnormalities.<br>AEs will be summarized by system organ class and preferred term, and assessed for seriousness, severity, and relationship to study treatment. |
| To assess the local reactogenicity of MAD0004J08 | • Proportion of participants with solicited local AEs at the injection site. |

| | |
|---|---|
| | Three solicited local AEs will be assessed: pain, swelling and redness at the injection site. Solicited local AEs will be summarized by preferred term and overall (any solicited AE). |
| To assess whether a single administration of MAD0004J08 elicits specific anti-drug antibodies (ADA) | • Proportion of tested participants who develop ADA. The first 60 randomized participants will be tested for ADA. |
| **Efficacy** | |
| **Efficacy objectives** | **Efficacy endpoints** |
| **Primary efficacy objective**<br>To demonstrate that MAD0004J08 shortens the time to clearance of SARS-CoV-2 from the upper respiratory tract (URT) | **Primary efficacy endpoint**<br>• Time to SARS-CoV-2 clearance in the URT.<br>Time to clearance is defined as the number of days from the day of Visit 1 (baseline) to the day the 1st nasopharyngeal swab testing negative is taken, followed by a subsequent negative swab taken at least 24 hours later. A swab will be considered negative if SARS-CoV-2 is undetectable by RT-PCR.<br>The primary endpoint is censored at Day 28. |
| **Key secondary efficacy objective**<br>To demonstrate that MAD0004J08 reduces the proportion of clinically relevant outcomes | **Key secondary efficacy endpoint**<br>• Proportion of patients experiencing at least one of the following failure events of the composite efficacy outcome:<br>o $SpO_2 < 94\%$<br>o Newly established or increased dose home oxygen therapy (increased home oxygen therapy only applies to patients with underlying conditions other than COVID-19 requiring such therapy, e.g., COPD)<br>o Hospitalization<br>o Death |
| **Secondary efficacy objectives** | **Secondary efficacy endpoints** |
| To assess the impact of MAD0004J08 on SARS-CoV-2 virus in the URT | • Proportion of participants with SARS-CoV-2 clearance in the URT at each visit.<br>• SARS-CoV-2 viral load (number of copies) in nasopharyngeal swab, as measured by RT-PCR at each visit. |
| To assess the impact of MAD0004J08 on the clinical course of COVID-19 | • SPO2% at each visit and lowest SpO2 % post baseline.<br>• Proportion of participants with SpO2 % < 94%.<br>• Proportion of participants with newly established or increased dose home oxygen therapy increased home oxygen therapy only applies to |

| | |
|---|---|
| | patients with underlying conditions other than COVID-19 requiring such therapy, e.g., COPD).<br>• Area under the curve (AUC) of COVID-19 total symptom score (range: 0-24).<br>• Proportion of participants requiring hospitalization.<br>• Cumulative time of hospital stay in days.<br>• Proportion of hospitalized participants requiring supplemental oxygen therapy.<br>• Cumulative time of hospitalized oxygen therapy in days.<br>• Proportion of participants admitted to intensive care unit (ICU).<br>• Cumulative time of ICU stay in days.<br>• All-cause mortality. |
| To assess the serum concentration of MAD0004J08 | • MAD0004J08 serum concentration. |
| **Exploratory** | |
| ***Exploratory objectives*** | ***Exploratory endpoints*** |
| Participants will be asked to agree to additional exploratory measurements as part of the informed consent process. If the participant does not agree, additional exploratory endpoints will not be assessed on his/her biological samples.<br>Exploratory endpoints are not mandatory and may be reported separately from the clinical Study Report. | |
| To assess the new Diesse4-Covid19 test to detect serum SARS-CoV-2 IgG | • Endpoints will be outlined in a separate document. |
| To assess the neutralizing power of MAD0004J08 vs. SARS-CoV-2 strains | • Endpoints will be outlined in a separate document. |
| To explore additional aspects of scientific and clinical interest | • Other exploratory endpoints based on measurements carried out in the stored biological samples may be added for research purposes. Endpoints will be outlined in separate documents. |

# 5 STUDY DESIGN

## 5.1 Overall Design

This clinical trial is designed as a randomized, stratified, placebo-controlled, double-blind, multi-center seamless adaptive study.

Informed consent must be taken from patients before any study-related assessments or procedures are performed. Consent may be provided by the patient's legal/authorized representative should the patient be incapable of doing so.

Participants must be at least 18 years of age with recently diagnosed (≤ 3 days from 1st positive nasopharyngeal swab taken) asymptomatic to moderately severe COVID-19 not requiring hospitalization at baseline. Patients with comorbidities will be allowed to participate in the study if all inclusion and exclusion criteria are met.

The trial is designed in two stages:

In Stage-1, participants meeting inclusion/exclusion criteria will be randomized (1:1:1 ratio) to one of the following three study treatments:

- MAD0004J08 400 mg, single dose
- MAD0004J08 100 mg, single dose
- Placebo, single dose

When a predefined number of primary endpoint events (see below for definition) is reached, the data will be analyzed following a pre-planned interim analysis plan. Based on the results of the interim analysis and pre-defined criteria, the DMC will recommend whether the study should progress to Stage-2, and if so, recommend one of the two MAD0004J08 doses for Stage-2. Alternatively, the DMC will recommend stopping the study either for futility or for efficacy, following pre-defined criteria. The final decisions on study progress and dose will be made by a subgroup of the SC.

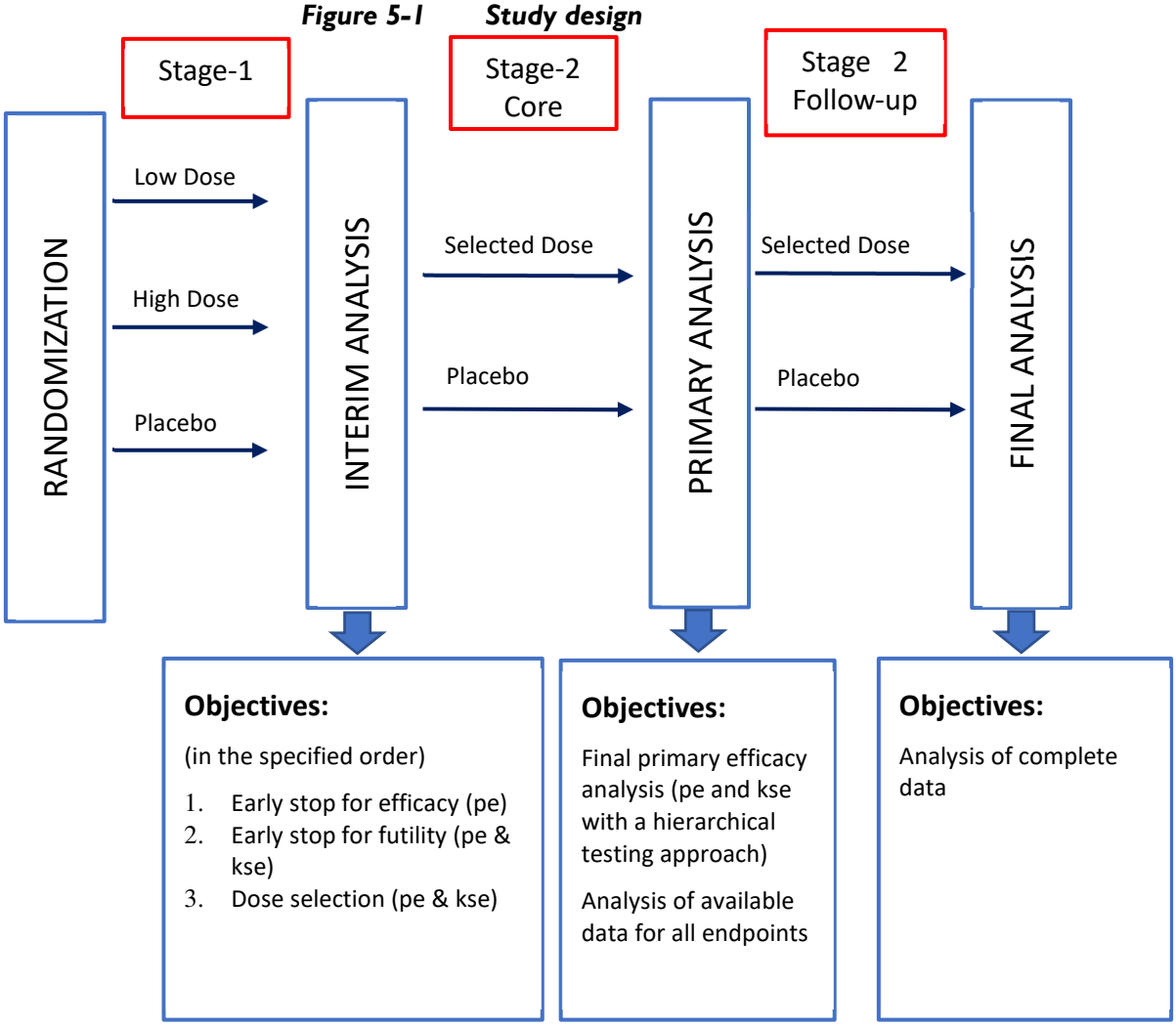
If the study continues, Stage-2 will start and new participants meeting inclusion/exclusion criteria will be randomized (1:1 ratio) to one of two treatments:

- MAD0004J08, dose level selected in Stage-1, single dose
- Placebo, single dose

Participants in all groups will receive standard of care for COVID-19 as deemed appropriate by the treating physician and the Investigator.

Figure 5-1 below illustrates the trial design and when decisions are made.

***Figure 5-1 Study design***




*pe=primary endpoint*
*kse=key secondary endpoint*

Twelve (12) study visits and 2 telephone calls are scheduled for each participant over approximately 168 days. Additional ad-hoc visit(s) may be necessary to confirm eradication of SARS-CoV-2 from the URT following the 1st negative nasopharyngeal swab (see the Schedule of Activities (SoA) in Section 1.3 for details).

Due to the need to minimize time between diagnosis and intervention, screening procedures, baseline procedures, randomization and administration of study treatment will typically occur on the same day (Day 1, Visit 1) at the study center. However, if the ≤3-day limit is maintained between the day the 1st positive swab is taken and the day IMP is administered, Visit 1 can occur over two days (Days 0 and 1).

At Visit 1 (baseline) all participants will undergo testing for serum IgA and IgG vs. the spike (S) protein, and IgG vs. nucleocapsid (N) protein: participants testing negative to all three antibodies at

baseline are referred to as seronegative; participants testing positive to one or more of the three antibodies at baseline are referred to as seropositive.

Visits from Day 3 to Day 21 (Visits 2 to 9) will be conducted by study staff at the participant's home, unless the participant is hospitalized. Visits from Day 28 to Day 168 (Visits 10 to 12) will be conducted at the study center.

Participants requiring hospitalization during the study period are to be hospitalized at the same study center where Visit 1 was conducted. Visits and procedures occurring during hospitalization will be conducted as per protocol to the extent the participant's condition allows it. Once a participant is discharged from the hospital, subsequent home and center visits will resume as per protocol.

Nasopharyngeal swabs will be taken at each visit. An additional swab may be taken at an ad hoc visit at least 24 hours after the 1st negative swab to confirm eradication of SARS-CoV-2 from the URT. Swabs will be tested for SARS-CoV-2 viral load by reverse transcriptase polymerase chain reaction (RT-PCR).

This is an event-driven study. The event is the 1st nasopharyngeal swab testing negative for SARS-Cov-2 by RT-PCR followed by an additional negative nasopharyngeal swab taken at least 24 hours thereafter for an individual participant.

Safety and efficacy endpoints will be analyzed as appropriate in two primary target populations and three time-windows:

Primary target populations: 1) all randomized participants (ALL), 2) seronegative randomized participants (SEROneg).

Time windows (defined at an individual participant level): 1) baseline (Visit 1) to end of Stage-1 or dropout (interim analysis), 2) baseline (Visit 1) to end of Stage-2 or dropout (primary analysis), 3) baseline (Visit 1) to end of study (Visit 12) or dropout (final analysis).

The interim and primary time windows are variable, whereas the final time window is fixed unless the participant drops out from the study.

Study oversight and recommendations on progress from Stage-1 to Stage-2 will be under the direction of a Data Monitoring Committee (DMC) composed of individuals with appropriate expertise. A Steering Committee (SC) will ensure transparent management of the study according to the protocol and make final decisions on study progress.

## 5.2 Rationale for Study Design

Randomized, double-blind, placebo-controlled trials represent the gold standard for clinical research. Randomization and blinding are key to minimizing most forms of bias, including selection, assessment, and analysis bias. The design supports the rigorous assessment of the safety and efficacy of MAD0004J08 combined with SoC.

Stratified randomization will be used to ensure balance across treatment groups for participants ≥65 years old, and younger participants with concomitant diseases, which are well known risk factors for progression to severe COVID-19 and are likely to have an impact on the time to viral clearance (the primary efficacy endpoint). Stratified randomization will also ensure that at least 30% of participants

are ≥65 years old as this is the age group most frequently impacted by COVID-19 (see Section 7.3 for details on randomization and stratification).

The double-dummy blinding technique will be used to maximize the level of blinding in the trial (see Section 7.3 for details on blinding).

Seamless adaptive designs allow combination of two or more traditional development phases and pre-planned changes during the study based on the outcome of one or more interim analyses. These designs are increasingly used in drug and vaccine development with the goal of making best use of the data and minimize sample size. In our trial, Phase II dose selection (Stage-1) progresses seamlessly to Phase III confirmatory evaluation (Stage-1 + Stage-2). The adaptation consists in the selection of only one of the two MAD0004J08 dose levels tested in Stage-1 for assessment in Stage-2.

See Section 6.5 for the rationale of the study population.

## 5.3 Justification for Dose

The dose of MAD0004J08 (100 mg) was calculated in order to exceed a neutralizing titer in serum of 1/100. Since most COVID-19 convalescent patients have a neutralizing titer ranging from 1/20 to 1/320, it is assumed that a titer exceeding 1/100 will provide sufficient neutralizing potency to eliminate the virus from the body. Considering that MAD0004J08 has a neutralization potency of 3 ng/mL (i.e., 3 µg/L or 3 µg/Kg), it was assumed that a neutralization titer of 1/100 can be achieved with 0.3 mg/kg. Therefore, 21 mg would be sufficient to achieve a titer of 1/100 in a person weighing 70 kg. The proposed dose of 100 mg exceeds the titer of 1/100 by 5-fold, while the proposed titer of 400 mg exceeds the titer of 1/100 by 20-fold.

## 5.4 Justification for comparator

Placebo is considered an appropriate and ethically acceptable comparator as all patients will also receive standard of care treatments for COVID-19.

## 5.5 Definition of end of study

A participant is considered to have completed the study if he/she has completed all required phases of the study including the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

The end of the study is defined as the date of last scheduled procedure shown in the SoA for the last participant in the trial globally.

The participation of the patient in the study regularly ends:

- At the end of the follow-up (Visit 12).
- In case of death.

# 6 STUDY POPULATION

## 6.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all the following criteria:

**Inclusion criteria**

1. Signed written informed consent taken before any study procedure from any patient capable of giving consent, or, when the patient is incapable of doing so, by his or her legal/authorized representative.
2. Age ≥18 years. At least 30% of participants will be ≥ 65 years old.
3. First nasopharyngeal swab testing positive for SARS-CoV-2 by RT-PCR taken no more than 3 days before randomization (Visit 1). Results of "rapid" semiquantitative tests are not acceptable.
4. Asymptomatic to moderately symptomatic outpatients with no need for immediate hospitalization: grade 1, or grade 2 or grade 3 of Clinical Severity Scale.
5. No childbearing potential (post-menopause, surgically-induced, or pharmacologically-induced sterility) or, if of childbearing potential, negative urinary pregnancy test (women) and commitment to use at least 2 forms of contraception for at least 168 days from administration of study drug (men and women).

## 6.2 Exclusion criteria

1. Severe or critical COVID-19: grade 4 or grade 5 of clinical severity scale.
2. Current hospitalization and/or hospitalization or emergency room visit in the past 14 days.
3. Need for immediate hospitalization for any reason in the investigator's opinion.
4. Severe liver disease as determined by values of ALT and/or AST >5x upper limit of normal (ULN) and/or history of liver cirrhosis.
5. Severe renal disease as determined by estimated creatinine clearance (CcCl) <30 mL/min or serum creatinine >2 mg/dL (>176.8 μmol/L) or ongoing renal dialysis.
6. Absolute neutrophil count (ANC) < 1000/μL.
7. Demyelinating and connective tissue disease.
8. Active tuberculosis or suspected active bacterial, fungal, viral, or other infection (besides COVID-19).
9. Any condition that in the Investigator's opinion may be negatively affected by the study treatments and/or study procedures.
10. Any condition, including psychiatric disorders, alcohol, or substance abuse, which in the Investigator's opinion may interfere with completion of the study procedures.
11. Any condition with life expectancy <6 months in the Investigator's opinion.
12. Ongoing or planned pregnancy.

13. Ongoing breast feeding.
14. History of life-threatening event in the 1 month before Visit 1.
15. History of surgery in the 1 month before Visit 1.
16. History of treatment with blood components in the 6 months before Visit 1.
17. History of cancer treated with chemotherapy in the 6 months before Visit 1.
18. History of solid organ transplant at any time before Visit 1.
19. History of severe and/or serious allergic reaction to monoclonal antibodies or any component of MAD0004J08, including anaphylaxis at any time before Visit 1.
20. Treatment with an investigational drug or vaccine within 5 half-lives or 30 days (whichever is longer) of randomization.
21. Treatment at any time with monoclonal antibodies bamlanivimab, bamlanivimab + etesevimab combination, and casiribimab + imdevimab combination.

Receipt of approved vaccine(s) vs. COVID-19 is NOT an exclusion criterion, i.e., is compatible with enrolment in the study if all inclusion and exclusion criteria are met.

## 6.3 Definition of participants <65 years old at risk for COVID-19 progression and hospitalization (for strata definition purposes)

All participants ≥ 65 years old are considered at risk for COVID-19 progression and hospitalization.

A participant < 65 years old will be considered at risk for complications of COVID-19 (stratum #2, see Section 7.3.1) if he/she suffers from at least one of the following conditions [57] NOT MEETING EXCLUSION CRITERIA at Visit 1:

- Body mass index ≥ 35 kg/m$^2$.
- Cardiovascular disease including hypertension
- Chronic lung disease including asthma
- Type I or type II diabetes mellitus
- Chronic kidney disease, including requiring dialysis
- Chronic liver disease
- Diseases associated with immunosuppression (e.g., cancer treatment, bone marrow or organ transplant, poorly controlled HIV/AIDS, sickle cell anemia thalassemia, prolonged use of immune weakening medications).

IMPORTANT: at risk patients of any age meeting one or more exclusion criteria must not be enrolled in the trial.

As part of the informed consent process, participants will be made aware that, because of the exceptional circumstances, AIFA has granted use of alternative unapproved mAbs in non-hospitalized patients with mild to moderate COVID-19 at risk of disease progression. The benefits and risks of such alternatives will be discussed. It will be emphasized that participants are at liberty to choose the alternative mAbs, if available locally and recommended by their doctor, instead of entering this trial.

## 6.4 Screening Failures

A subject who signed an Informed Consent Form (ICF) but is subsequently found to be ineligible prior to randomization will be considered a screening failure. Screening failures will be entered on the corresponding section of the electronic Case Report Form (eCRF). A minimal set of screening failure information is required to ensure transparent reporting of screening failure participants, to respond to queries from regulatory authorities and to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements. Minimal information should include demography, screening failure details, eligibility criteria and any serious adverse event (SAE).

Screen failures may not be rescreened.

## 6.5 Scientific rationale for study population

A short interval (3 days or less) between the day the first swab testing positive is taken and the day MAD0004J08 is administered is required in this study because clinical evidence is emerging suggesting that mAbs targeting SARS-CoV-2 may be most effective shortly after infection [11, 12]. Vice versa, bamlanivimab has not shown clinical benefit in hospitalized COVID-19 patients [34].

A population restricted to participants without antibodies vs. SAES-CoV-2 at baseline (referred to as "seronegative") receiving IMP was selected as primary population for the efficacy endpoints in addition to all randomized participants receiving IMP. The reason for this choice is that the absence of detectable antibodies vs. SARS-CoV-2 (seronegative status) in most patients indicates an early stage of disease. In a comprehensive systematic review of clinical studies, the mean or median time to seroconversion ranged from 12 to 15 days post symptom onset for IgG [35]. Also, seronegative patients generally have a higher viral load than seropositive patients, and high viral load has been associated with a stronger response to the casiribimab + imdevimab mAb combination compared to low viral load [12].

Older adults and younger adults with concomitant risk for COVID-19 complications are allowed in the study as these categories are at increased risk for progression to severe COVID-19, which may be prevented by an intervention capable of shortening time to clearance from the virus (see Section 6.3 for definition of at risk for COVID-19 complications).

Exclusion criteria are meant to ensure that the positive risk-benefit balance of MAD0004J08 (see Section 3.3) is maintained and that any negative impact on endpoint assessment and compliance is minimized.

# 7 STUDY INTERVENTION

## 7.1 Study Intervention Administration

### 7.1.1 Study intervention description

Participants will be randomized (1:1:1 ratio) to receive MAD0004J08 or matching placebo solution as described in Section 7.1.2. MAD0004J08 and placebo in their final formulation and presentation are referred to as Investigational Medicinal Products (IMP) or study drugs.

| *MAD0004J08* | |
|---|---|
| Investigational product | MAD0004J08, human monoclonal Antibody (mAb), 2.5 mL 2R vial |
| Manufacturer (active substance) | Menarini Biotech, via Tito Speri 12, 00071 Pomezia (Rome), Italy |
| Manufacturer (finished product) | Istituto Biochimico Italiano Lorenzini, via Fossignano 2, 040011 Aprilia (Latina), Italy |
| Pharmaceutical form | Solution for injection |
| Dose | Low dose: 100 mg - single dose<br>High dose: 400 mg - single dose |
| Administration route | Intramuscular |

| Placebo | |
|---|---|
| Investigational product | Placebo matching to MAD0004J08, 2.5 mL 2R vial |
| Manufacturer | Istituto Biochimico Italiano Lorenzini, via Fossignano 2, 040011 Aprilia (Latina), Italy |
| Pharmaceutical form | Solution for injection |
| Dose | Not applicable |
| Administration route | Intramuscular |

Along with the study intervention, patients in all three treatment arms will receive SoC as deemed appropriate by the treating physician and the Investigator.

The composition of the investigational products is summarized in the table below.

***Table 7-1 Composition of investigational medicinal products***

| | **MAD0004J08** | **Placebo** |
|---|---|---|
| ***Drug substance***<br>***Nominal amount*** | MAD0004J08<br>42.5 ± 2.5 mg / mL<br>100 mg ± 10 mg / vial | None |
| ***Buffer #1***<br>***Nominal amount*** | Sodium phosphate monobasic monohydrate<br>1.89 g/L<br>4.4 mg / vial | none |
| ***Buffer # 2***<br>***Nominal amount*** | Sodium phosphate dibasic anhydride<br>0,92 g/L<br>2.2 mg / vial | none |
| ***Saline solution***<br>***Nominal amount*** | Sodium Chloride<br>8,70 g/L<br>20.4 mg/vial | Sodium Chloride<br>9 g/L<br>22.5 mg/vial |
| ***Volume per vial*** | 2.35 ml + 0.18 ml overfilling | 2.5 ml + 0.2 ml overfilling |
| ***Appearance*** | Clear, water-like | Clear, water-like |

### 7.1.2 Dosing and administration

In Stage-1, participants will be randomized (1:1:1 ratio) to one of the following three study treatments:

- MAD0004J08 400 mg IM, single dose
- MAD0004J08 100 mg IM, single dose
- Placebo IM, single dose

In Stage-2 participants will be randomized (1:1 ratio) to one of the following two study treatments:

- MAD0004J08 400 mg or 100 mg IM, single dose (based on outcome of Stage-1)
- Placebo IM, single dose

Individual participant study treatment packs will be provided. Each study treatment pack will contain 4 study treatment 2R vials. The content of the vials will be different in the different study arms as follows.

- Vials for the MAD0004J08 400 mg treatment will have the following composition:
  - Vial 1: MAD0004J08 100 mg

  - Vial 2: MAD0004J08 100 mg
  - Vial 3: MAD0004J08 100 mg
  - Vial 4: MAD0004J08 100 mg

- Vials for the MAD0004J08 100 mg treatment will have the following composition:
  - Vial 1: MAD0004J08 100 mg
  - Vial 2: placebo
  - Vial 3: placebo
  - Vial 4: placebo

- Vials for the placebo treatment will have the following composition:
  - Vial 1: placebo
  - Vial 2: placebo
  - Vial 3: placebo
  - Vial 4: placebo

Syringes and needles will be provided separately from the participant study treatment packs.

Each enrolled and randomized patient will receive two IM injections as follows:

- 1st injection with one syringe filled with the content of 2 vials (5.0 mL in total).
- 2nd injection with the other syringe, filled with the content of the remaining 2 vials (5.0 mL in total).

A different syringe must be used for each injection. Any 2 vials of the study treatment pack may be used for the 1st and 2nd injection.

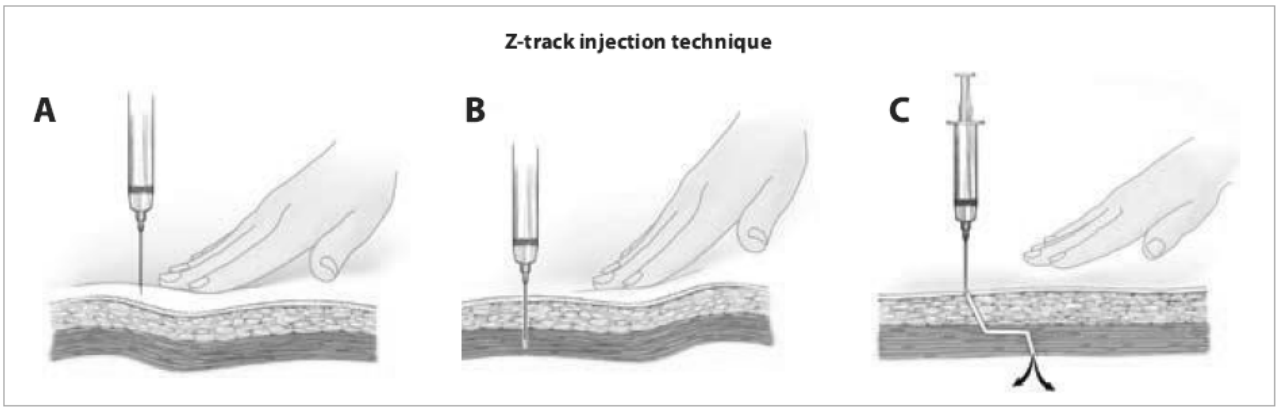
Vials must never be taken from a different treatment pack without guidance from the Contract Research Organization (CRO). If a treatment pack or a single vial is lost or broken, the study staff must contact immediately the CRO contact person and he/she will indicate which reserve study treatment pack can be used.

The first 5 mL injection will be administered in the ventrogluteal region of one buttock; the second 5 mL injection will be administered in rapid sequence in the ventrogluteal region of the other buttock. If for whatever reason the ventrogluteal region is not considered appropriate for injection, the dorsogluteal region can be used. During and after each injection it is recommended to massage the area to prevent and relieve pain.

The Z-track injection technique illustrated in the Figure below [36] is recommended. Briefly:

1. Pull or push the skin 2-3 cm away from the injection site with the non-dominant hand.
2. Pierce the skin at 90° and depress the plunger slowly (1-2 minutes); if resistance occurs, pause, then resume depressing the plunger
3. Withdraw the needle, then release the skin.

***Figure 7-1 Z-track injection technique***




The recommendations on IM injection technique provided above stem from the vast experience gained on medications requiring large IM volumes (> 3 mL), including ceftazidime, cefuroxime, ertapenem, penicillin G benzathine, and fulvestrant (Faslodex) [36-38]. In particular, Fasodex is approved by the US FDA in hormone receptor-positive metastatic breast cancer postmenopausal women who have failed antiestrogen therapy at the 500 mg dose, administered as 2 injections of 5 mL each on Days 1, 14, 28 and monthly thereafter [38]. This is the same number of injections and volume as for MAD0004J08 in this trial. Large clinical trials have shown that with the appropriate technique, injection site reactions are infrequent and mostly mild [36].

## 7.2 Preparation/Handling/Storage/Accountability

### 7.2.1 Acquisition and accountability

MAD0004J08 and matching placebo will be provided by the Sponsor. Upon arrival of investigational products at the site, the pharmacist should check them for any damage and verify their proper identity, quantity, integrity of seals and report any deviations, including storage temperature. Upon receipt, all study treatments must be stored according to the label instructions in a secured location. Site staff designated by the Principal Investigator will maintain accurate records of the product's delivery to the study site, the inventory at the site, the use by each participant and the return to the Sponsor or alternative disposition of unused products. Monitoring of drug inventory will be performed by the field monitor during the study conduct and a copy of the accountability log will be provided by the Investigators at the completion of the study.

### 7.2.2 Formulation, appearance, packaging and labeling

The composition and appearance of MAD0004J08 and reference product is described in Table 7-1.

The primary packaging will consist of 2R vials. Primary packaging labels will report:

- Sponsor name
- Study number
- Investigator's name
- Site No.
- Batch No.
- Patient ID

- Dispensed on [DATE]
- Vial Number

Individual participant study treatment packs will be provided. Each study treatment pack will contain four study treatment 2R vials. The content of the vials will be different in the different study arms as described above (see Section 7.1.2).

Participant study treatment packs (Secondary packaging) will have labels reporting all the information requested according to Annex 13 to Good Manufacturing Practice (published by the Commission in the rules governing medicinal products in the European Community, Volume 4) as follows:

1. Pharmaceutical dosage form, route of administration, quantity of dosage units
2. The batch and/or code number to identify the contents and packaging operation
3. A study reference code allowing identification of the study, site, Investigator and Sponsor if not given elsewhere
4. The study subject kit number (and randomization number)
5. The name of the Investigator
6. Instructions for use (reference may be made to a leaflet or other explanatory document intended for the person administering the product)
7. "For clinical study use only" or similar wording
8. The storage conditions
9. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity.

Labels will be in local language.

### 7.2.3 Product storage and stability

The investigational products will be stored at 2-8°C in a dry locked place, sheltered from light.

## 7.3 Methods of Treatment Assignment – Randomization and Blinding

### 7.3.1 Randomization and stratification

In Stage-1, eligible participants will be randomly assigned to one of three treatments with an allocation ratio of 1:1:1 as described above.

In Stage-2, eligible participants will be randomly assigned to one of two treatments with an allocation ratio of 1:1 as described above.

Randomization will be stratified in three strata as follows:

- ≥ 65 years of age (yoa) (stratum #1)
- < 65 yoa at increased risk for COVID-19 disease progression and hospitalization (stratum #2)
- < 65 yoa not at risk for COVID-19 disease progression and hospitalization (stratum #3).

See Section 6.3 for definition of at risk for COVID-19 disease progression and hospitalization.

At least 30% of the study participants will be ≥ 65 yoa (stratum #1).

Stratification by SEROpos / SEROneg status was not implemented because the results of the relevant test will not be available by the randomization time.

The randomization numbers will be generated using procedures that ensure that treatment assignment is unbiased. Randomization lists will be produced using a validated system that automates the assignment of randomization numbers.

Participants who fulfill all inclusion criteria and none of the exclusion criteria will be randomized via an Interactive Web Response System (IWRS) that assigns the participant a randomization number linked to a treatment arm. Staff is to use the IWRS to obtain the study treatment to be given to the patient.

### 7.3.2 Blinding

The study will be double-blind, i.e., all patients, site personnel, monitors, Sponsor, CRO, and study teams will be blinded to the treatment given to each patient.

The double-dummy technique will be used to mask the treatments, i.e., all patients will receive two injections of study drug of 5.0 mL each, the content of which will be different in each treatment arm, as outlined above (see Section 7.1.2).

Statistical analyses and preparation of regulatory documents conducted during the study will be carried out by subject matter experts not otherwise involved in the conduct of the study.

The DMC will be unblinded. Stopping rules for futility or efficacy and criteria for dose selection will be based on pre-defined criteria that will be detailed in the statistical analysis plan (SAP).

Individual patient unblinding during the trial may occur in case of patient emergencies or medically important adverse events. The code breaking procedure is managed by means of an on-line specific utility implemented in the eCRF and is available for the Investigator and Pharmacovigilance staff only. Sealed envelopes will also be provided as back-up in the unlikely case of system unavailability.

If during the study the Investigator needs to break a code (for emergency reasons), he/she shall follow the procedure implemented in the system clicking on an *ad hoc* icon present on the toolbar. In case of an individual patient unblinding, an automatic email will be sent to the study team notifying the occurrence of a code break reminding the Investigator to discontinue the patient from the study.

## 7.4 Concomitant Therapy

Concomitant treatment with mAbs bamlanivimab, and casiribimab + imdevimab combination is not permitted.

Otherwise, participants will receive standard of care (SoC) treatment for COVID-19 as deemed appropriate by the treating physician and the Investigator

Participants will receive treatment for other concomitant conditions as deemed appropriate by the treating physician and the Investigator.

All previous and concomitant treatments, including non-prescription medications, will be recorded at each visit as described in Section 9.5.12 below.

### 7.4.1 Rescue medication

Not applicable to this study.

# 8 TREATMENT DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

## 8.1 Discontinuation of Study Intervention

Not applicable: patients will be treated with a single dose of study treatment.

## 8.2 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study, if he/she and/or the Investigator decides to do so, at any time and for any reason. Possible reasons for participant withdrawal are as follows:

- Withdrawal of consent.
- Any clinical condition that in the Investigator's opinion could become dangerous for the participant or prevent the good conduct of the clinical trial.
- Lack of cooperation/compliance of the participant.
- Occurrence of adverse events for which the study medication is contraindicated or treatment with a medication that is not permitted as a concomitant medication.
- Loss to follow-up.

The reason for participant discontinuation or withdrawal from the study will be recorded on the electronic case report form (eCRF).

If possible, patients should be assessed using the procedure described in Section 9.2.5 (Discontinuation visit).

## 8.3 Loss to Follow-Up

A participant will be considered lost to follow-up if he/she misses one or more visits and cannot be contacted by the study center. Before a participant is deemed lost to follow-up, the Investigator or authorized designee must make every effort to regain contact with the participant, (including at least 3 telephone calls). These contact attempts should be documented in the participant's medical records.

All participants who are lost to follow up should be recorded by the Investigator in the appropriate pages of the eCRF.

# 9 STUDY ASSESSMENTS AND PROCEDURES

## 9.1 Logistics and study set-up

This will be a multicenter study. The list of study centers and contact details will be included in a separate document.

As described below, 12 study visits and 2 telephone calls are scheduled for each participant. Ad-hoc visit(s) may be necessary.

For each study center there will be a hospital team and a field team. Field study personnel may be hired or contracted through a commercial vendor. Field study personnel will be considered an integral part of the study team and undergo the same training on the protocol and study procedures as hospital study personnel.

### 9.1.1 Identification of candidate participants

It is critical that the study treatment be administered by the 3rd calendar day after the day the nasopharyngeal swab leading to COVD-19 diagnosis was taken for the reasons explained in Section 6.5. Semiquantitative "rapid tests" do not qualify for this study. Furthermore, only patients testing positive for the first time will be considered for enrolment (i.e., repeat positive tests will be dismissed). Each center will set up a procedure for early identification of study candidates which is adequate and efficient for the local set up.

Patients who potentially qualify for the study and are interested in participating will be asked to reach the study center using the dedicated transportation organized for the study (see below). Patients will be informed that there is no guarantee that they will actually enter the study whether or not they meet all eligibility criteria.

### 9.1.2 Study center visits

Visit 1 will take place at the study center. A car/van equipped for COVID-19 patients will pick up the candidate patient at home and bring him/her back home at the end of the visit. All study personnel and support staff (e.g., drivers) involved in the transportation will use adequate personal protection equipment (PPE) for COVID-19 in compliance with local regulations. Vehicles will be sanitized in compliance with local regulations.

At the beginning of Visit 1, before any study procedure is started, the study staff will review the Informed Consent Form (ICF) with the patient. The patient will sign the ICF if he/she agrees to participate in the study. During Visit 1, inclusion and exclusion criteria will be reviewed and, if the patient qualifies for and agrees to participate, all baseline procedures (see Section 1.3), including nasopharyngeal swab, will be conducted and study treatment will be administered. The participant will remain at the study center for at least 4 hours after study drug administration.

Every effort will be made to complete all Visit 1 procedures (see below) on the same day. However, if necessary, Visit 1 may span over 2 days as long as the study treatment is administered within the 3rd calendar day after the day the swab leading to COVID-19 diagnosis was taken. In this case, the participant will be brought home for the night and then back to the center the following morning, always by an appropriately equipped car/van.

As mentioned above, candidates may not be randomized even if meeting inclusion/exclusion criteria should the maximum daily number of randomized participants sustainable by the center be reached.

Visits 10 (Day 28), 11 (Day 56) and 12 (Day 168) will also occur at the study center. If the participant has tested negative for SARS-CoV-2, he/she will be asked to use normal means of transportation. If the participant has yet to test negative for SARS-CoV-2, the same special transportation described for Visit 1 will be used.

### 9.1.3 Home visits

All visits from Visit 2 (Day 3) to Visit 9 (Day 21) will be conducted at the patient's home by field study personnel unless the patient requires hospitalization (see below). At least one qualified (MD or trained nurse) field study team member is required for home visits. Field study personnel will travel in vans or cars equipped for transportation of nasopharyngeal swabs and blood samples. All field study personnel and supporting staff (e.g., drivers) will use adequate PPE for COVID-19 in compliance with local regulations. Vehicles will be sanitized in compliance with local regulations.

### 9.1.4 Hospitalizations

As described in Section 6, candidates may be enrolled in the study only if not hospitalized nor requiring hospitalization at baseline. However, hospitalization may be necessary at some point during the study.

Should hospitalization be required, every effort should be made to hospitalize the participant at the study center where Visit 1 took place. If hospitalization is required by the participant or by a health care provider other than a study staff-member, the participant or a family member or friend or neighbor must immediately alert the study personnel that hospitalization is needed. During hospitalization all scheduled visits and procedures will occur at the study center hospital, conducted by the hospital-based study team, to the extent the participant's conditions allow. Once the participant is discharged from the hospital, subsequent visits will resume as planned.

If for whatever reason a participant is hospitalized in a different hospital, data required for the study will be taken to the greatest extent possible.

## 9.2 Individual study visits

The SoA in Section 1.3 provides a summary outline of visits and procedures.

A total of 12 visits are scheduled for each participant: Visit 1 (Day 1 or Days 0-1: screening/baseline/ randomization), Visit 2 (Day 3), Visit 3 (Day 5), Visit 4 (Day 7), Visit 5 (Day 9), Visit 6 (Day 11), Visit 7 (Day 13), Visit 8 (Day 15), Visit 9 (Day 21), Visit 10 (Day 28), Visit 11 (Day 56 ± 7), Visit 12 (Day 168 ± 7).

Visits 1, 10, 11 and 12 will occur at the Study center; Visits 2 to 9 will occur at the participant's home, unless the participant is hospitalized.

Visits 1 to 10 are to occur on the scheduled day. For Visits 11 and 12, a window of ± 7 days is allowed. Should a visit be skipped or delayed for whatever reason the following visit should occur on the originally scheduled day.

### 9.2.1 Visit 1 (Day 1 or Day 0-1)

Visit 1 will occur at the study center. As described above, typically Visit 1 will be completed in one day (Day 1). If necessary and if the ≤ 3-day limit is ensured between the day the swab leading to diagnosis of COVID-19 is taken and the day the study drug is administered, Visit 1 may occur over two days, referred as Days 0 and 1.

Visit 1 includes screening and baseline procedures, randomization, and study drug administration.

The following procedures will be conducted (numbering as per Schedule of Activities, Section 1.3):

1. Informed consent, to be taken before any study procedure, including screening
2. Demographics and medical history
3. Full physical examination including calculation of Body Mass Index (BMI)
4. Vital signs: body temperature (BT), respiratory rate (RR), heart rate (HR), diastolic blood pressure (DBP), and systolic blood pressure (SBP)
5. Electrocardiogram (ECG)
6. Peripheral capillary oxygen saturation ($SpO_2$)
7. Chest imaging (ultrasound or X-rays)
8. Arterial blood gas analysis (hemogasanalysis) to calculate the $PaO_2/FiO_2$ ratio i.e., the ratio of arterial oxygen partial pressure ($PaO_2$ in mmHg) to fractional inspired oxygen ($FiO_2$ expressed as a fraction)
9. COVID-19 Clinical Severity Scale
10. Solicited local AE recording
11. Unsolicited AE recording
12. Previous and concomitant medications recording
13. Blood samples for routine safety tests: results must be available before randomization and dosing
14. Urine sample for pregnancy test (for women of childbearing potential): results must be available before randomization and dosing
15. Review of inclusion / exclusion criteria

If the participant meets all inclusion and exclusion criteria and is enrolled, the following procedures will be conducted:

16. Blood sample for measurement of serum anti-SARSC-CoV.2 antibodies
17. Blood sample for measurement of anti-drug antibodies (ADA) and MAD0004J08 concentration, limited to the first 60 randomized participants
18. Blood sample for exploratory research (to be taken only if participant provided dedicated informed consent)
19. Nasopharyngeal swab
20. Randomization and administration of study drug (IMP)
21. COVID-19 symptom and oxygen intake questionnaire.

Study personnel will monitor each participant for at least 4 hours after administration of study drug.

### 9.2.2 Visit 2 (Day 3), Visit 3 (Day 5), Visit 5 (Day 9), Visit 6 (Day 11), Visit 7 (Day 13), Visit 8 (Day 15), Visit 9 (Day 21)

These visits will occur at the participant's home unless the participant is hospitalized.

The following procedures will be conducted (numbering as per Schedule of Activities, Section 1.3):

4. Vital signs: BT, RR, HR, DBP and SBP
6. $SpO_2$
10. Solicited local AE recording
11. Unsolicited AE recording
12. Previous and concomitant medication recording
19. Nasopharyngeal swab
21. COVID-19 symptom and oxygen intake questionnaire.

### 9.2.3 Visit 4 (Day 7)

This visit will occur at the participant's home unless the participant is hospitalized.

The same procedures as Visit 2 will be conducted, with the addition of the following (numbering as per Schedule of Activities, Section 1.3):

13. Blood sample for routine safety tests

17. Blood samples for measurement of ADA and MAD0004J08 concentration for a subgroup of participants (first 60 randomized participants)

18. Blood sample for exploratory research (to be taken only if participant provided dedicated informed consent)

21. COVID-19 symptom and oxygen intake questionnaire.

### 9.2.4 Visit 10 (Day 28), Visit 11 (Day 56 ±7), Visit 12 (Day 168 ±7)

These visits will occur at the study center.

The following procedures will be conducted (numbering as per Schedule of Activities, Section 1.3):

3. Symptom/history-driven physical examination

4. Vital signs: BT, RR, HR, DBP, and SBP

5.Electrocardiogram (ECG)

6. $SpO_2$

10. Solicited local AE recording (only visit 10)

11. Unsolicited AE recording

12. Previous and concomitant medications recording

13. Blood samples for routine safety tests

14. Urine pregnancy test, limited to women of childbearing potential

17. Blood sample for measurement of ADA and MAD0004J08 concentration, limited to the first 60 randomized participants.

18. Blood sample for exploratory research, limited to visit 10, to be taken only if participant provided dedicated informed consent) (only visit 10)

19. Nasopharyngeal swab

21. COVID-19 symptom and oxygen intake questionnaire.

### 9.2.5 Discontinuation visit

If a participant discontinues prematurely (i.e., before Visit 12), three documented attempts will be made to schedule an *ad hoc* discontinuation visit. If the participant is cleared from SARS-CoV-2 (2 consecutive negative swabs) or hospitalized, this visit will occur at the study center, and procedures will be the same as for Visit 12, to the extent the participant's condition allow it. If the participant is positive for SARS-CoV-2 and quarantining at home, this visit will occur at home, and procedures will be the same as for Visit 12.

### 9.2.6 Ad hoc visits

As described above, the 1st nasopharyngeal swab testing negative for SARS-CoV-2 must be confirmed by a subsequent negative nasopharyngeal swab to be taken at least 24 hours thereafter.

Visits 2 to 8 are separated by two days from one another. Hence, the following visit will be used for the confirmatory nasopharyngeal swab, with no need for an ad-hoc visit. However, should the 1st negative swab occur at Visit 8 or later, an ad-hoc home visit will be scheduled as soon as possible starting 24 hours after the 1st negative result to obtain an additional nasopharyngeal swab.

For the ad-hoc visit, PPE should be used as if the participant were still positive for SARS-CoV-2.

If the confirmatory nasopharyngeal swab gives positive results, the previous swab will no longer be considered negative, and the procedure will be repeated when then next negative swab occurs.

An ad hoc visit will also be conducted when a hospitalized participant for whatever reason cannot undergo a nasopharyngeal swab at a scheduled visit. A nasopharyngeal swab will be taken during an ad hoc visit as soon as the patient can undergo the swab again in the Investigator's judgement. Thereafter, nasopharyngeal swabs will be conducted as per protocol.

## 9.3 Study calls

Study staff will contact each participant by phone twice, on Day 42 (±7) and Day 112 (±7).

The following procedures will be conducted (numbering as per Schedule of Activities, Section 1.3):

11. Unsolicited AE recording

12. Previous and concomitant medication recording.

## 9.4 Daily recordings of hospitalization events

If a participant is hospitalized during the study, the investigator or designated study personnel will complete the hospitalization event questionnaire each day, at approximately the same time from the day of admission to the day of dismissal (included).

Hospitalization is defined as ≥24 hours stay in a hospital. Emergency room stay will be counted as hospitalization if it lasts for ≥ 24 hours.

## 9.5 Assessments and procedures

The SoA in Section 1.3 and Section 9.2.1 provides a summary outline of assessments and procedures by visit.

### 9.5.1 Informed consent and other consents

The Investigator or other authorized study personnel will explain the informed consent form (ICF) to each participant. This ICF will include collection of ADA and MAD0004J08 concentration (only for the first enrolled 60 subjects). Thereafter, the participant will be asked to review and sign the ICF at Visit 1 before any study procedure, including screening.

Each participant will also be asked to sign a separate consent authorizing the sponsor to collect, store and test samples and to use remaining specimens originally taken for mandatory testing for exploratory endpoints and other research not described in the protocol. Refusal to sign this additional consent does not preclude enrolment if the participant signs the main ICF.

A detailed description of the informed consent process is provided in Section 11.1.1.

### 9.5.2 Demography and medical history

The following demographic information will be collected at Visit 1: date of birth, sex, ethnicity.

Medical history will be collected at Visit 1 through interview and review of available documentation and documented in the medical history eCRF page.

As part of the medical history the participant will be asked when the transmission of COVID-19 might have occurred. This information will be included in the eCRF as a date (dd-mmm-yyyy). The best approximation should be included using day 1 and day 15 of a month if the participant does not recall the exact day. If the participant has no recollection of an event possibly responsible for transmission, an appropriate box should be ticked in the e-CRF. If the participant indicates two or more events in which the transmission could have occurred, the most recent event should be used. This information may be included in subgroup analyses (Section 10-5.11).

### 9.5.3 Physical examination

A complete physical examination will be performed at Visit 1 before dosing. This examination excludes pelvic, rectal, and breast examinations unless clinically indicated. The complete physical examination includes calculation of the Body Mass Index (BMI), calculated as follows:

$$BMI = body\ weight\ in\ kg/(height\ in\ m)^2$$

A history and symptom-directed physical examination will be performed at other on-site visits.

Investigators should pay special attention to clinical signs related to COVID-19 and to ongoing medical conditions.

Significant findings that were present prior to the signing of informed consent must be included in the Medical History page of the eCRF. Significant new findings that begin or worsen after informed consent must be recorded in the Adverse Event page of the eCRF.

### 9.5.4 Vital signs

Vital signs will be measured at Visit 1 before dosing and at each subsequent study visit.

Vital signs to be measured are body temperature (°C), heart rate (beats / minute), respiration rate (breaths / minute), diastolic and systolic blood pressure (mmHg).

The participant should be in a sitting position and should be given the time to relax before vital signs are measured.

Properly calibrated and maintained local equipment will be used to measure body temperature and blood pressure.

### 9.5.5 Electrocardiogram (ECG)

ECG will be measured at Visit 1 before dosing and at Visit 10, Visit 11 and Visit 12.

Properly calibrated and maintained ECG local equipment will be used. Standard procedures for ECG measurement will be followed.

### 9.5.6 Pulse oximetry for peripheral capillary oxygen saturation ($SpO_2$)

Peripheral capillary oxygen saturation ($SpO_2$) will be measured at Visit 1 before dosing and at each subsequent study visit.

$SpO_2$ (%) will be measured by using a commercial pulse oximeter.

### 9.5.7 Chest imaging (ultrasound or X-rays)

Chest ultrasound or chest X-rays will be conducted at Visit 1 before dosing to allow completion of the COVID-19 Clinical Severity Scale.

Properly calibrated and maintained local equipment will be used. Standard procedures will be followed.

### 9.5.8 Arterial Blood Gas Analysis for $PaO_2/FiO_2$

Arterial blood gas analysis (hemogasanalysis) will be conducted to calculate the $PaO_2/FiO_2$ ratio i.e., the ratio of arterial oxygen partial pressure ($PaO_2$ in mmHg) to fractional inspired oxygen ($FiO_2$ expressed as a fraction).

$PaO_2/FiO_2$ is required to allow completion of the COVID-19 Clinical Severity Scale.

Properly calibrated and maintained local equipment will be used. Standard procedures will be followed.

### 9.5.9 COVID-19 Clinical Severity Scale

Clinical severity will be assessed via the COVID-19 Clinical Severity Scale at Visit 1 before dosing.

The COVID-19 Clinical Severity Scale reported below is a 5-grade categorical scale. It is a modified version of the scale reported in NIH Coronavirus Disease 2019 (COVID-19) Treatment Guidelines [39].

***Table 9-1 COVID-19 Clinical Severity Scale***

**Grade 1. Asymptomatic COVID-19**

The patient meets **ALL** of the following criteria:

1. No symptoms or signs of COVID-19 (see below)
2. $SpO_2 \geq 94$ % on room air at sea level
3. No evidence of lower respiratory disease by clinical assessment, i.e., no shortness of breath or dyspnea
4. No chest imaging suggestive of lower respiratory disease.

**Grade 2: Mild COVID-19**

The patient meets **ALL** of the following criteria:

1. One or more of the following COVID-19 signs and symptoms:
    i. fever (≥37.5 ºC)
    ii. cough
    iii. sore throat
    iv. malaise
    v. headache
    vi. muscle pain
    vii. vomiting
    viii. diarrhea
    ix. loss of taste
    x. loss of smell
2. $SpO_2 \geq 94$ % on room air at sea level
3. No evidence of lower respiratory disease during clinical assessment, i.e., no shortness of breath or dyspnea
4. No chest imaging suggestive of lower respiratory disease.

**Grade 3: Moderate COVID-19.**

The patient meets **ALL** of the following criteria:

1. $SpO_2 \geq 94\%$ on room air
2. Evidence of lower respiratory disease during clinical assessment i.e., shortness of breath or dyspnea AND/OR chest imaging suggestive of lower respiratory disease with lung infiltrates covering ≤50% of lung surface.

**Grade 4. Severe COVID-19.**

The patient meets **ANY** of the following criteria:

1. $SpO_2 < 94$ on room air at sea level
2. Chest imaging with evidence of lung infiltrates covering >50% of lung surface.

**Grade 5. Critical COVID-19**

The patient meets **ANY** of the following criteria:

1. Acute respiratory distress syndrome defined by $PaO_2/FiO_2$ <300 mmHg with positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) ≥5 $cmH_2O$
2. Sepsis or septic shock
3. Multiple organ dysfunction.

### 9.5.10 Solicited local adverse events

The grading scale used in this study to assess local solicited AEs described below is derived from the FDA Center for Biologics Evaluation and Research (CBER) guidelines on toxicity grading scales for healthy adult volunteers enrolled in preventive vaccine clinical trials [40].

Three solicited local AEs at the injection site will be recorded and graded by the study staff at Visits 1 to 10: pain, redness, and swelling. Two sets of recordings will be carried out each time, one for each side at the site of injection.

***Table 9-2 Solicited local AE grading scale***

| AE | Grade | | Description |
|---|---|---|---|
| Pain at injection site | 1 | mild | Does not interfere with activity |
| | 2 | moderate | Interferes with activity |
| | 3 | severe | Prevents activity |
| | | | |
| Redness at injection site | 1 | mild | > 2.0 cm to 5.0 cm |
| | 2 | moderate | > 5 cm to 10.0 cm |
| | 3 | severe | >10 cm |
| | | | |
| Swelling at injection site | 1 | mild | 2.5 – 5 cm and does not interfere with activity |
| | 2 | moderate | 5.1 - 10 cm or interferes with activity |
| | 3 | severe | >10 cm or prevents daily activity |
| | | | |

For solicited local AEs, relatedness to study drug is assumed (see Section 9.6 for definitions). Seriousness will be assessed as described in Section 9.6.

### 9.5.11 Unsolicited adverse events

Unsolicited Adverse events will be collected at each visit and phone call throughout the study as described in Section 9.6. Participants will be encouraged to contact the study personnel any time between visits to report AEs as they see fit.

### 9.5.12 Previous and concomitant medications

Information on previous and concomitant medication, including vaccines and non-prescription medications, will be collected at each visit and phone call throughout the study.

"Previous" refers to discontinued medications taken in the past year at Visit 1 and since the last visit for all other visits.

The information to be collected includes trade name, date of start and (where appropriate) end of intake, dosing schedule.

### 9.5.13 Routine blood safety tests

Venous blood will be drawn for routine safety tests at Visit 1 before dosing, Visit 4, Visit 10, Visit 11 and Visit 12.

At visit 1, results must be available before randomization and dosing.

Details on blood volumes, processing and shipment are provided in the study manual. The study centers' local laboratories will be used for blood safety tests.

The following parameters will be measured:

1. Complete blood cell count
2. Bilirubin (total and direct)
3. Aspartate aminotransferase (AST)
4. Alanine aminotransferase (ALT)
5. Creatinine
6. Prothrombin time (INR)
7. Partial thromboplastin time (aPTT)
8. Lactate dehydrogenase (LDH)
9. Creatine phosphokinase (CPK)
10. Erythrocyte sedimentation rate
11. C-reactive protein (CRP)
12. Serum glucose
13. Electrolytes: sodium, potassium, calcium, chorine

### 9.5.14 Urine pregnancy test

Urine will be collected from women of childbearing potential at Visit 1 for a rapid pregnancy test. Results must be available before randomization and dosing. A urine rapid pregnancy test will be also performed at Visit 10, Visit 11, and Visit 12.

Commercial kits will be used. Details on collection and processing are provided in the study manual.

### 9.5.15 Review of inclusion and exclusion criteria

At Visit 1, once procedures 1 to 14 have been carried out and results are available, inclusion and exclusion criteria reported in Sections 6.1 and 6.2 will be reviewed. If the participant meets all the criteria and is enrolled in the study, Study 1 will be completed with procedures 16 to 18 (see below) and study drug (IMP) will be administered (procedure 19).

### 9.5.16 Measurement of Anti-SARS-CoV-2 antibodies

Venous blood will be drawn to measure anti-SARS-CoV-2 antibodies at Visit 1 before dosing.

A central laboratory will be used for all tests to detect andi-SARS-CoV2 antibodies. Details on the selected commercial tests, blood volumes, processing, and shipping are provided in the study manual.

#### 9.5.16.1 Tests to assess baseline seronegative / seropositive status

Three assays will be used to determine each participant's status at baseline as seronegative or seropositive, as follows:

1) Antibody test to measure serum concentration of IgG vs. the spike (S) protein of SARS-CoV-2.

2) Antibody test to measure serum concentration of IgG vs. the nucleocapsid (N) protein of SARS-CoV-2.

3) Antibody test to measure serum concentration of IgA vs. the spike (S) protein of SARS-CoV-2.

A participant testing negative to all three tests Visit 1 will be classified as seronegative. A participant testing positive to one or more of the three tests at visit 1 Visit 1 will be classified as seropositive.

#### 9.5.16.2 Evaluation of new test

The experimental assay DIESSE ELISA KIT will be evaluated as one of the exploratory objectives of this study.

### 9.5.17 Measurement of anti-drug antibodies (ADA) and MAD0004J08 concentration

A sample of venous blood will be drawn in the first 60 randomized participants to measure anti-drug antibodies (ADA) vs. MAD0004J08 and MAD0004J08 concentration at Visit 1 before dosing, Visit 4, Visit 10, Visit 11, and Visit 12.

A central laboratory will be used for this measurement. Details on processing and shipping are provided in the study manual.

### 9.5.18 Samples for exploratory research

At Visit 1, Visit 4 and Visit 10, two samples of venous blood will be taken and stored for exploratory endpoints and future research, as follows:

- One 9 mL sample for assessment of serum neutralizing power vs variant strains
- One 7 mL sample for other exploratory measurements.

These samples will only be taken if the participant gives permission by signing the dedicated informed consent form. A participant who does not grant permission to take exploratory samples may still enter the study if he/she agrees to and signs the main informed consent for.

Details on processing, and shipping are provided in the study manual.

### 9.5.19 Nasopharyngeal swab and SARS-CoV-2 viral load determination

#### 9.5.19.1 Nasopharyngeal swab

A nasopharyngeal swab will be taken at Visit 1 and at each subsequent study visit. If necessary (See Section 9.2.6) additional nasopharyngeal swab(s) will be taken at ad hoc visit(s).

Flexible nasopharyngeal nylon flocked swabs will be used.

Details on the procedure are reported in the study manual.

If a patient is hospitalized and nasopharyngeal swab cannot be carried out on one or more planned visits (e.g., the patient is intubated), the procedure will be skipped. A nasopharyngeal swab will be conducted during an *ad hoc* visit as soon as the patient can undergo the swab again in the Investigator's judgement. Thereafter, nasopharyngeal swabs will be conducted as per protocol.

#### 9.5.19.2 Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) test for SARS-CoV-2 detection

A central laboratory will be used for the RT-PCR quantitative assessment of SARS-CoV-2 viral load in nasopharyngeal swabs. Details on the selected commercial test processing and shipping are provided in the study manual.

### 9.5.20 Randomization and IMP administration

Randomization and administration of the study drug (IMP) as described in Section 7.1.2 will occur at Visit 1, once all inclusion and exclusion criteria have been reviewed and met and the decision is made to enroll the participant in the study.

After administration of the study drug, each participant will remain at the study center and be observed for AEs for at least 4 hours.

Preparation, handling, storage and accountability of IMP are described in Section 7.2.

This is a single-dose treatment hence no IMP will be administered after Visit 1.

### 9.5.21 COVID-19 symptom and oxygen intake questionnaire

COVID-19 symptoms [39] will be scored by the study staff at each visit.

The symptoms are as follows:

1. Feeling feverish
2. Cough
3. Sore throat
4. Fatigue (malaise)
5. Headache
6. Muscle pain
7. Gastrointestinal symptoms
8. Shortness of breath with exertion
9. Loss of taste
10. Loss of smell

Each of the symptoms # 1-8 will be scored on a 4-point ordinal scale (0=absent, 1=mild, 2=moderate, 3=severe). The total score will range from 0 (best) to 24 (worst).

Symptoms # 9 and # 10 will be scored as yes/no and assessed separately.

In addition, the participant will be asked whether that day oxygen therapy was started or, if the participant was already receiving oxygen therapy before the start of the study for conditions other than COVID-19 (e.g., COPD), whether the dose and/duration and/or frequency of oxygen therapy was increased. This information will be used for the key secondary efficacy composite endpoint.

### 9.5.22 Hospitalization events questionnaire

Hospitalization is defined as ≥24 hours stay in a hospital. Emergency room stay will be counted as hospitalization if it lasts for ≥ 24 hours.

Dates of hospital admission and discharge will be recorded in the eCRF.

Each day of hospitalization, the following information will be recorded in the hospitalization events section of the eCRF:

- Supplemental oxygen (yes/no)
- Non-invasive ventilation or a high flow oxygen device (yes/no)
- Intensive care Unit (ICU) stay (yes/no)
- Intubation (yes/no)
- Mechanical ventilation (yes/no)
- Extra corporeal membrane oxygenation (ECMO) (yes/no)
- Additional organ support (e.g., pressors, renal replacement) (yes/no, type)

## 9.6 Adverse Events (AE) and Serious Adverse Events (SAE)

Adverse events (AEs) will be reported by the participant, or, when appropriate, by a caregiver, or the participant's legally authorized representative at each scheduled visit and phone call. Participants and caregivers / legal representatives will be encouraged to contact the study center at any time between visits and calls to report AEs.

In this study two types of AEs will be captured: unsolicited AEs and solicited local AEs. The latter are a set of three predefined AEs at the injection site, i.e., pain, redness and swelling, that will be assessed by the study staff through Visit 10 (see Section 9.5.10). All definitions and procedures described in this section apply to both types of AEs, unless stated otherwise.

The Investigator and qualified designees are responsible for detecting, assessing, documenting, and recording AEs and SAEs and for following up AEs that are serious, considered related to the study treatments or study procedures, or that caused the participant to discontinue the study treatment or the study.

### 9.6.1 Definitions

#### 9.6.1.1 Adverse event (AE)

An AE is any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment. An AE can therefore

be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

**Events Meeting the AE Definition**

- Any abnormal laboratory test results (e.g., hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements, eye symptom or findings), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition, except COVID-19 (see below).
- New conditions detected or diagnosed after study start even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

**Events NOT Meeting the AE Definition**

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Lack of efficacy per se will not be reported as an AE. Such instances will be captured in the efficacy assessments. Hence, worsening of COVID-19 symptoms as detected by the symptom scores will not be recorded as AE unless it qualifies as SAE.

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events eCRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's eCRF. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

### 9.6.1.2 Serious adverse event (SAE)

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

- The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect.

f. Other situations

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

#### 9.6.1.3 Adverse Event of Special Interest (AESI)

An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., regulators) might also be warranted.

In this study the following events are considered AESI:

- Anaphylactic / anaphylactoid reactions.

AESIs are to be reported as stated in Section 9.6.3.

#### 9.6.1.4 Adverse Drug Reaction (ADR) or Adverse Reaction (AR)

An adverse reaction is any noxious and unintended response to a medicinal product related to any dose of the product. In accordance with ICH E2A, the definition of an adverse reaction implies a reasonable possibility of a causal relationship between the adverse event and the Investigational Medicinal Product (IMP). An adverse reaction, in contrast to an adverse event, is characterized by the fact that a causal relationship between a medicinal product and an occurrence is suspected. It could also be related to the administration procedure when the procedure is an essential part of the IMP administration.

#### 9.6.1.5 Unexpected Adverse Reaction (UAR)

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

#### 9.6.1.6 Serious Adverse Reaction (SAR)

Serious adverse reactions (SARs) are defined as all noxious and unintended responses to an IMP related to any dose administered that result in death, are life-threatening, require inpatient hospitalization or prolongation of existing hospitalization, result in persistent or significant disability or incapacity, or are a congenital anomaly or birth defect. Except for the relatedness (causality), the definitions of SAEs apply.

During clinical investigations, adverse events may occur which, if suspected to be medicinal product-related (AR), might be significant enough to lead to important changes in the way the medicinal product is developed (e.g., change in dose, population, needed monitoring, consent forms). This is particularly true for reactions which, in their most severe forms, threaten life or function. Such reactions should be reported promptly to regulators.

Therefore, special medical or administrative criteria are needed to define reactions that, either due to their nature ("serious") or due to the significant, unexpected information they provide, justify expedited reporting.

#### 9.6.1.7 Suspected Unexpected Serious Adverse Reaction (SUSAR)

An adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction.

### 9.6.2 Recording and follow-up of AEs and SAEs: Content

The Investigator must record all adverse events in the Adverse Event Log provided in the participant's CRF with the following information:

1. Description of Adverse event
2. Date of onset (time can be recorded, if applicable)

3. Seriousness
4. Severity (intensity)
5. Causal relationship to IMP ("relatedness") (SAEs)
6. Outcome with date and/or time

Care must be taken not to introduce bias when detecting AE and SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

The Sponsor or an appointed Sponsor representative will assess expectedness of SAEs based on reference information in the Investigator Brochure.

#### 9.6.2.1 Description of Adverse Event

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a patient suffers from the same adverse event more than once and the patient recovers in between the events, the adverse events should be recorded separately. If an adverse event changes in seriousness and/or severity, a worst-case approach should be used when recording the event, i.e., the highest seriousness and severity and the longest duration of the event (exception: an adverse event with onset after enrolment but before the first IMP administration (i.e., a pre-treatment adverse event), which changes in severity (intensity) after IMP administration, must be recorded as two separate events. The initial adverse event should be recorded with outcome "not recovered" and the date and time of outcome is when the severity changed. The second adverse event should be recorded with date and time of onset when the intensity changed.

A procedure is not an AE: the reason for conducting the procedure is an AE. Hospitalization is not an AE: the reason for hospitalization is and AE. Death is not an AE: the cause of death is an AE (exception: sudden death of unknown cause is an AE).

#### 9.6.2.2 Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the adverse event is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken, or the examination was performed.

For pre-existing clinically significant conditions (diagnosed or observed as a result of the screening procedures) becoming worse after IMP administration, the date of onset is the date the worsening began.

#### 9.6.2.3 Seriousness

Each AE must be evaluated for seriousness base on the definitions reported above. If an AE is assessed as serious, expedited reporting is mandatory.

#### 9.6.2.4 Severity or Intensity

The severity (intensity) of each unsolicited AE will be classified using the following 3-point scale:

Mild: Awareness of signs or symptoms, but no disruption of usual activity.

Moderate: Event sufficient to affect usual activity (disturbing).

Severe: Inability to work or perform usual activities (unacceptable).

The severity of each solicited local AE will be classified using the same 3-point scale (mild-moderate-severe), but with different definitions. Definitions are provided in Section 9.5.10.

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification is provided:

- The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache).
- This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

#### 9.6.2.5 Causal Relationship to IMP

The Investigator should make an assessment of the relationship between study treatment and each occurrence of each AE/SAE.

The causality assessment will be determined using a two-level scale: related or not related.

An AE/SAE is considered related to study intervention if there is a reasonable possibility that the study intervention contributed to the AE.

Not-related means there is no reasonable possibility that the AE is causally related to administration of the study intervention. There are other more likely causes for the AE.

The Investigator will use clinical judgement to determine the relationship.

Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.

There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report. However, it is very important that the Investigator always assess causality for every event before the initial transmission of the SAE data.

The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Solicited AEs are considered related by definition. Hence, assessment of causal relationship will not be required for these events.

#### 9.6.2.6 Expectedness (SAE)

The Sponsor or CRO defines the expectedness of SAEs.

Expected SAEs are events consistent with the Investigator's Brochure of MAD0004J08.

#### 9.6.2.7 Outcome

The outcome of AEs/SAEs with date and/or time (as appropriate) must be reported by the Investigator.

The outcome of each SAE must be reported to the Sponsor or CRO, even if this extends beyond the SAE reporting period (i.e., after the final study visit).

For analysis purposes, the outcome for SAE will be determined on the final study visit.

Outcome of all AE will be classified as one of the following:

- Resolved
- Resolved with sequelae
- Ongoing
- Death
- Unknown

### 9.6.3 Recording and Follow-up of AEs and SAEs: Timing

All unsolicited AEs and SAEs will be collected from the time of signing of the Informed Consent Form (ICF) until participation in study has ended. This includes AEs that begin before the start of study treatment but after signing of the ICF.

Solicited AEs will be collected from Day 1 to Day 28 (inclusive).

All SAEs will be recorded and reported to the Sponsor or designee immediately and in any case within 24 hours. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

Care will be taken not to introduce bias when detecting AEs and SAEs. Open-ended and non-leading verbal questioning of the participant are preferred to inquire about AE occurrences.

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of the study treatment under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Ethics Committees (ECs), and Investigators.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file and will notify the EC, if appropriate according to local requirements.

#### 9.6.3.1 Expedited Reporting of SAEs and SUSARS

The primary mechanism for reporting an SAE by the Investigator to the Sponsor or delegate (e.g., CRO) will be the electronic data collection tool.

The site will enter the SAE data into the electronic system as soon as it is identified and not later than 24 hours of his/her knowledge of the occurrence of the SAE.

All SAE (related and unrelated) must be reported to the Sponsor or delegate throughout the study.

The Investigator must not wait to collect additional information to fully document the event before notifying the CRO Safety team of an SAE. The initial notification should include at least the following:

- Protocol number and name and contact number of the Investigator
- Participant ID number (and initials and date of birth, if available)
- Date participant received study drug
- SAE and date of event onset
- Current status of participant

NOTE: the reporting must be done anyway even if one or more of the above items are missing.

The Investigator is responsible for expedited safety report submission to the Sponsor or delegate and the Sponsor or delegate reports to regulatory authorities within specific time periods of being notified of the event. Therefore, it is important that the Investigator submit additional information requested as soon as it becomes available.

The Sponsor must expedite the reporting to all concerned Investigator(s)/institutions(s), to the EC(s), where required, and AIFA of all adverse drug reactions (ADRs) that are both serious and unexpected.

The Sponsor shall ensure that all relevant information about SUSARs that are fatal or life-threatening is recorded and reported to the competent authorities and ECs concerned as soon as possible and no later than 7 days after first knowledge by the Sponsor, and that relevant follow-up information is subsequently communicated within an additional eight days.

All other SUSARS shall be reported to the competent authorities and EC(s) concerned as soon as possible no later than of 15 days after first knowledge by the Sponsor.

The Sponsor will notify the DMC of all SUSARS within the same timelines the report is send to Investigators, health authorities, EC and other relevant parties. Any follow-up report will be sent with the same timelines.

If the electronic system is unavailable, the site may use the paper SAE data collection tool instead of the electronic data collection tool, in order to report the event within 24 hours of becoming aware. In such cases the paper SAE form should be completed by the Investigator or authorized delegate and scanned and emailed or faxed to the Sponsor or CRO. The Investigator is responsible for ensuring an adequate transmission of the fax and will store the distribution confirmation in the study file.

In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE report form sent by overnight mail or courier service.

Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE CRF pages within the designated reporting time frames.

After the study is completed at a given site, if used, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.

If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form.

Contacts for SAE reporting and for all safety personnel are contained in the Team Contact List which will be maintained by the study Sponsor.

#### 9.6.3.2 Other events requiring Expedited Reporting

The Investigator must report the following events by scanning and emailing, or faxing the appropriate form to the local medical monitor within 24 hours of becoming aware of the event:

- Withdrawal of consent during the study for medical reasons
- Emergency unblinding.
- Protocol violation affecting the safety of a participant.
- Any event other than SAE that, in the opinion of the Investigator, precludes further administration of the study drug
- Pregnancy

#### 9.6.3.3 Non-Expedited Reporting

In cases where reporting is not required immediately (see above) the Investigator shall report within the appropriate time frame, taking account of the specificities of the trial and of the SAE, as well as possible guidance in the protocol or the IB.

Expedited reporting of reactions which are serious but expected will ordinarily be inappropriate. Expedited reporting is also inappropriate for SAE from clinical investigations that are considered not related to study product, whether the event is expected or not. Similarly, non-serious adverse reactions, whether expected or not, will ordinarily not be subject to expedited reporting.

AEs, including SAEs, should be recorded by the Sponsor and the Investigator from the signature of informed consent to the end of the trial unless otherwise provided for in the protocol.

Once a year throughout the clinical trial, the Sponsor shall provide to member states in whose territory the clinical trial is being conducted, AIFA (Italy) for this trial, and the EC(s) with a listing of all suspected serious adverse reactions which have occurred over this period and a report of the subjects' safety.

### 9.6.4 Pregnancy

Details of all pregnancies in female participants will be collected throughout the study.

If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined below.

Pregnancy is not considered an AE or SAE. However, abnormal pregnancy outcomes, for example, spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy, are considered SAEs.

#### 9.6.4.1 Male participants with partners who become pregnant

The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy.

The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported including fetal status (presence or absence of anomalies) and indication for the procedure.

#### 9.6.4.2 Female participants who become pregnant

The Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a participant's pregnancy.

The participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, including fetal status (presence or absence of anomalies) or indication for the procedure.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

A spontaneous abortion (occurring at <20 weeks gestational age) or still birth (occurring at >20 weeks gestational age) is always considered to be an SAE and will be reported as such.

Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating in the study will be withdrawn from the study and will follow the standard discontinuation process.

### 9.6.5 Hypersensitivity Reactions

Many drugs, including biologic agents, carry the risk of systemic hypersensitivity reactions.

If such a reaction occurs, details describing each symptom should be provided to the Sponsor.

Study centers must have appropriately trained medical staff and appropriate medical equipment available to treat hypersensitivity reactions.

Participants who experience a systemic hypersensitivity reaction should be treated per the local standard of care.

### 9.6.6 Product complaints

A product complaint is any written, electronic, or oral communication that concerns deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a study treatment.

The Sponsor collects product complaints on study treatment and drug delivery systems used in clinical studies in order to ensure the safety of study participants, monitor quality, and facilitate process and product improvements.

Investigators and participants will be instructed to contact the Investigator as soon as possible if they have a complaint or problem with the intervention so that the situation can be assessed.

AEs/SAEs that are associated with a product complaint will also follow the processes outlined above.

#### 9.6.6.1 Time period for detecting Product Complaints

Product complaints will be reported to the Sponsor within 24 hours after the Investigator becomes aware of the complaint.

#### 9.6.6.2 Follow-up of Product Complaints

Follow-up applies to all participants, including those who discontinue study intervention.

The Investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the product complaint.

### 9.6.7 Overdose

There is no known antidote for an overdose of MAD0004J08.

In the event of an overdose, the Investigator should:

- Contact the CRO immediately
- Closely monitor the participant for any AE/SAE and laboratory abnormalities
- Provide supportive care as necessary
- Document the quantity of the excess dose in the CRF.

# 10 STATISTICAL CONSIDERATIONS

This section of the protocol summarizes the planned statistical analysis approach for the study.

The details of the statistical analysis will be provided in the Statistical Analysis Plan (SAP), which will be finalized before the first clinical database lock for the study and treatment unblinding. If, after the study has begun, but prior to unblinding, changes are made to the primary objective/ hypothesis or to the statistical methods related to them, then the protocol will be amended. Changes to secondary and exploratory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be reported in the SAP and Clinical Study Report (CSR) of the study. Additional exploratory analyses of the data not described in the protocol will be conducted as deemed appropriate and will be clearly identified in the SAP and the CSR of the study.

This is an adaptive confirmatory phase II/III trial with two doses of MAD0004J08 and two primary populations, i.e., the whole population (ALL) and the targeted population of subjects who are SARS-CoV-2 seronegative at baseline (SERONeg). The part of the study before the interim analysis will also be referred to as Stage-1, while the part after the interim analysis will be referred to as Stage-2.

The study is based on the approach described in Di Scala & Glimm, 2011 [42], that combines interim decision making on dose selection based on Bayesian predictive power with adjustment methods that control the type I error rate of the final efficacy decision (selected dose versus placebo) at the prespecified study-wise $\alpha$ level.

At the interim analysis, this approach allows for the selection of one dose on the basis of efficacy (and safety, see below) results observed, and a stop for futility or efficacy. At the final analysis, this approach allows for testing of efficacy of the selected dose of MAD0004J08.

At the end of Stage-1, safety data will also be assessed for the purpose of dose selection and, since the method described by Di Scala & Glimm does not require selecting the most efficacious dose at the interim analysis, in principle the dose which is less efficacious but safer could be selected.

Furthermore, as the study has two primary populations, the approach is applied independently in each of them. Therefore, in principle, at the interim analysis, based on the Bayesian predictive power, two different doses could be selected for the ALL and the SERONeg populations. This finding is considered unrealistic because of biological plausibility. However, in this situation, the decision on dose selection for the two populations will involve a deeper evaluation of the safety data and other external considerations.

The testing strategy is described in Section 10.2.

## 10.1 Planned analyses

Because of the pressing need for COVID-19 treatments, three pre-defined sets of formal statistical analyses will be conducted referred to as interim, primary, and final analysis.

**1) Interim analysis**

This analysis will be performed at the end of Stage 1, i.e., as soon as possible (collected data have been cleaned and all key decisions necessary for the statistical analysis have been made in the final Data

Review Meeting(s)) after the pre-defined number of events for the interim efficacy analysis is taken for both populations (see Section 10.3.1).

The following endpoints will be analyzed:

- Primary endpoint
- Key secondary efficacy endpoint
- Primary and selected secondary safety endpoints (they will be defined in the SAP).

To perform this analysis, the database will be locked.

**2) Primary analysis**

This analysis will be performed as soon as possible after the pre-defined number of events for the primary efficacy analysis is taken for both populations (see Section 10.3.1).

All study endpoints will be analyzed on the available frozen data.

**3) Final analysis**

The final analysis will be conducted when the complete six-month follow-up data have been taken.

All study endpoints will be analyzed on the available frozen data.

**Additional considerations**

The primary efficacy analysis is also an interim analysis considering that the study will continue under the same blinding modality (i.e., double-blind condition). However, this will be the analysis where all key efficacy decisions are made, so no multiplicity adjustment is planned for the final analysis on complete data.

No unblinded data will be shared with personnel involved in the study conduct at any time during and after the interim analysis until study completion. Further details will be provided in the SAP.

Monitoring of unblinded safety data (including AEs, SAEs, and selected laboratory measurements) will occur throughout the study and will be conducted by the DMC members. Details of the safety reviews, including the frequency and approximate timing, will be specified in the DMC charter.

The DMC will also be responsible for evaluating results of the interim analysis conducted at the end of Stage-1, and based on predefined criteria, to make recommendations regarding the continuation of the study in Stage-2 and dose selection.

A predefined subgroup of the SC composed of senior members not otherwise involved in the study will be responsible for final decisions on stopping for futility or efficacy or dose selection.

Unblinding details will be specified in the unblinding plan section of the SAP or in a separate unblinding plan document.

## 10.2 Testing strategy

This is an event-driven study. In this context the "event" is the 1st nasopharyngeal swab testing negative for SARS-Cov-2 at RT-PCR for an individual participant. SARS-Cov-2 clearance from the

URT however should be confirmed by a second negative nasopharyngeal swab taken at least 24 hours after the first negative result.

The primary endpoint is time to the first SARS-CoV-2 negative nasopharyngeal swab.

The key secondary endpoint is the proportion of patients experiencing at least one of the events of the composite outcome (see Section 4).

Figure 5-1 in Section 5.1 shows the trial design and how decisions are made.

Within each of the two primary populations (ALL and SERONeg), the testing strategy can be summarized as follows:

Interim analysis (end of Stage-1):

1. Early stop for efficacy will be based on a frequentist test of the primary endpoint. The multiplicity of testing will be taken care of with the O'Brien Fleming approach and a hierarchical ordering of the doses. The statistical test for the key secondary endpoint (performed at the nominal alpha level which has been set for the interim analysis) will have an inferential value only if the primary endpoint is statistically significant (hierarchical testing with no impact on multiplicity).

2. If efficacy cannot be claimed, the second question is whether the study should be closed for futility. Early stop for futility will occur if each primary population meets its futility rule. Futility must be shown in both populations in order to stop the study. The futility rule will be based on the predictive probabilities for the primary and key secondary endpoints. The futility analysis has no impact on multiplicity of testing.
3. If the study is not stopped for either efficacy or futility, a single dose will be selected for Stage-2. Dose-selection will be based on a linear combination of predictive probabilities for the primary and key secondary endpoints. This selection has an impact on multiplicity of testing that will be taken into account in the primary efficacy analysis (end of Stage-2).

Primary analysis (end of Stage-2 / Core):

4. The primary efficacy analysis will be based on a frequentist test of the primary efficacy endpoint and the alpha level of this test is adjusted for the two efficacy analyses (interim and final) through the O'Brien Fleming approach and for the dose selection analysis (interim) through the Dunnett's method as described by Di Scala and Glimm [42]. The statistical test for the key secondary endpoint will have an inferential value only if the primary endpoint is statistically significant at the nominal alpha level which has been set for the primary analysis. All secondary endpoints will be analyzed without adjustment for multiplicity.

Final analysis (end of Stage-2 / Follow-up):

5. The primary efficacy analysis will not be repeated. The analysis of all secondary endpoints, including the key secondary endpoint, will be repeated.

The multiplicity of testing due to the two primary populations (ALL and SEROneg) is accounted for by the Bonferroni method. Strategies to be followed for decision making (stop for efficacy, select dose) in case of discordant results between the two populations are discussed below.

The main elements of the testing strategy are as follows:

a. At both interim and primary analyses, each of the two populations will be treated separately, as if two independent studies were conducted.
b. The trial is event driven, that is both interim and primary analyses will be carried out after the prespecified numbers of events for each of the two stages and each of the two populations have been observed (see Section 10.3.1 on sample size determination).
c. The study sample size can be adjusted at any time, based on blinded data (i.e., overall number of events, overall number of dropouts, and proportion of SEROneg patients).
d. A log-rank test will be used for efficacy claims on the primary endpoint with the O'Brien Fleming α-spending rule to account for the multiplicity of testing at the interim and the primary analyses. At the primary analysis, the nominal stage-2 α-level of the log-rank test will be adjusted with the Bonferroni method to take into account the interim treatment selection (see next point). Details on the primary analysis are provided in Section 10.5.5.
e. In the interim analysis, in addition to the efficacy analysis conducted with the O'Brien Fleming approach and a hierarchical ordering of the tests for the doses (see above):

   e.1. The study can be closed for futility, with non-binding futility bounds, based on the Bayesian predictive probabilities computed for the primary endpoint and the key secondary endpoint. Details on this analysis are provided in Section 10.5.4.2.

   e.2. Bayesian predictive probabilities will be calculated, combining the primary and key secondary endpoints described in Section 4, for dose selection. Details on this computation are provided in Section 10.5.4.

f. In the interim and primary analyses, the key secondary endpoint will be tested only if the primary endpoint is statistically significant at the same α level which is used for the primary endpoint (hierarchical approach).
g. In case of discordant results between the two primary populations (ALL and SEROneg) for dose selection or for efficacy stopping, decisions will be made on a case-by-case basis considering study results (secondary endpoints and safety findings) as well as external elements, such as regulatory requirements, cost of goods, , need to define the risk benefit ratio for the general population, etc.

## 10.3 Sample size determination

The sample size has been computed with PASS 2020, v20.0.2 and nQuery v 8.0.0.0 software.

### 10.3.1 Primary analysis

The study is powered to assess interim decisions and final decision on the efficacy of the selected dose of MAD0004J08 in both pre-defined populations (ALL and SEROneg).

To account for the multiplicity of testing due to the presence of multiple populations, a simple Bonferroni adjustment is applied, i.e., each of the two populations is tested at the two-sided 0.025 α level.

There are two additional adjustments of the α level that are needed, the first to take into account the possibility to stop the study early for efficacy if at least one treatment is statistically superior to the control group and the second to select the treatment dose at the end of Stage-1.

The possibility to stop the study for futility does not have any impact on the overall type I error rate of the final efficacy decision and therefore on sample size.

The decision to stop the study early for efficacy will be based on a conventional group-sequential α-spending rule for the primary endpoint. An O'Brien-Fleming α-spending approach is used for this purpose and the interim analysis is planned when approximately 40% of the total expected events occur in each of the two populations. The O'Brien-Fleming method is conservative with respect to the interim decision; the choice of this method is justified by the fact that an early stop for efficacy can only be justified with clear-cut evidence from the primary endpoint.

As described in Section 10.2, the adjustment for the treatment selection will be based on the Bonferroni approach, which splits the Stage-2 α level into 2 equal parts (we have to split the α level provided by O'Brien-Fleming method for the final analysis).

In summary, the study-wise α level of 0.05 (two-sided) is split into 2 equal parts to take into account the two populations (ALL and SEROneg). Each 0.025 nominal α level (two-sided) is split into $\alpha_1$ = 0.00016 and $\alpha_2$ = 0.02494 (rounded to 0.024 for sample size computation) for the interim and primary analyses, respectively, according to the O'Brien-Fleming approach conducted when 40% of expected events have been observed; finally, the Stage-2 $\alpha_2$ level is split into 2 equal parts to take into account the dose selection that occurred at Stage-1. In conclusion, for the purpose of sample size computation, the nominal $\alpha_{final}$ level to conduct the primary efficacy analysis in each of the two primary populations has been set to 0.012 (two-sided).

Viral dynamics in SARS-CoV-2 infected patients are still to be fully determined. Based on recent papers [43-48], the median time to negative conversion of the nasopharyngeal swab for SARS-CoV-2 is expected to range between 10 and 12 days in the placebo group. For sample size computation, it is assumed to be 12 days.

In summary, the following assumptions are made:

- Median time from 1st SARS-COV-2 positive nasopharyngeal swab taken to 1st SARS-CoV-2 negative nasopharyngeal swab (referred to as "time to negative swab") in the placebo group: 12 days in both the ALL and the SEROneg populations
- Median time to negative swab in the selected dose of MAD0004J08 group of the ALL population: 9 days (*Hazard Ratio (HR)=1.33*)
- Median time to negative swab in the selected dose of MAD0004J08 group of the SEROneg population: 8 days (*HR=1.5*)
- Proportion of patients belonging to the SEROneg population: 50%
- Power: 80%
- $\alpha_{final}$ level: 0.012 (two-sided)

- Drop-out rate: 10% for the first 28 days (this includes any kind of data loss)
- Follow up time: 28 days.

Study follow up of participants is actually 6 months. However, since nasopharyngeal swab testing is frequent only up to Day 28, for the purpose of the analysis of the primary endpoint, follow-up time is considered 28 days.

With these assumptions, for conducting a log rank test for two treatment comparison on the primary endpoint, 336 patients are needed in each treatment group of the ALL population in the primary efficacy comparison with the selected dose vs. placebo. These patients account for approximately 168 patients in each treatment group of the SEROneg population, for whom a bigger treatment effect is assumed (see above).

Considering that the interim analysis is planned when 40% of the total number of events are observed, which roughly corresponds to 40% of the sample size, the study sample size is expected to be approximately as follows:

- 402 patients (134 per each of the initial three treatment arms) will be enrolled in Stage-1 with an allocation rule of 1:1:1.
- After dose selection, an additional 404 patients (i.e., 336-134=202 in the selected dose arm and 202 in the placebo arm) will be enrolled in Stage-2 with an allocation rule of 1:1.

Therefore, the estimated total number of patients for the study is 806.

As stated above, the sample size is driven by the target number of events which are expected to be 546 (259+287) in the primary analysis for the ALL population and 277 (129+148) in the same analysis for the SEROneg population.

If no treatment selection were planned, i.e., all three treatment arms were taken to the final analysis, the number of expected events in the final analysis would have been 833 (259+287+287) in the ALL population and 425 (129+148+148) in the SEROneg population and 40% of them accounts for 334 events in the former population and 170 in the latter.

In conclusion, the interim analysis is carried-out when 334 events are observed in the ALL population and 170 in the SEROneg population and the final analysis is carried-out when 546 and 277 events are observed in the two populations, respectively.

The criteria to claim efficacy at the interim analysis are very stringent: with a nominal $\alpha_1$ level of 0.0002, the study will have 80% power to detect a median difference between treatments of approximately 5.5 days (HR=1.85) in the ALL population and 7 days (HR=2.4) in the SERO-population.

Both targets (i.e., pre-defined number of events for the ALL and the SEROneg populations) must be reached to trigger the interim and the primary analyses, so the sample size indicated above is just an estimation.

The Sponsor may adjust the size of the study based on blinded review(s) of the total number of negative swabs for SARS-CoV-2 occurring during the study, the actual number of dropouts, and the proportion of SEROneg patients.

### 10.3.2 ADA analysis

Sample size for the ADA and MAD0004J08 concentration (60 randomized participants) was not based on statistical considerations.

### 10.3.3 Safety analysis

For the safety objective, with 134 subjects per group and 268 subjects when combining both active dose groups at the interim analysis and 336 subjects per group at the final analysis, the study will have the following probability of observing at least 1 AE for a given true rate of that AE:

***Table 10-1 Probability of Observing at least 1 AE for Given True Event Rates***

| Assumed True Event Rate of an AE | N=134 (Interim Analysis-Single Dose) | N=268 (Interim Analysis-Combined Doses) | N=336 (Primary and Final Analyses-Single Dose) |
|---|---|---|---|
| 0.1% | 0.13 | 0.24 | 0.29 |
| 0.2% | 0.24 | 0.42 | 0.49 |
| 0.4% | 0.42 | 0.66 | 0.74 |
| 0.6% | 0.55 | 0.80 | 0.87 |
| 0.8% | 0.66 | 0.88 | 0.93 |
| 1.0% | 0.74 | 0.93 | 0.97 |
| 5.0% | >0.99 | >0.99 | >0.99 |
| 10.0% | >0.99 | >0.99 | >0.99 |

These probabilities apply to both the primary safety endpoint (participants with severe and/or serious AEs) and to the secondary endpoints (participants with any AE).

### 10.3.4 Key secondary endpoint

Maintaining unchanged the main assumptions that have been made for the primary endpoint (i.e., power=80%, alpha level=0.012 (two-sided), drop-out rate≅10%), the treatment difference that a study with 336 participants (168 in the SEROneg population) per treatment arm would be able to detect on the key secondary endpoint at the primary analysis has been computed. The treatment difference between proportions of participants experiencing the composite endpoint depends on the expected proportion of participants experiencing such endpoint in the placebo arm. The results are illustrated in Table 10-2 below for placebo proportions ranging from 10% to 30%.

***Table 10-2 Detectable Treatment Differences on the Key Secondary Endpoint***

| Assumed True Proportion of Patients Experiencing the Composite Endpoint in Placebo Arm | Detectable Δ (*) | Proportion of Patients Experiencing the Composite Endpoint in Active Arm (**) |
|---|---|---|
| **ALL population** | | |
| 10% | 7.0% | 3.0% |
| 20% | 10.0% | 10.0% |
| 30% | 12.0% | 18.0% |
| **SEROneg population** | | |
| 10% | 9.5% | 0.5% |
| 20% | 14.0% | 6.0% |
| 30% | 17.0% | 13.0% |

(*) In a study with 336 enrolled participants (168 in the SEROneg population) per treatment group, assuming power=80%, 2-sided alpha=0.012, dropout rate≅10% and using a chi-squared test for two proportions with continuity correction.
(**) Derived from previous column.

## 10.4 Analysis sets

For analysis purposes, the following patient sets are defined for both ALL and SEROneg populations:

- *Full Analysis Set (FAS)*

All randomized participants who received the study treatment and provided baseline and at least 1 post baseline assessment of the primary endpoint. Subjects will be analyzed according to the treatment group to which they have been randomized.

- *Safety Analysis Set (SA set)*

All randomized participants who received the study treatment. Subjects are analyzed according to the treatment they actually received.

- *Per Protocol Analysis Set (PPAS)*

All subjects of the FAS who complied with the Inclusion/Exclusion criteria of the protocol, had no major protocol violations (to be defined before unblinding), and received study treatment as scheduled.

- *ADA and MAD0004J08 concentration analysis set (based on 60 participants)*

All randomized participants who received a dose of MAD0004J08 and have at least one post-treatment ADA result.

## 10.5 Statistical analysis of the primary and key secondary endpoint

### 10.5.1 General approach

All variables will be analyzed descriptively (N, N missing, mean, median, SD, Min, Max for continuous variables and observed counts and percentages for categorical variables) by treatment group and study visit, as available.

Additional statistical methodology, such as details on statistical models, treatment of missing data and adjustments for covariates, will be described in the SAP.

### 10.5.2 Analysis of the primary efficacy endpoint

The primary efficacy estimand, which is used for the analysis of the primary efficacy endpoint, will be based on the elements described in Table 10-3.

***Table 10-3*** **Primary Efficacy Estimand**

| |
|---|
| **Population:**<br>Full analysis set. Subjects will be analyzed according to the randomized treatment (intention to treat). |
| **Endpoint:**<br>Time to event, which is SARS-CoV-2 clearance from the URT.<br>A participant is considered having reached the event, i.e., SARS-CoV-2 clearance from the UTR, when SARS-CoV-2 measured by RT-PCR is undetectable in a nasopharyngeal swab, which is to be confirmed by a subsequent negative nasopharyngeal swab taken at least 24 hours after the 1st negative result. Otherwise, a participant is defined as not having reached the event.<br>Time to event is defined as the number of days from baseline (Day 1) to the day of the 1st negative nasopharyngeal swab. |
| **Intercurrent events**:<br>Potential intercurrent events may include: 1) withdrawal from the study prior to having met the criteria for the primary efficacy endpoint, 2) hospitalization, or 3) death. For withdrawals unrelated to study treatment, absence of data following withdrawal will be treated as censored observations at the time of exiting the study; COVID-19 or treatment-related withdrawals and deaths will be censored at Day 28 even if the event occurred before. All efforts will be made to ensure that hospitalized patients undergo regular nasopharyngeal swabs. If no swab is taken during and/or after hospitalization, these patients will be censored at Day 28. If the participant is still not cleared from SARS-CoV-2 on Day 28, the time to clearance will be censored at Day 28. |
| **Summary measure:** Data will be summarized in terms of difference in median time to SARS-CoV-2 clearance and hazard ratio. |

The null hypothesis for efficacy testing is $H_0$: $\log(Hazard\ Ratio) = 0$ and it is tested as a two-sided hypothesis at the pre-defined $\alpha_{final}$ level (see Section 10.5.5) or as a one-sided hypothesis at the predefined $\frac{\alpha_{final}}{2}$ level.

The statistical method for the primary analysis will be a time-to-event analysis using the stratified log-rank test, with the protocol defined stratification factor in 3 levels based on age and presence / absence of risk factors. Time to event curves will be displayed using the Kaplan-Meier method.

The stratified Cox proportional hazard regression model with treatment group as a fixed effect and adjusting for the protocol defined stratification factor will be used for sensitivity assessment.

As additional sensitivity analyses, due to anticipated heterogeneity in risk of disease progression, the following baseline variables will be included in the Cox proportional hazard regression model:

- Presence of risk factors for disease progression and hospitalization as described in Section 6.3.
- Severe obesity (body mass index [BMI] of 40 or higher).

– Presumed time since contracting the virus.

Other sensitivity analyses will be based on different strategies for treating the intercurrent events. These strategies will be described in the SAP. All analyses described above will be repeated on the PPAS for sensitivity purposes.

### 10.5.3 Analysis of the key secondary endpoint

The key secondary endpoint is the proportion of patients experiencing at least one of the events of the composite efficacy outcome at any time after dosing, which is defined as follows:

- $SpO_2 < 94\%$
- Newly established or increased dose home oxygen therapy (increased home oxygen therapy only applies to patients with underlying conditions other than COVID-19 requiring such therapy, e.g., COPD)
- Hospitalization
- Death.

This endpoint will be analyzed by means of a test for dichotomous variables (Cochran-Mantel-Haenszel test) stratified by the protocol defined stratification factor in 3 levels based on age and presence / absence of risk factors.

The α-level for testing will be the same used for the primary endpoint. This test will have confirmatory value only if the primary endpoint is statistically significant at the pre-defined α-level.

The stratified logistical regression model with treatment group as a fixed effect and adjusting for the protocol defined stratification factor will be used for sensitivity assessment.

### 10.5.4 Interim analysis and decision making

The interim analysis is performed when 40% of the total planned events for the primary endpoint are observed in the two primary populations, i.e., when at least 334 events are observed in the ALL population and at least 170 in the SERONeg population. Both targets must be reached to trigger the interim analysis.

As discussed in Section 10.1, interim decision making will encompass decisions on:

- Stopping trial for efficacy
- Stopping trial for futility
- Dose selection

These topics will be addressed in the following three Sections.

The assumption is that positive values of the test statistics reflect the superiority of the active treatment vs. placebo.

#### 10.5.4.1 Early Stop for efficacy

Early stop for efficacy is based on the conventional group-sequential O'Brien and Fleming α-spending rule for the primary endpoint. Then multiplicity of testing for the two doses is taken care of by using a hierarchical testing approach. Testing will start with the highest dose at the significance level $\alpha_1 =$

0.00016 (according to the O'Brien Fleming α-spending rule). The lowest dose will be tested at the same alpha level only if the first test is statistically significant.

Decision on study closure for efficacy will be mandatory only if statistically significant results are obtained in both dose groups, both primary populations and both primary and key secondary endpoints. In all other cases, the final decision on study closure for efficacy will be based on a wider evaluation of the study data and external considerations (see Section 10.2).

The key secondary endpoint will be submitted to statistical testing only if the test for the primary endpoint is statistically significant at the specified alpha level (hierarchical testing).

#### 10.5.4.2 Bayesian predictive power for futility

For the futility analysis purpose, both the primary and the key secondary endpoints will be considered.

In the interim analysis, the chance of study success, i.e., the chance of showing a statistically significant result on the primary endpoint in the primary analysis, will be measured via the Bayesian predictive probabilities for the primary and the key secondary endpoints that will be described in the next section (Section 10.5.4.3).

Denoting these probabilities with $Pr_{j,\ primary\ endpoint}$ and $Pr_{j,\ key\ secondary\ endpoint}$, the trial is to be stopped for futility if:

$$max_j\left(Pr_{j,primary\ endpoint}\right) < predefined\ threshold_{primary\ endpoint}$$

and

$$max_j\left(Pr_{j,key\ secondary\ endpoint}\right) < predefined\ threshold_{key\ secondary\ endpoint}$$

The pre-specified thresholds for futility are the same, i.e., 10% for both the maximum predictive probability (over the two doses) of the primary endpoint and the maximum predictive probability of the key secondary endpoint.

Both thresholds must be met in both primary populations (ALL and SEROneg) in order to close the study for futility.

If futility is shown only on one of the two populations, the study will continue.

#### 10.5.4.3 Bayesian predictive power for dose selection

At the interim analysis, the procedure described below is applied considering both the primary and the key secondary endpoints. Decision for dose selection only concerns the two doses of MAD0004J08; the placebo arm is continued to the end of the trial and is not subject to any interim decision. In order to guarantee type I error control against possible non-prespecified changes in the recruitment rate after the interim analysis, the method requires that only one dose is carried forward into Stage-2.

*Primary endpoint*

Let's indicate with $\theta_{ij}$ the log-hazard ratios between treatment dose $j$ ($j$=1,2) and the placebo arm at stage $i$ ($i$=1,2) for the primary endpoint.

In the Bayesian framework, the log-hazard ratios $\theta_{ij}$ are considered random variables with prior distributions and inference on treatment effect is usually based on the posterior distributions, which can be defined as the conditional distributions of the $\theta_{ij}$ given the observations from Stage-1 of the study.

In the present protocol, testing is conducted in a frequentist manner: the posterior distribution will be used only to compute the predictive distribution, which expresses the probability of obtaining future observations using all information acquired until the end of Stage-1. It is computed by combining mathematically the original distribution of the observations and the posterior distribution.

Dose selection will be based on the predictive distributions of the estimates $\hat{\theta}_{ij}$ of $\theta_{ij}$ that can be derived from the posterior distributions.

The decision about the dose to be continued in Stage-2 will be based on the Bayesian predictive distribution. The details for computing this distribution and the predictive power are provided in Appendix II-A.

*Key secondary endpoint*

For the key secondary endpoint, $p_j$ represents the true probability of experiencing a composite event in treatment dose *j* and $p_{placebo}$ the corresponding probability in the placebo group.

At Stage-1, for each of the two treatments *j*, we obtain the estimates $\hat{p}_{1j}$ and $\hat{p}_{1placebo}$ while at Stage-2, we obtain the estimates $\hat{p}_{2j}$ and $\hat{p}_{2placebo}$ and $\hat{p}_{(1+2)j}$ and $\hat{p}_{(1+2)placebo}$, where $\hat{p}_{(1+2)j}$is the weighted average of $\hat{p}_{1j}$and $\hat{p}_{2j}$ and $\hat{p}_{(1+2)placebo}$is the weighted average of $\hat{p}_{1placebo}$ and $\hat{p}_{2placebo}$ with weights that are proportional to the sample size of Stage-1 and Stage-2.

The decision about the dose to be continued in Stage-2 will be based on the Bayesian predictive distribution and power for dichotomous outcomes.

The details for computing this distribution and the predictive probability are provided in Appendix II-B based on the book by Berry et al [49] and the paper by Harari et al [50].

The described predictive probabilities, denoted by $Pr_{je}$, where *e=primary endpoint or key secondary endpoint*, will be computed at interim for both the primary and the key secondary endpoints and for both doses (i.e., four predictive probabilities will be computed; the predictive probabilities of the key secondary endpoint correspond to virtual tests of the key secondary endpoint, that in reality will be tested only if the primary endpoint has reached statistical significance).

To select the treatment dose to be continued in Stage-2, evidence from the primary and the secondary endpoints is combined via the utility function:

$$util_j = w_j \times Pr_{j\ primary\ endpoint} + (1 - w_j) \times Pr_{j\ key\ secondary\ endpoint}$$

where the weights $w_j$ are equal to 2/3, so that in the final decision for dose selection the primary endpoint will count for 2/3 and the key secondary endpoint will count for 1/3.

The dose having max utility will be indicated as the dose to be selected for Stage-2. However, as anticipated at the beginning of Section 10, other considerations, mainly related to safety results or to discordant results between the two primary populations, could drive to a different decision.

### 10.5.5 Final decision making

As described in Sections 10.5.4.1 and 10.5.4.3, at interim:

- $l_{11}$, $l_{12}$ (i.e., the statistics of the log-rank test for the two doses) are computed and testing starts with the highest dose (highest dose vs. placebo), followed by the test of the other dose (lowest dose vs. placebo) only if the former test is statistically significant.
- If the study is not closed for efficacy, futility is computed as described in Section 10.5.4.2 and, if the study is not closed for futility, the less performing treatment (less efficient or less safe) is dropped using the approach described in Section 10.5.4.3.

In the primary analysis, $l_{2s}$ (the regular statistic for the log-rank test computed for the selected dose) is computed. $H_0$ is rejected if $l_{2S} \geq \Phi^{-1}(1 - \frac{\alpha - \alpha_1}{2})$, where $\Phi^{-1}(.)$ is the normal quintile, α=0.025 (because of the two primary populations), and $\alpha_1$ is the α-level spent at the interim analysis. Thus, the "stage-2 alpha" is split by 2 according to the Bonferroni method.

The Bonferroni approach guarantees control of the type I error rate without the requirement that $l_{2s}$ be stochastically independent of $l_{12}$ and $l_{12}$.

### 10.5.6 Estimation issues

It is well known that selecting promising treatment(s) based on the observed data leads to overestimation of the treatment effect in the selected treatment arm(s) and to an underestimation of the treatment effect in the dropped arm(s). However, each additional event observed after the interim analysis reduces the conditional bias, and the bias at the primary and final analyses is smaller than at the interim analysis.

Bruckner et al. [51] have shown that the bias of the maximum likelihood estimator (MLE) of the log-hazard ratio in the Cox proportional hazards model is small when the number of treatments is <6 and decreases when the number of observed events increases.

Since in the present study there are only two treatment arms in addition to the placebo arm and the number of expected events is high (approximately 84% and 86% of subjects are expected to reach the event within 28 days in the ALL and SEROneg populations, respectively), the primary estimate of the treatment effect will be based on uncorrected estimators (such as the MLE in the Cox regression model).

As a sensitivity analysis, the treatment effect in terms of hazard ratio will be computed using the shrinkage estimator of the scaled log-hazard ratios (LR shrinkage estimator), since this has been proven to reduce both bias and mean squared error [51]. This estimator tends to overcorrect the bias and so it will be used as a conservative estimator of the treatment effect, knowing that it underestimates the true effect.

### 10.5.7 Analysis of the other secondary efficacy endpoints

All secondary analyses will be performed in the FAS and the PPAS. All secondary endpoints will be tested at the 5% significance level, without adjustment for multiplicity. These tests will not have a confirmatory meaning. 95% CIs will be computed for each secondary endpoint.

SARS-CoV-2 viral load (number of copies) in nasopharyngeal swab, as measured by RT-PCR, will be log transformed. A mixed model for repeated measures (MMRM) will be used with fixed terms for study visit, treatment, interaction between visit and treatment, protocol defined stratification factor and the baseline viral load as continuous covariate [52]. Details on the model will be given in the SAP. Geometric means over time with their 95% confidence intervals will be estimated.

Spearman's rank correlation coefficient will be used to assess correlations between viral load and clinical variables at each study visit.

For the COVID-19 related symptoms (see Section 9.5.21), the total symptom score will be computed for each subject at each visit as the sum of the symptoms excluding the last two (range: 0-24). The area under the total symptom score curve (AUC) assessed through Day 28 via the linear trapezoidal method will be analyzed by means of an ANOVA model, with terms for treatment and protocol defined stratification factor or by a non-parametric equivalent test stratified by the protocol defined stratification factor, depending on the actual distribution of this variable. Evaluations of the questionnaire for the remaining visits and responses to the remaining two questions (loss of appetite, and changes in taste and smell) will be summarized by treatment.

The lowest $SpO_2$ % value observed from baseline to Day 28 will be analyzed by means of an ANCOVA model, with terms for treatment and protocol defined stratification factor and the $SpO_2$ % value at baseline as the covariate. $SpO_2$ % values at visits after Day 28 will be summarized by treatment.

Proportions of patients needing hospitalization (defined as ≥ 24 hours of hospital and/or emergency room care), oxygen therapy, or admission to ICU will be compared among treatment groups by using logistic regression stratified by the protocol defined stratification factor.

The proportion of subjects with $SpO_2$ % < 94% and the proportions of patients with SARS-CoV-2 clearance in the URT will be compared with the same logistic regression model.

Duration (number of days) of hospitalization, oxygen therapy, ICU stay will be modelled using Poisson regression stratified by the protocol defined stratification factor with an offset for number of days of observation.

Proportion of deaths will be descriptively analyzed. Full details of all secondary analyses will be in the SAP.

### 10.5.8 Safety and reactogenicity analyses

#### 10.5.8.1 Unsolicited Adverse Events

AEs will be categorized according to the Medical Dictionary for Regulatory Activities (MedDRA) terms.

All safety analyses will be performed in the SA set. Intercurrent events will be disregarded. Missing safety data will not be imputed, with the exception of missing dates, which will be imputed according to rules described in the SAP. No other missing information will be imputed in the safety analysis.

Adverse events happening between signature of informed consent and randomization will be only listed.

The primary safety endpoint is the proportion of participants reporting severe (Grade 3) and/or serious unsolicited AEs. Treatment comparison on this proportion will be primarily based on the Clopper-Pearson 95% CI.

Standard statistical analyses will be carried out on unsolicited treatment-emergent AEs (TEAEs), SAEs, severe TEAEs, and related (as perceived by the Investigator) TEAEs/SAEs.

Descriptive summary statistics will include counts and percentages of participants with the indicated endpoint and the associated Clopper-Pearson 95% CIs.

#### 10.5.8.2 Local solicited Adverse Events (Reactogenicity)

Three kinds of AEs at each injection site (i.e., pain, redness, and swelling) will be recorded and graded by severity through Day 28 as reported in Section 9.5.10.

The worst (highest) grade for each solicited event at each time point will be used for the analysis.

Standard statistical analyses will be carried out on solicited AEs, severe solicited AEs, and serious solicited AEs. All solicited AEs are considered related.

#### 10.5.8.3 Other safety measurements

Safety parameters that will be assessed include, but are not limited to, safety laboratory parameters, ECGs, and vital signs at all relevant study visits. These will be listed and summarized using standard descriptive statistics. Since local laboratories will be used, data will be standardized to SI units for the statistical analysis. Additional analysis will be performed if warranted upon review of the data.

#### 10.5.8.4 Anti-Drug Antibodies (ADA) and MAD0004J08 concentration

Treatment-emergent anti-drug antibodies (TEADA) will be assessed and analyzed in the ADA analysis set (first 60 randomized participants).

Treatment-emergent ADAs are defined as participants:

- with a ≥2-fold (1 dilution) increase in titer compared with the minimum required dilution if no ADAs were detected at baseline (treatment-induced ADA)

or

- with a ≥4-fold (2 dilutions) increase in titer compared with baseline if ADAs were detected at baseline (treatment-boosted ADA).

The frequency and percentage of participants with preexisting ADA and who are TEADA positive to MAD0004J08 will be tabulated. The distribution of titers and frequency of neutralizing antibodies (if assessed) for the TEADA positive participants will also be tabulated. The relationship between the

presence of antibodies, MAD0004J08 concentration and efficacy response or safety data will be assessed. Additional details will be provided in the SAP.

### 10.5.9 Baseline descriptive analysis

Demographic characteristics as well as other relevant baseline characteristics (e.g., presence of comorbidities, presumed time since contracting the virus) will be tabulated overall, at treatment group level and by each of the three protocol defined strata.

### 10.5.10 Exploratory analyses

The exploratory objectives anticipated in the protocol are to assess the new Diesse4-Covid19 test to detect serum SARS-CoV-2 IgG, and the neutralizing power of MAD004J08 vs. SARS-COv-2 strains. The endpoints related to these objectives, as well as other exploratory endpoints and the respective statistical analyses of the data collected in patients who gave their informed consent, will be described in separate documents.

Exploratory analyses may not be reported in the CSR.

### 10.5.11 Subgroup analyses

This study is not powered for subgroup analyses; therefore, all subgroup analyses will be treated as exploratory. Subgroup analyses will be conducted for the primary endpoint on the FAS and PPAS.

Subgroups may include:

- Protocol defined strata
- Presumed time since contracting the virus
- Baseline severity of COVID-19
- High risk status at baseline for COVID-19 progression and hospitalization.

Treatment group differences will be evaluated within each category of the subgroup regardless of whether the interaction is statistically significant. Definitions for the levels of the subgroup variables, the analysis methodology, and any additional subgroup analyses will be defined in the SAP.

# 11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

## 11.1 Regulatory/Ethical and study oversight considerations

### 11.1.1 Informed Consent process

Written informed consent will be taken prior to conducting any study-related procedures.

Participants must be informed that their participation is voluntary, i.e., they are free to refuse to participate and may withdraw their consent at any time and for any reason during the study and the storage period of biological samples.

The investigator or authorized designee will explain the study to the participant or his/her legally authorized representative and answer all questions regarding the study. The investigator or

authorized designee will conduct the consent discussions on an individual basis with each participant. Adequate time will be allowed for all questions to be addressed.

Participants will be required to sign a statement of informed consent that meets the requirements of national and local regulations, ICH guidelines, and the regulations of the EC(s) as applicable. The informed consent will be taken by the use of a written consent form (ICF) approved by the EC.

Other consents may be presented to the participant which are optional and if not signed, would not exclude the participant from the study:

- Consent to collect, store and test samples for research not described in the protocol.
- Consent to allow any remaining specimens originally taken for mandatory testing to be used for research not described in the protocol.
- Consent to genetic testing

Sample testing will be in line with the consent of the participant.

The medical record and/or CRF must include a statement that written informed consent was taken before the participant was enrolled in the study and the date the written consent was taken. The authorized person obtaining the informed consent must also sign the ICF.

If there is a change to the ICF during the conduct of the study, participants must be re-consented to the most current version of the ICF.

Any withdrawal of consent for sample testing will be documented in the eCRF.

The original ICF will be kept on file at the study center. A copy of the ICF must be provided to the participant or the participant's legally authorized representative prior to conducting any study-related procedures.

If participants are asked to consent to optional exploratory research using additional samples and/or the remainder of mandatory samples, a separate consent will include text that addresses the use of additional samples and/or remaining mandatory samples for optional exploratory research. Refusal to consent to exploratory research does not preclude consenting to participate in the study.

In accordance with the European Medicines Agency (EMA) "Guidance on the management of clinical trials during the covid-19 (coronavirus) pandemic version 4 04/02/2021", the following specific aspects of the consent procedure will be taken into account:

- If written consent by the trial participant is not possible (for example because of physical isolation due to COVID-19 infection), consent may be given orally by the trial participant (Art 2(j) of Directive 2001/20/EC) in the presence of an impartial witness. In such cases, the witness is required to sign and date the informed consent form and the Investigator is expected to record how the impartial witness was selected.
- In addition, it could be considered that the trial participant and the person obtaining consent sign and date separate informed consent forms.

In either case, all relevant records should be archived in the Investigator's site master file. A correctly signed and dated informed consent form should be taken from the trial participant later, as soon as possible.

### 11.1.2 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, and the Sponsor. The study will comply with GDPR 2016/679 requirements. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the EC and Regulatory Agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing EC, Institutional policies, Sponsor requirements and local regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the CRO working on behalf of the Sponsor. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. Only the study center will be able to link the study ID number to the patient's identity. The study data entry and study management systems used by clinical sites and by the CRO research staff will be secured and password protected.

### 11.1.3 Future use of stored specimens

If the participant agrees by signing the dedicated ICF, 16 mL of venous blood (one 7 mL sample and one 9 mL sample) will be drawn at Visit 1, Visit 4, and Visit 10 and stored under the supervision of the Sponsor. These specimens as well as any specimen left over from mandatory testing may be used for exploratory endpoints and for future research. The identity of the participant will never be disclosed.

### 11.1.4 Study oversight

The study Sponsor, the EC(s), the institution(s) through which the research is performed, and all members of the Principal Investigator's clinical team and the national regulatory authority share responsibility for ensuring the safety of participants in this trial.

The Principal Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the EC(s) in accordance with the requirements, policies, and procedures established by the EC(s).
- Notifying the EC(s) of SAEs or other significant safety findings as required by EC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of ICH and GCP guidelines, regulations of the EC(s), and all other applicable country and local regulations.

- Closely monitoring study participants and taking whatever measures necessary to ensure their safety. The Principal Investigator may delay an individual's study treatment administration or pause study treatment administration altogether if he/she is concerned that the study drug might place a participant or participants at significant risk.
- Determining seriousness, severity and causality with respect to the study drug for each AE.

Where specified, the responsibilities of the Principal Investigator may be delegated to other medically qualified Investigators (designees).

The Sponsor has an institutional responsibility to ensure participant safety and is ultimately accountable for safety oversight. Local medical monitors and the DMC play an important role in this regard and support the Sponsor.

The local medical monitor is the Sponsor's representative and is a physician in his/her country of residence. The local medical monitor:

- Reviews the safety of the product in a specific region and, in conjunction with the Sponsor, determines expectedness of AEs.
- Is responsible for safety oversight in-country and plays an important role in the reporting of SAEs and pregnancies, as described in the protocol.
- In consultation with the CRO/Sponsor, may assess the severity and causality for AEs and may upgrade the degree of severity and causality determined by the Principal Investigator or designee

The EC has institutional responsibility for the safety of research participants. The EC has the authority to terminate, suspend or require changes to a clinical trial.

The national regulatory authority, AIFA, has the authority to terminate, suspend or require changes to a clinical trial.

#### 11.1.4.1 Data Monitoring Committee (DMC)

The Sponsor will form a DMC, whose primary goals will be as follows:

- preserve the safety of study participants.
- evaluate results of the interim analysis conducted at the end of Stage-1 and based on predefined criteria make one of the following recommendations regarding the continuation of the study:
    - Stop for futility.
    - Stop for efficacy.
    - Select low or high dose for continuation to Stage-2.

The DMC will be provided unblinded results, which will be generated by statistician(s) not otherwise involved in the study.

The stopping rules for futility and efficacy and the criteria for dose selection are specified in the protocol and will be further detailed in the SAP, which will be released before the database for the interim analysis is locked.

Information that may unblind the study during the analyses will not be reported to study sites or the blinded study team until the study has been completed.

The DMC will consist of members external to the study and to the Sponsor.

The DMC members will not have data entry/validation responsibilities or direct contact with the site(s) or testing facilities.

Details on the DMC composition, responsibilities, frequency of meetings, and procedures will be included in the DMC Charter.

#### 11.1.4.2 Steering Committee

A Steering Committee (SC) will be appointed by the sponsor and may include investigators, clinical experts not directly involved in the clinical trial and staff from the Sponsor. The SC will oversee the scientific integrity of the trial, the scientific validity of the study protocol, assessment of study quality and conduct as well as the scientific quality of the final study report.

A predefined subgroup of the SC composed of senior members not otherwise involved in the study will be responsible for final decisions on stopping for futility or efficacy or dose selection. The decision will take into account the recommendations from the DMC. This subgroup will receive a summary of unblinded results. A list of the unblinded Sponsor representatives will be kept and pre-specified procedures for ensuring study blinding will be followed.

The details of the SC and its role will be defined in the SC charter.

#### 11.1.4.3 Clinical monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is compliant with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP E5), and with applicable regulatory requirements.

The Investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information. All information on the eCRFs must be traceable to these source documents in the patient's file. The Investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).

Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and of data that will be used for all primary variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific Monitoring Plan (MP). No information in source documents about the identity of the patients will be disclosed.

In accordance with the EMA's "Guidance on the management of clinical trials during the COVID-19 (coronavirus) pandemic version 4 04/02/2021", a risk-based approach to monitoring will be implemented focusing on processes that are critical to ensure the rights, safety and well-being of trial participants and the integrity of the trial (and trial data), as well as the extra burden that introduction of these processes may put on site staff and facilities. The MP will be drafted in accordance with these considerations in order to strike an acceptable balance between appropriate oversight and the capacity of the trial site. Critical data for which source data verification (SDV) needs to be performed will be

identified in the MP. Results of adjusted monitoring/review measures and their impact will be reported to the Sponsor in monitoring reports and in the clinical study report, where applicable.

Adjusting monitoring activities may include a combination of the following:

a) On-site monitoring

Cancelling or postponing of on-site monitoring visits and extending of the period between monitoring visits are likely to be necessary.

To the extent on-site monitoring remains feasible, it should take into account national, local and/or organizational social distancing restrictions, the urgency (e.g., source data verification can often be postponed) and the availability of site staff and should only be performed as agreed with trial sites.

Additional measures regarding on-site monitoring may include limited, targeted on-site monitoring identifying higher risk clinical sites, if not already applicable for the trials of concern.

The on-site monitoring plan will need to be adapted and alternative measures (like those outlined in b), c) and d) below) put in place or relied on to a greater extent if already present.

b) Centralized monitoring and central review of data collected Centralized monitoring is an established method under ICH GCP E6. 5.18.3 (Addendum). In the context of the pandemic, the role of centralized monitoring has an increasing importance. Centralized monitoring of data acquired by electronic data capture systems (e.g., eCRFs, central laboratory or ECG / imaging data, ePROs etc.) that are in place or could be put in place provides additional monitoring capabilities that can supplement and temporarily replace on-site monitoring through a remote evaluation of ongoing and/or cumulative data collected from trial sites, in a timely manner.

c) Off-site monitoring Additional off-site monitoring activities could include phone calls, video visits, e-mails or other online tools in order to discuss the trial with the investigator and site staff. These activities could be used to get information on the clinical trial progress, to exchange information on the resolution of problems, review of procedures, trial participant status as well as to facilitate remote site selection and investigator training for critical trials.

d) Remote source data verification In addition to the above mentioned, established methods (a-c), and taking into account the continuing nature of the COVID-19 pandemic and the need to ensure the quality of clinical trial data and to protect the rights, safety and well-being of the participants in the EU/EEA, remote source data verification (rSDV) can be justified in clinical trials. Remote SDV can be considered only during the COVID-19 pandemic related public health crisis and when in line with EU and national law (or temporary national emergency measures)12 . Remote SDV may be considered for trials:

- involving COVID-19 treatment or prevention;
- investigating serious or life-threatening conditions;
- where the absence of SDV for critical data may likely pose unacceptable risks to participants' safety or the reliability/integrity of trial results;

- involving particularly vulnerable participants such as children or those temporarily (e.g. trials in emergency situations) or permanently (e.g. trials in patients with advanced dementia) incapable of giving their informed consent or
- in pivotal trials.

Remote SDV should focus on the quality control of critical data such as primary efficacy data, important safety data. Important secondary efficacy data may be monitored simultaneously, provided this does not result in a need to access additional documents and therefore in an increased burden for trial site staff. The sponsor will determine the extent and nature of remote SDV that they consider needed for each trial under this exceptional situation and will carefully weigh it against the extra burden that introduction of any alternative measures would put on site staff and facilities.

In the case of these trials, principal investigators should make their own determination as to whether or not the situation at their clinical site allows any of the following options for remote SDV:

- Sharing pseudo-anonymized copies of trial related source documents with the monitor; this may be done electronically where manageable by the site staff;
- Direct, suitably controlled remote access to trial participants' electronic medical records;
- Video review of medical records with clinical site team support, without sending any copy to the monitor and without the monitor recording images during the review. For COVID-19 trials starting now, when remote SDV is foreseen, it should be described in the initial protocol application (and informed consent form). In case of ongoing trials, introduction of remote source data verification should be submitted, in line with national law or temporary national emergency measures, via a substantial amendment. These provisions should be in line with the principles of necessity and proportionality and in a way that protects trial participants' rights and should not place any disproportionate burden on site staff as determined by the investigator and trial site staff.

Remote SDV can be carried out only in agreement with the investigators who should not be put under undue pressure to accept remote SDV and should always give priority to the care to be given to trial participants and other patients.

Remote SDV should not be carried out if adequate data protection, including data security and protection of personal data even if pseudonymised, is not ensured.

### 11.1.5 Quality Assurance and Quality Control

Following written Standard Operating Procedures, monitors will verify that the clinical trial is conducted, and data generated, documented (recorded), and reported in compliance with the protocol, ICH GCP E6 and applicable regulatory requirements.

Given the current situation, on-site audits may, in general, be avoided or postponed. Audits should only be conducted if permitted under national, local and/or organizational social distancing restrictions. On-site audits as well as remote audits can be considered, after agreement with the Investigator and if the audits are assessed as essential, e.g., triggered audits with the purpose of investigating serious deviations from the trial protocol or from the applicable legislation.

### 11.1.6 Data handling and record keeping

#### 11.1.6.1 Data collection and management responsibilities

**Data collection**

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Designated Investigator staff will enter the data required by the protocol into the eCRF using fully validated software that conforms to 21 CFR Part 11 requirements. Designated Investigator site staff will not be given access to the electronic data capture system until they are trained.

Web-based software will be used, and no installation procedure is needed. Each site will be authorized by the administrator to access the eCRF. Each site-qualified personnel will be allowed to access the eCRF by means of a 'login mask' requiring user ID and password and may read, modify, and update only the information entered at his or her site and according to their profile. Each page reports site code and patient code.

On-line validation programs will check for data discrepancies and generate automatic warning messages, allowing the Investigator to confirm or correct the data entered. The Investigator will certify that the data entered in the eCRF are complete and accurate.

After database lock, the Investigator will receive a CD-ROM of patient data for archiving at the investigational site.

**Database management and quality control**

The CRO working on behalf of the Sponsor will review the data entered in the eCRF by investigational staff for completeness and accuracy and instruct site personnel to make any necessary corrections or additions. The data manager will perform the cleaning session by reviewing the warning messages raised by on-line checks and by running post-entry checks by means of validation programs and data listings specific for the study. The occurrence of any protocol deviations will also be evaluated.

If clarifications are needed, the data manager will raise queries through the web application. Designated Investigator site staff will be required to respond to queries and to make the relevant corrections, if needed.

Data collection and query flows, as well as the on-line and off-line checks, are detailed in the Data Management Plan and Data Validation documents.

Concomitant and prior medications entered in the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical (ATC) classification system. Medical history/current medical conditions and AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA).

The database will be locked after all the above actions have been completed and the database has been declared complete and accurate.

#### 11.1.6.2 Study records retention

The Investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (ICH E6 Section 8) and as required by applicable regulations and/or guidelines. The Investigator/institution should take measures to prevent accidental or premature destruction of these documents.

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

### 11.1.7 Protocol deviations

A protocol deviation is any noncompliance with the clinical study protocol or GCP requirements. The noncompliance may be on the part of the subject, the Investigator, or the study site staff. Corrective actions are to be developed by the site and implemented promptly following protocol deviations.

It is the responsibility of the site to prevent any protocol deviations and of the site staff and monitors to use continuous vigilance to identify and report deviations. All deviations must be addressed in study source documents.

The COVID-19 situation is likely to introduce more protocol deviations than normal. The Sponsor will manage such protocol deviations in accordance with standard procedures. The Sponsor will perform an analysis of the number and type of deviations periodically to assess whether a protocol amendment or other modifications are needed.

### 11.1.8 Insurance

The Sponsor certifies that it has taken out a liability insurance policy covering this clinical trial. This insurance policy is in accordance with local laws and requirements. The insurance of the Sponsor does not relieve the Investigator and the collaborators from any obligation to maintain their own liability insurance policy. An insurance certificate will be provided to the EC and/or regulatory authorities.

### 11.1.9 Publication and data sharing policy

Study information from this protocol will be posted on publicly available clinical trials registers including clinicaltrials.gov, EudraCT, AIFA's Osservatorio Nazionale sulla Sperimentazione Clinica dei Medicinali (OsSC) before enrolment of participants begins and updated in line with the requirements of each register.

The final study report will include all available safety data, clinical assessments, and concomitant medications through the final study visit.

Interim study reports may be generated for the Emergency Use Authorization (EUA) dossier in agreement with regulators.

The database will be locked prior to unmasking and preparation of the interim and final study reports when all of the relevant data have been entered, reviewed, and all queries related to the data have been addressed.

Modifications or additions to the analyses will be included in the relevant statistical analysis plan.

Any decisions to deviate from the planned analyses described in the protocol and in the statistical analysis plan will be described in detail in the final study report.

The interim and final clinical study report will be reviewed and approved by the Sponsor signatory and the Principal Investigator.

Summaries of the results of the study will also be posted on the relevant websites.

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

### 11.1.10 Conflict of interest policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

### 11.1.11 Financial disclosure

Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study.

### 11.1.12 Protocol Amendments

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor and EC. Only amendments that are required for patient safety may be implemented prior to EC approval.

## 11.2 Study and center start and closure

### 11.2.1 Study start

The study start date is the date on which the first trial center opens for recruitment of participants.

### 11.2.2 Study center closure

The sponsor reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the sponsor. Study centers will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause, and enough notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the EC or local health authorities, the sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the investigator.
- Discontinuation of further study intervention development.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the Investigators, EC(s), and regulatory authorities of the reason for termination or suspension, as specified by applicable regulatory requirements. The Investigator shall promptly inform the participants and assure appropriate participant therapy and/or follow-up.

## 11.3 Protocol Amendment history

No protocol amendment has been made as of the release date of this protocol.

# 12 References

1. NIH Overview of COVID-19: Epidemiology, Clinical Presentation, and Transmission https://files.covid19treatmentguidelines.nih.gov/guidelines/section/section_9.pdf. Last updated : July 17th, 2020.
2. Walsh KA et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020; 81:357-371.
3. Yan X et al. Duration of SARS-CoV-2 viral RNA in asymptomatic carriers. Critical Care 2020; 24:245-247.
4. Andreano E et al. Human monoclonal antibodies for discovery, therapy, and vaccine acceleration. Curr Opin Immunol 2019; 59:130-134.
5. Griffin MP et al. Single-Dose Nirsevimab for Prevention of RSV in Preterm Infants. N Engl J Med 2020; 383:415-425.
6. Wang CY et al. Effect of Anti-CD4 Antibody UB-421 on HIV-1 Rebound after Treatment Interruption. N Engl J Med 2019; 380:1535-1545.
7. Sok D et al. HIV Broadly Neutralizing Antibodies: Taking Good Care of the 98. Immunity 2016; 45:958-960.
8. Gaudinski MR et al. Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody mAb114 targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study. Lancet 2019; 393:889-898.
9. Jiang S et al. Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses. Trends Immunol 2020; 41:355-359.
10. Baum A et al. REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. Science 10.1126/science.abe2402 (2020).
11. Chen P et al. SARS-CoV-2 neutralizing antibody LY-Co555 in outpatients with COVID-19. New Eng J Med October 28 2020, 2020 DOI: 10.1056/NEJMoa2029849.
12. Weinreich DM, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with COVID-19, New Eng J Med December 17 2020, DOI:10./NEJMoa2035002.
13. Bamlanivimab EUA Letter of Authorization, November 10, 2020 https://www.fda.gov/media/143602/download.
14. Casirivimab + Indeviimab EUA Letter of Authorization, November 21, 2020 https://www.fda.gov/media/143891/download .
15. Andreano E et al. Extremely potent human monoclonal antibodies from convalescent COVID-19 patients. bioRxiv preprint doi: https://doi.org/10.1101/2020.10.07.328302. Posted October 2020, not peered reviewed.
16. Korber B, er al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell 2020:S0092-8674(0020)30820-30825.
17. Bastard P, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020, 370.
18. Zuo Y, et al.: Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med 2020.
19. Zalevsky et al. Enhanced antibody half-life improves in vivo activity. Nature biotechnology 2010, 28:157-159.

20. Schlothauer et al. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. Protein Eng Des Sel 2016, 29:457-466.
21. Lee WS, et al. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat Microbiol 2020, 5:1185-1191.
22. Ackerman ME, et al. Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcγR2a and FcγR2b. Journal of virology 2013, 87:5468-5476.
23. Mackness BC, et al. Antibody Fc engineering for enhanced neonatal Fc receptor binding and prolonged circulation half-life. mAbs 2019, 11:1276-1288.
24. Butler AL, Fallon JK, Alter G: A Sample-Sparing Multiplexed ADCP Assay. 2019, 10.
25. Ackerman ME, et al. Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. PLoS pathogens 2016, 12:e1005315-e1005315.
26. Karsten CB, et al. A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis. Journal of immunological methods 2019, 471:46-56.
27. Baum A, et al. REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. Science 2020.
28. Imai M, et al.: Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. 2020, 117:16587-16595.
29. Rogers TF, et al.: Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. 2020, 369:956-963.
30. Sia SF, et al.: Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 2020, 583:834-838.
31. Singh S et al. Monoclonal antibodies – a review. Curr Clin 2018; 13:85-99 Pharmacol Monoclonal antibodies.
32. Meyler's Side Effects of Drugs (16th edition) 2016; 1100-1102.
33. Arvin AM et al. A perspective on potential antibody-dependent enhancement of SARS-Cov-2. Nature 2020:584; 353-363.
34. ACTIV-3/TICO LY-CoV555 Study Group. A neutralizing monoclonal antibody for hospitalized patients with COVID-18. New Engl J Med Dec 22 2020, DOI: 10.1056/NEJMoa2033130.
35. Post N et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. Pre-print MedRsViv doi: https://doi.org/10.1101/2020.08.25.2017880.
36. Hopkins U et al. Large-voIume IM injections: a review of best practices. Oncology Nurse Advisor Jan-Feb 2013:32-37.
37. Owens R. Clinical experience with fulvestrant (Falsodex): a nurse perspective. Eur J Oncol Nurs 2004; 8 (Suppl): S89 – S94.Zimmermann PG. Revisiting IM injections. Am J Nurs 2010; 110: 60-61.
38. Faslodex US FDA Package Insert, May 20th, 2020.
39. NIH Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/ (downloaded on 19 Oct 2020).
40. US Food and Drug Administration. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Rockville, MD: Center for Biologics Evaluation and Research; September 2007 https://www.fda.gov/regulatory-

information/search-fda-guidance-documents/covid-19-developing-drugs-and-biological-products-treatment-or-prevention).

41. Division of Acquired Immunodeficiency Syndrome (DAIDS) AE Grading Table, corrected Version 2.1, July 2017 https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf.
42. Di Scala L and Glimm E. Time to event analysis with treatment arm selection at interim. Statist. Med. 2011, 30: 3067-3081.
43. Zou L et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *NEJM*, 382(12), March 2020.
44. He X et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. MedRxiv preprint doi: https://doi.org/10.1101/2020.03.15.20036707.
45. Wolfel R et al. Virological assessment of hospitalized cases of coronavirus disease 2019. MedRxiv preprint doi: https://doi.org/10.1101/2020.03.05.20030502.
46. Liu Y et al. Viral dynamics in mild and severe cases of COVID-19. Lancet online Mar 2020 https://doi.org/10.1016/ S1473-3099(20)30232-2.
47. Gautre P et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, *International Journal of Antimicrobial Agents* (2020), doi: https://doi.org/10.1016/j.ijantimicag.2020.105949.
48. Hung IFN et al. Triple combination of interferon beta-1b, lopinavir– ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet Published online May 8, 2020.
49. Berry S et al. Bayesian Adaptive Methods for Clinical Trials. New York: Chapman & Hall, 2010.
50. Harari O et al. Utilizing Bayesian predictive power in clinical trial design. Pharmaceutical Statistics 2020;1-16. Httsps://doi.org/10.1002/pst.2073.
51. Brückner M, Titman A, and Jaki T. Estimation in multi-arm two-stage trials with treatment selection and time-to-event endpoint. Statist. Med. 2017, 36: 3137–3153.
52. Rose CE et al. A. Comparison of Methods for Analyzing Viral Load Data in Studies of HIV Patients. PLoS ONE 2015; 10(6): e0130090. doi:10.1371/journal.pone.0130090.
53. Gottlieb RL et a. Effect of bamlanivimab as monotheraly or in combination with etesevimab on viral load in patients with mild to moderate COVID-19. JAMA 2021
54. Conditions of use casirivimab and imdevimab (REGN-COV2) https://www.ema.europa.eu/en/documents/referral/regn-cov2-antibody-combination-casirivimab/imdevimab-covid-19-conditions-use-conditions-distribution-patients-targeted-conditions-safety_en.pdf
55. Parere CTS AIFA su anticorpi monoclonali (04 Feb 2021). https://www.aifa.gov.it/documents/20142/1289678/parere_cts_monoclonali_04.02.2021.pdf/68737075-6f07-2a43-7f94-0bc55f2e38f1
56. EMA issues advice on use of REGN-COV2 antibody combination (casirivimab / imdevimab) European Medicines Agency https://login.microsoftonline.com/organizations/oauth2/v2.0/authorize?response_type=id_token&scope=openid%20profile&client_id=5e3ce6c0-2b1f-4285-8d4b-75ee78787346&redirect_uri=https%3A%2F%2Fteams.microsoft.com%2Fgo&state=eyJpZCI6IjA0NjhlMTU4LTVhZTAtNGUzZS1hNDg1LTFkNTFmNjhmNDQ1OCIsInRzIjoxNjA4MDQxODQ0LCJtZXRob2QiOiJyZWRpcmVjdEludGVyYWN0aW9uIn0%3D&nonce=ef713a9a-7a22-426b-bf6d-8c42dafe17af&client_info=1&x-client-SKU=MSAL.JS&x-client-Ver=1.3.4&prompt=select_account&safe_rollout=apply%3A8a2fcdf7-d1dd-4a19-8929-

a91bb4fef2e3&client-request-id=ce294b1f-2513-4e71-86de-2296361a68fd&response_mode=fragment

57. European Medicines Agency. Conditions of use, conditions for distribution and patients targeted and conditions for safety monitoring addressed to member states for unauthorized product available for use; casirivimab and indevimab (REG-COV2). https://www.ema.europa.eu/en/documents/referral/regn-cov2-antibody-combination-casirivimab/imdevimab-covid19-article-53-procedure-conditions-use-conditions-distribution-patients-targeted_en.pdf

# 13 Appendices

## 13.1 APPENDIX I. Contraceptive Guidance

### 13.1.1 Women

#### 13.1.1.1 Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

#### 13.1.1.2 Woman Not of Childbearing Potential (WOCBP)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with either
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy, or
    c. Documented bilateral oophorectomy.

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

Note: Determination can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female is defined as women with:
    a. 12 months of amenorrhea for women >55, with no need for FSH
    b. 12 months of amenorrhea for women >40 years old with FSH ≥40 mIU/mL and no other medical condition such as anorexia nervosa and not taking medications during the amenorrhea (e.g., oral contraceptives, hormones, gonadotropin releasing hormone, anti-estrogens, selective estrogen receptor modulators (SERMs), or chemotherapy that induced amenorrhea)

#### 13.1.1.3 Participation in the study

Women of childbearing potential may participate in this study.

Women of childbearing potential must test negative for pregnancy prior to initiation of treatment as indicated by a negative urine pregnancy test at the screening visit.

Women of childbearing potential who are completely abstinent or in a same sex relationship, as part of their preferred and usual lifestyle, must agree to either remain abstinent or stay in a same sex relationship without sexual relationships with males.

All other women of childbearing potential must agree to use two forms of effective contraception, where at least one form is highly effective (less than 1% failure rate), for the entirety of the study.

Abstinence or contraception must continue for 90 days after the intervention.

#### 13.1.1.4 Acceptable methods of contraception

Highly effective methods of contraception (less than 1% failure rate) include, but are not limited to:

- combination oral contraceptives
- implanted contraceptives, or
- intrauterine devices.

Effective methods of contraception include but are not limited to diaphragms with spermicide or cervical sponges.

#### 13.1.1.5 Unacceptable methods of contraception

Use of male and female condoms as a double barrier method is not considered acceptable due to the high failure rate when these barrier methods are combined.

Barrier protection methods without concomitant use of a spermicide are not an effective or acceptable method of contraception.

Periodic abstinence (e.g., calendar, ovulation, symptom-thermal, post-ovulation methods), declaration of abstinence just for the duration of a trial, and withdrawal are not acceptable methods of contraception.

### 13.1.2 Men

Men, regardless of their fertility status, must agree to either remain abstinent (if this is their preferred and usual lifestyle) or use condoms as well as one additional highly effective method of contraception (less than 1% failure rate) or effective method of contraception with non- pregnant women of childbearing potential partners for the duration of the study and until their plasma concentrations are below the level that could result in a relevant potential exposure to a possible fetus, predicted to be 90 days after the dose.

Men with pregnant partners should use condoms during intercourse for the duration of the study and until the end of estimated relevant potential exposure to the fetus, predicted to be 90 days after the dose.

### 13.1.3 Other Guidance

Men should refrain from sperm donation for the duration of the study and until their plasma concentrations are below the level that could result in a relevant potential exposure to a possible fetus, predicted to be 90 days after the dose.

## 13.2 Appendix II. Statistical analysis, details

### A. Predictive power calculation for the log hazard-ratio

As stated in Section 10.5.4.3, the decision about the dose to be continued in Stage-2 will be based on the Bayesian predictive distribution, which is defined as the conditional distribution of $\begin{pmatrix}\hat{\theta}_{21}\\ \hat{\theta}_{22}\end{pmatrix}$ given $\begin{pmatrix}\hat{\theta}_{11}\\ \hat{\theta}_{12}\end{pmatrix}$. These probabilities will also be used for the futility analysis (see Section 10.5.4.2).

We denote with $l_{ij}$ the statistic of the log-rank test for the single dose $d_j$ ($j$=1,2) and the single time $t_i$ ($i$=1,2 for Stage-1 and Stage-2). For our purpose, it is more convenient to use $\tilde{l}_{2j}$ instead of $l_{2j}$, where:

$$\tilde{l}_{2j} = \frac{i_{2j} \times l_{2j} - i_{1j} \times l_{1j}}{\sqrt{i_{2j}^2 - i_{1j}^2}}$$

is the so-called independent increment structure.

In our notation:

$$i_{ij} = \sqrt{\sum_{k=1}^{d_i} p_{kj} \times (1 - p_{kj})}$$

$$I_{ij} = i_{ij}^2$$

$d_i$ = number of uncensored events that occurred until $t_i$

$t_i$ = time of interim ($i$=1) and final analysis ($i$=2)

$t_k^*$ = time at which an event has occurred in group $j$ or placebo

$$p_{kj} = \frac{r_{kj}}{(r_{kj} + r_{kplacebo})} \; if \; the \; event \; occurred \; in \; group \; j \; or \; placebo \; at \; time \; t_k^* \, , 0 \; otherwise$$

$r_{kj}$ = number of patients at risk in group $j$ at time $t_k^*$

It is to be noted that $l_{i1}$ and $l_{i2}$ are treated separately, in the sense that the total number of patients at risk is set equal to $r_{kj} + r_{kplacebo}$ at time $t_k^*$ for the comparison of the placebo arm and treatment dose j.

For the primary endpoint, the estimates of the log-hazard ratios at Stage-1 and Stage-2 are as follows:

$$\hat{\theta}_{1j} = \frac{l_{1j}}{i_{1j}}$$

and

$$\hat{\theta}_{2j} = \frac{\tilde{l}_{2j}}{\sqrt{I_{2j} - I_{1j}}}$$

Under a number of simplifying assumptions that are described by Di Scala and Glimm [42], it can be shown that asymptotically:

$$\begin{pmatrix}\hat{\theta}_{21}\\ \hat{\theta}_{22}\end{pmatrix} | \begin{pmatrix}\hat{\theta}_{11}\\ \hat{\theta}_{12}\end{pmatrix} \sim N\left(\begin{pmatrix}\hat{\theta}_{11}\\ \hat{\theta}_{12}\end{pmatrix}, \boldsymbol{I}_1^{-1} + \begin{pmatrix}(I_{21}-I_{11})^{-1} & v\\ v & (I_{22}-I_{12})^{-1}\end{pmatrix}\right) \qquad \text{(A1)}$$

where:

$$\boldsymbol{I}_1^{-1} = \begin{pmatrix} I_{11}^{-1} & v_{11,12} \times i_{11}^{-1} \times i_{12}^{-1} \\ v_{11,12} \times i_{11}^{-1} \times i_{12}^{-1} & I_{12}^{-1}\end{pmatrix}$$

$$v_{11,12} = \frac{\sum_{k=1}^{d_1} p_{k1} \times p_{k2}}{\sqrt{\sum_{k=1}^{d_1} p_{k1} \times (1-p_{k1}) \times \sum_{k=1}^{d_1} p_{k2} \times (1-p_{k2})}}$$

$$v = \frac{v_{21,22}^*}{\sqrt{(I_{21}-I_{11}) \times (I_{22}-I_{12})}}$$

$$v_{21,22}^* = \frac{\sum_{k=1}^{d_2} p_{k1} \times p_{k2} \times \frac{r_{kplacebo}}{r_{k1}+r_{k2}+r_{kplacebo}} - \sum_{k=1}^{d_1} p_{k1} \times p_{k2} \times \frac{r_{kplacebo}}{r_{k1}+r_{k2}+r_{kplacebo}}}{\sqrt{i_{21}^2 - i_{11}^2} \times \sqrt{i_{22}^2 - i_{12}^2}}$$

Considering the two treatments *j* separately (*j=1,2*), the predictive probability that the final log-rank statistic lies above some fixed threshold *c* is obtained from (A1) as follows:

$$\Pr\left(\tilde{l}_{2j} > c | \hat{\theta}_{1j}\right) = \Phi\left(\frac{i_{1j}}{i_{2j}} \times \left(c - \sqrt{I_{2j} - I_{1j}}\hat{\theta}_{1j}\right)\right) \qquad \text{(A2)}$$

where:

$\Phi(.)$= cumulative distribution function of the standard normal distribution

$c = \Phi^{-1}(1-(\alpha-\alpha_1)/2)$

$\Phi^{-1}(.)$ is the standard normal quantile.

$i_{1j}$ and $I_{1j}$ (see above) can be computed considering that, for each dose j, $d_1 = d_{1j} + d_{1placebo}$ are observed; $i_{2j}$ and $I_{2j}$ can also be computed considering that $d_2 = d_{2j} + d_{2placebo} + d_1$ can be estimated as the planned number of events minus the observed number of events in the non-considered active treatment arm.

The predictive probability in (A2) represents the probability of a successful trial (i.e., a statistically significant trial) given the interim results and a non-informative Normal prior for the true log-hazard ratios $\theta_j$, *j=1,2*.

In Sections 10.5.4.2 and 10.5.4.3, the marginal predictive probabilities in (A2) are indicated with $Pr_{j,primary\ endpoint}$.

**B. Predictive power calculation for dichotomous outcomes**

Let's indicate with $y_j \sim Binom(\frac{n_{stage1}}{2}, p_j)$ the total number of events observed in the treatment group $j$ of $n_{Stage1}/2$ patients checked for a dichotomous outcome and $y_{placebo} \sim Binom(\frac{n_{stage1}}{2}, p_{placebo})$ the corresponding number of events in the placebo group.
Here $p_j$ represents the true probability of experiencing a composite event in treatment dose $j$ and $p_{placebo}$ the corresponding probability in the placebo group.

For each of the two treatments $j$, at Stage-1, we obtain the estimates $\hat{p}_{1j}$ and $\hat{p}_{1placebo}$ while at Stage-2, we could obtain the estimates $\hat{p}_{2j}$ and $\hat{p}_{2placebo}$ and $\hat{p}_{(1+2)j}$ and $\hat{p}_{(1+2)placebo}$ where $\hat{p}_{(1+2)j}$ is the weighted average of $\hat{p}_{1j}$and $\hat{p}_{2j}$ and $\hat{p}_{(1+2)placebo}$is the weighted average of $\hat{p}_{1placebo}$ and $\hat{p}_{2placebo}$ with weights that are proportional to the sample sizes of Stage-1 and Stage-2.

In contrast to the analysis of time to event data, the statistics $\hat{p}_{1j}$ and $\hat{p}_{2j}$ and corresponding statistics for the placebo group can be calculated from observations before and after the interim analysis.

Berry et al [49] and Harari et al [50] have shown that, for each treatment dose $j$, the posterior predictive distribution $\hat{p}_{2j}|\hat{p}_{1j}$ can be approximated as follows:

$$\hat{p}_{2j}|\hat{p}_{1j} \sim N\left(\hat{p}_{1j}, \frac{\frac{n_{stage2}}{2} \times \hat{p}_{1j} \times (1-\hat{p}_{1j})}{\frac{n_{stage1}}{2} \times \left(\frac{n_{stage1}}{2} + \frac{n_{stage2}}{2}\right)}\right) \quad \text{(B1)}$$

and a similar expression can be obtained for the posterior predictive distribution $\hat{p}_{2placebo}|\hat{p}_{1placebo}$ of the placebo group.

At Stage-2, rejection of the null hypothesis for treatment dose $j$ would be obtained if:

$$Z_j = \frac{\left(\hat{p}_{(1+2)placebo} - \hat{p}_{(1+2)j}\right)}{\sqrt{\frac{\hat{p}_{(1+2)j} \times (1-\hat{p}_{(1+2)j})}{(n_{stage1}+n_{stage2})/2} + \frac{\hat{p}_{(1+2)placebo} \times (1-\hat{p}_{(1+2)placebo})}{(n_{stage1}+n_{stage2})/2}}} > z_{1-\alpha}$$

Based on (B1), the Bayesian predictive probability that the Z statistics lies above $z_{1-\alpha}$ is as follows (assuming large values are desirable):

$$\Pr\left(Z_j > z_{1-\alpha} | (\hat{p}_{1placebo} - \hat{p}_{1j}\right) = \phi\left(\frac{\hat{p}_{1placebo} - \hat{p}_{1j} - z_{1-\alpha} \times \sigma_{\hat{\Delta}}}{\sigma_{\hat{\Delta}|(\hat{p}_{1placebo} - \hat{p}_{1j})}}\right) \quad \text{(B2)}$$

with:
*Φ(.)*= cumulative distribution function of the standard normal distribution

$$\sigma^2_{\hat{\Delta}} = \frac{\hat{p}_{1placebo} \times (1-\hat{p}_{1placebo})}{\frac{n_{stage1}}{2} + \frac{n_{stage2}}{2}} + \frac{\hat{p}_{1j} \times (1-\hat{p}_{1j})}{\frac{n_{stage1}}{2} + \frac{n_{stage2}}{2}}$$

$$\sigma^2_{\hat{\Delta}|(\hat{p}_{1placebo} - \hat{p}_{1j})} = \frac{\frac{n_{stage2}}{2} \times \hat{p}_{1placebo} \times (1-\hat{p}_{1placebo})}{\frac{n_{stage1}}{2} \times \left(\frac{n_{stage1}}{2} + \frac{n_{stage2}}{2}\right)} + \frac{\frac{n_{stage2}}{2} \times \hat{p}_{1j} \times (1-\hat{p}_{1j})}{\frac{n_{stage1}}{2} \times \left(\frac{n_{stage1}}{2} + \frac{n_{stage2}}{2}\right)}$$

In Sections 10.5.4.2 and 10.5.4.3, the predictive probabilities in (B2) are indicated with $Pr_{j,key\ secondary\ endpoint}$.