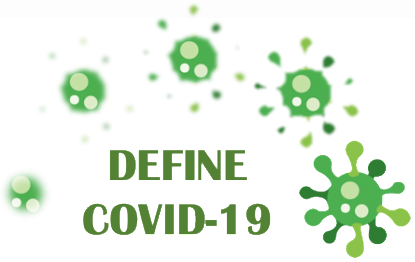
DEFINE

Evaluating therapies for COVID 19

PROTOCOL BOOKLET

Academic and Clinical Central Office for Research and Development



DEFINE

Evaluating therapies for COVID 19

Co-sponsors	The University of Edinburgh & Lothian Health Board ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Funder	Life Arc
Chief Investigator	Professor Kev Dhaliwal Professor of Molecular Imaging & Healthcare Technology Consultant in Respiratory Medicine, Tuberculosis /Infection and Interventional Medicine Centre for Inflammation Research University of Edinburgh
Sponsor Reference	AC20063
IRAS Number:	282934
EudraCT Number	2020-002230-32
REC Number	20/SS/0066
ISRCTN Number	ISRCTN14212905
NCT Number	NCT04473053

The DEFINE Clinical Trial was set up as a platform trial, with one main protocol and intervention specific protocols (Appendix Protocols). By the time the trial completed in 2025, there had been three interventions approved as part of the clinical trial (treatment arms 2-4) along with standard care (treatment arm 1). Details of each are provided in the table below.

Treatment arm 1	Standard care relevant to the disease stage at entry (e.g. antipyretic/analgesics, cough linctus, oxygen, CPAP, intubation and ventilation with/without prone positioning, ECMO, cardiac support including ionotropes/intra-aortic balloon pump).
Treatment arm 2	Standard care and TD139 (**Appendix 1**)
Treatment arm 3	Standard care and Nafamostat (**Appendix 2**)
Treatment arm 4	Standard care and SARS-CoV-2 VSTs (**Appendix 3**)

For the purposes of upload to the relevant registries, all relevant versions of the protocol and appendicies have been provided in this single document.

Treatment arms 1-3 were run in parallel, with participants randomly allocated to a particular treatment arm. The relevant versions of the documents current whilst these arms were recruiting are as follows:

PROTOCOL	VERSION AND DATE
Main Protocol	Version 4 20 Jan 2021
Appendix 1 Protocol	Version 3 28 Oct 2020
Appendix 2 Protocol	Version 4 20 Jan 2021

Treatment arm 4 ran independently of the other arms of this platform, the protocol versions relevant to that intervention were:

PROTOCOL	VERSION AND DATE
Main Protocol	Version 7 10 Jun 2024
Appendix 1 Protocol	Version 6 10 Jun 2024

Please note:

All signature pages/details have been redacted for privacy purposes prior to upload

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PROTOCOL APPROVAL SIGNATURE PAGE

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CONTENTS

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development
AE	Adverse Event
AES	Advanced Electronic Signature
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
CI	Chief Investigator
CRF	Case Report Form
CSR	Clinical Study Report
CT	Computed tomography
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CXR	Chest X ray
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator Brochure
ISF	Investigator Site File
MHRA	Medicines and Healthcare products Regulatory Agency
MV	Mechanical Ventilation
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
QA	Quality Assurance

REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
WOCBP	Woman of childbearing potential

INTRODUCTION

1.1 BACKGROUND AND SYNOPSIS

COVID-19 is a community acquired pneumonia caused by infection with a novel coronavirus, SARS CoV2 and is a serious condition with high mortality in hospitalised patients. Urgent investigation of potential treatments for this condition is required.

This protocol describes an overarching and adaptive trial designed to provide safety, pharmacokinetic (PK)/ pharmacodynamic (PD) information and exploratory biological surrogates of efficacy which may support further development and deployment of candidate therapies in larger scale trials of SARS-CoV2 positive patients receiving normal standard of care.

Given the spectrum of clinical disease, community based infected patients or hospitalised patients can be included. SARS-CoV2 positive patients will be divided into cohorts, a) community b) hospitalised patients with new changes suggestive of a viral pneumonitis on a chest x-ray (CXR) or a computed tomography (CT) scan or requiring supplemental oxygen and c) hospitalised requiring assisted ventilation. Participants may be recruited from all three of these cohorts, depending on the experimental therapy, its route of administration and mechanism of action. The relevant cohort(s) for any given therapy will be detailed in the therapy-specific appendix.

Candidate therapies can be added to the protocol and previous candidates removed from further investigation as evidence emerges. Candidate assets will be novel or repurposed products that are first-in-COVID-19. The trial will be monitored by an independent Data Monitoring Committee (DMC) to ensure patient safety.

This is an experimental medicine platform trial encompassing early phase studies and as such formal sample size calculations are not appropriate.

Each candidate therapy will include a small cohort of patients.randomised to candidate therapy or existing standard of care management dependent on disease stage at entry. Cohort numbers will be defined in the protocol appendices and can be changed by the trial management group as the study progreses. To enable additional sites to participate in this study, certain assessments that require specialised equipment or techniques to address secondary endpoints will be optional.

1.2 RATIONALE FOR STUDY

This early phase trial platform aims to support promising novel and repurposed therapeutic assets but without prior information on use in COVID-19, to determine safety, PK-PD profile and determine exploratory biological markers of efficacy in small cohorts of COVID-19 patients. The results of these studies are intended to provide initial safety, pharmacokinetic and pharmacodynamic data (or equivalent surrogates) and experimental medicine data to support further development in follow on clinical studies in COVID-19 patients.

A major limitation in the design of many early clinical trials is the limited amount of mechanistic data from patients with COVID-19. Mechanisms have been inferred from animal models, related infections or clinical syndromes.

There is a clear and urgent need to pursue experimental medicine studies in humans to establish a solid mechanistic basis for rapid evaluation, including in existing clinical trial platforms against COVID-19 (e.g DoH RECOVERY)

The trial platform will be as flexible as possible to ensure a broad range of patients can be recruited and candidate therapies can be added or removed as evidence emerges. The interim trial results will be monitored by an independent DMC to evaluate any patient safety signals.

As COVID-19 follows a variable clinical path in individual patients, the protocol is designed to enable inclusion of patients across the disease stages. The trial is intended to provide mechanistic data from patients receiving standard of care therapy and from patients treated with the therapy candidates. The study will enable delivery of pharmacokinetic information and effects of standard of care and candidate agents on surrogate biomarkers of the disease process and the specific drug target.

2. STUDY OBJECTIVES

2.1 OBJECTIVES AND ENDPOINTS

The main study objectives and endpoints are listed below. All treatment specific endpoints will be in addition to these and will be included in the relevant appendices.

Objectives	Endpoints
Primary	
To evaluate the safety and tolerability of candidate agents as add-on therapy to SoC in patients with COVID-19.	Safety will be assessed using: • Haematological and biochemical safety laboratory investigations. • Physical examination • Vital signs (blood pressure/heart rate/temperature and respiratory rate) • Daily electrocardiogram (ECG) readings • Adverse events
Secondary	
To explore the PK/PD or appropriate surrogate of bioavailability of the proposed trial treatments in COVID-19 patients.	To explore the PK/PD of the proposed trial treatments.
Assess the response of key exploratory biomarkers during treatment period.	Evaluate the change from baseline values for key exploratory biomarkers of target engagement for each treatment. For each treatment, refer to the relevant appendix for specific biomarker endpoints. More information on the biomarkers chosen for analysis can be found in Section 6.5.
To evaluate the improvement or deteroriation of patients in each treatment arm.	Record changes to WHO ordinal scale and NEWS2 score
To evaluate the number of oxygen-free days.	Duration (days) of oxygen use and oxygen-free days.
To evaluate ventilator-free days and incidence and duration of any form of new ventilation use.	• Duration (days) of ventilation and ventilation-free days. • Incidence of any form of new ventilation use and duration (days) of new ventilation use.
Change in the ratio of the oxygen saturation to fraction of inspired oxygen concentration (SpO2/FiO2)	• SpO2/FiO2, measured daily from first dose to Day 15, hospital discharge, or death
To evaluate SARS-CoV-2 viral load.	Qualitative and quantitative polymerase chain reaction (PCR) determination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva samples while hospitalised on Days 1, 3, 5, 8, 11, 15. And oropharyngeal/nasal swab on the same days if tolerated.

To evaluate time to discharge	• Duration of total hospital stay • Duration to discharge following treatment
To evaluate the use of renal dialysis or haemofiltration for each treatment arm.	Record requirement for renal dialysis or haemofiltration

Biomarkers may include individual molecules, such as cytokines or analyses of cellular function eg flow cytometry, or tests of biological processes, eg thromboelastography or imaging.

3. STUDY DESIGN

All research staff involved in any aspect of the study will be required to adhere to local practice regarding use of personal protective equipment and other applicable safety protocols and guidelines relating to contact with COVID-19 patients and sample collection.

DEFINE is an open label randomised exploratory early interventional clinical experimental medicine platform trial. Candidate assets will be included in the appropriate phase (described in the treatment appendix) according to prior knowledge, with a view to progressing promising assets along the development pathway.

4. TARGET POPULATION

Patients with confirmed SARS CoV2 infection with relevant COVID-19 symptoms/signs will be recruited into this trial. As SARS CoV2 has a range of clinical manifestations, representatives of three target patient cohorts will be included in this trial. Participants may be recruited from one or more of these cohorts, depending on the experimental therapy under investigation. The relevant cohort(s) for any given therapy will be detailed in the drug-specific appendix.

While the study will approach patients with suspected COVID-19 and conduct screening assessments whilst the test results are outstanding, only patients who are confirmed COVID-19 positive will included in a treatment arm.

Cohort 1A	Community (primary care) patients with confirmed COVID-19
Cohort 1B	Community (primary care) patients with confirmed COVID-19 with new changes on CXR or CT scan compatible with COVID-19 and deemed 'high-risk' of hospitalisation or death*
Cohort 2A	Hospitalised confirmed COVID positive patients with new changes on CXR or new changes on CT compatible with COVID-19 but not requiring supplemental oxygen,
Cohort 2B	Hospitalised confirmed COVID positive patients with: new changes on CXR or new changes on CT compatible with COVID-19 and requiring supplemental oxygen ,
Cohort 3	Hospitalised patients with confirmed COVID-19 requiring assisted ventilation (including non-invasive and mechanical ventilation)

* High risk is defined as over 50 years of age with comorbidies.

4.3 INCLUSION CRITERIA

Inclusion criteria:

- Provision of informed consent from the patient or representative
- Aged at least 16 years
- If the patient is of child bearing potential*, the patient, and their partner(s), agree to use medically-accepted double-barrier methods of contraception (eg, barrier methods, including male condom, female condom or diaphragm with spermicidal gel) during the study (if allocated to a treatment arm) and for at least 90 days after termination of study therapy. A vasectomised partner would be considered an appropriate birth control method provided that the partner is the sole male sexual partner and the absence of sperm has been confirmed.
- COVID-19 positive test result within last 14 days

Exclusion criteria:

- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1.73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Women who are pregnant or breastfeeding.
- Participation in another clinical trial of an investigational medicinal product (CTIMP)
- Known hypersensitivity to the IMP or excipients (e.g. lactose)
- Concomittant use of treatments for COVID-19 that are not recognised as locally approved standard care.
- Significant electrolyte disturbance (hyperkalaemia K+ >5.0 mmol/L or hyponatraemia Na+ < 120mmol/L)
- Patient currently receiving potassium sparing diuretics that cannot be reasonably withheld
- Patient currently receiving anticoagulation or antiplatelet agents that cannot be reasonably withheld if randomised to Nafamostat
- Patients (or their partners) planning on donating sperm/eggs during the trial period
- Ongoing dialysis
- History of serious liver disease (Child Pugh score > 10)
- Severe uncontrolled diabetes mellitus
- In the Investigator's opinion, patient is unwilling or unable to comply with drug administration plan, laboratory tests or other study procedures.

* A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

4.4 TREATMENT ARMS

Initially randomisation will be between three treatment arms but more arms will be added as substantial amendments as experimental therapies are prioritised. There is no restriction on the number of treatment arms that will be recruiting at any one time.

Treatment arm 1	Standard care relevant to the disease stage at entry (e.g. antipyretic/analgesics, cough linctus, oxygen, CPAP, intubation and ventilation with/without prone positioning, ECMO, cardiac support including ionotropes/intra-aortic balloon pump).
Treatment arm 2	Standard care and TD139 (**Appendix 1**)
Treatment arm 3	Standard care and Nafamostat (**Appendix 2**)

Further details on each of these treatment options can be found in the relevant appendix.

Patient numbers are defined in each appendix. It will be possible to increase these numbers following advice from the Trial Management Group, Data Monitoring Group and Statistician. It will be possible to affect a minor increase to the total patient number of one or two arms, without seeking the aforementioned advice, solely to ensure all active arms recruit the minimum number of participants, This option is necessary because of the database design.

Other arms will be added when/if evidence emerges of a mechanistic basis for proceeding to experimental studies for specific candidate agents. Conversely, in some patient populations, not all study arms are appropriate (e.g. due to contraindications based on comorbid conditions or concomitant medication).

4.4.1 Concomitant Medications

Routinely used medications for the alleviation/treatment of symptoms of COVID-19 are permitted. Only locally and nationally approved therapies to treat SARS-CoV-2/COVID-19 will be permitted. Concomittant use of treatments for SARS-CoV-2/COVID-19 that are not recognised as locally approved standard care will not be permitted.

Medicines specifically contraindicated if used in association with specific IMPs are listed in the candidate specific appendix.

Any other medication (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrolment (including screening) or receives during the study must be recorded in the eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

4.4.2 Risk/Benefit Assessment

This study is designed to evaluate preliminary safety, pharmacokinetic and pharmacodynamic features of agents which have a likelihood of potential beneficial effects on either viral replication and/or elaboration of an inflammatory response which characterises this disease. All patients in this study will receive standard of care management relevant to their stage of COVID-19 disease at trial entry and may progress to higher level intervention including ventilatory support, if required during the trial period.

Patients will be kept under review with regular assessments of vital signs, oxygenation status, laboratory markers of organ system function and electrocardiogram (ECG) parameters enabling relevant intervention and step up care where required. Detailed information about the known and expected risks and reasonably expected adverse events (AEs) of each candidate agent may be found in the corresponding appendix for that agent. Given the urgent need for therapy for this condition, the potential benefit to public health from identification of agents which may ameliorate COVID-19 progression which may accrue from the early study of their biological effects in this study outweigh the potential risks of these interventions in the conditions applied during the study.

4.5 CO-ENROLMENT

Co-enrolment with a Clinical Trial of an Investigational Medicinal Product (CTIMP) will not be permitted. Co-enrolment with a clinical investigation of a Medical Device or a non-interventional clinical study will be considered on a study-by-study basis and in discussion with the relevant Chief Investigators and Sponsors and industrial collaborators. Such co-enrolment will be undertaken in line with the Sponsor co-enrolment policy (POL008).

Co-enrolment involving non-interventional research (including questionnaire or tissue only studies) will be allowed provided this is not expected to affect the outcomes of both studies or place undue burden upon participants and their families.

5. PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

The first approach will always be done by member of the clinical care team (including members of the research team embedded within the clinical team) who is a qualified nurse or doctor.

Clinical members of the research team will liaise with members of the clinical care team to identify potential participants. A member of the clinical care team (which may include embedded research nurses or clinicians), will make the first approach regarding screening for eligibility.

It is permissible for patients who are highly suspected of having COVID-19 to be screened and consented. If the patient testsSARS-CoV2 negative during or after screening/consent etc, they will be withdrawn from the study. **Only those patients who are confirmed SARS-CoV2 positive will be randomised to a treatment arm.**This strategy will help to expedite recruitment and therapy administration.

5.2 CONSENTING PARTICIPANTS

Due to the nature of this study and the progression of the disease under investigation, it is likely that participants will fall into one of three following categories:

- Capacity to provide ongoing consent for the duration of the study
- Lose capacity during the course of the study (following consent)
- Incapacity to provide consent from the outset of the study

Consent procedures for each of these categories is described below.

5.3 CONSENTING PARTICIPANTS WITH CAPACITY

After being given the participant information sheet, potential participants will have 24 hours (or less if appropriate) to decide whether to take part. All willing and potentially eligible participants will be asked to provide written informed consent which will be taken by an appropriately qualified member of the research team who is delegated to do so. The original will be retained by the research team, one copy will be filed in the medical notes and one copy will be provided to the participant. Where it is not feasible to obtain an ink signature from the participant this will be overcome using electronic methods. **It will be made very clear to the patients at consent that, if tests are outstanding, that patients will only be allocated to a treatment arm if they are confirmed SARS-COV2 positive.**

5.4 PARTICIPANTS WHO LOSE CAPACITY DURING THE TRIAL

There may be participants who have capacity to consent at the beginning of the trial, who then go on to become incapacitated over the course of the trial (i.e. due to progression of the infection). It will be explained to all participants at the time of consent that informed consent will be respected following loss of capacity and will not affect their ongoing participation in the study but may result in them being unable to continue with the study drug.

5.5 CONSENTING PARTICIPANTS WITH INCAPACITY

It is anticipated that some critically ill incapacitated patients will be included in this trial. The selection and enrolment of adults with incapacity will take place within the legal framework described in Adults with Incapacity (Scotland) Act 2000 and Medicines for Human Use (Clinical Trials) Regulations, 2004. As some of these patients will be ventilated via an endotracheal tube it is very unlikely that they will have the capacity to consent for themselves due to the use of iatrogenic sedation and/or the presence of incapacitating illness.

Therefore, typically, a personal legal representative (also known as guardian/welfare attorney/nearest relative) for each potential participant will be identified and approached by a member of the clinical care team. Options for consent are outlined below. Personal legal represenatives will have 24 hours (or less if appropriate) to decide whether to take part.

5.5.1.1 Consent in person

An appropriate member of the research team, who has been delegated the duty of obtaining consent, will approach the relevant individual to discuss the study. The appropriate participant information sheet (PIS) will be given to the personal legal representative. Sufficient time will be given to consider participation in the trial. The research team will be available to answer any questions or discuss any aspect of the trial.

Details of who the personal legal representative may be obtained from information on the Electronic Patient Record system used at site. . These details will not be recorded. The personal legal representative is identified routinely in the intensive care nursing record.

If the patient/personal legal representative chooses to proceed, written, informed consent will be obtained. The original consent form will be signed and two copies made. One copy will be given to the personal legal representative and one stored in the participant's medical notes along with a copy of the information sheet.

Where it is not feasible to obtain an ink signature from the personal legal representative this will be overcome using electronic methods.

5.5.1.2 Consent via Telephone

Every effort will be made to approach and consent the personal legal representative in person. If the personal legal representative is only contactable by telephone then the informed consent process is permitted via telephone, provided the following:

- The representative who is being telephoned has previously had the opportunity to discuss the clinical aspects of the patient's care with the clinical team.
- A member of the clinical team has sought permission for the personal legal representative to be contacted via telephone regarding potential involvement in the study
- The approach to discuss consent is clearly separate to any discussions made between the representative and the clinical team

A member of the research team will contact the personal legal representative by telephone to explain what the study entails and answer any questions they may have. The personal legal representative will be given ample time to read and consider the information sheet. If the personal legal representative chooses to enrol the patient onto the study, verbal consent will be obtained by a member of the research team who will sign the consent form. This will be witnessed. All efforts will be made to obtain a signature electronically or on a paper copy of the consent form mailed out. However, in the absence of this signature, a consent form completed by a member of the research team and witnessed by an independent member of staff will be acceptable. A copy of the information sheet and consent form will be sent to the personal legal representative.

5.5.1.3 Consent via Professional Legal Representative

A personal legal representative should be identified and consulted unless it is not reasonably practicable to contact a personal legal representative before the decision to enter the adult into the trial is made.

Every attempt will first of all be made to identify the personal legal representative of the patient, this will include searching electronic health records or similar and speaking to the clinical team. In non Covid-19 circumstances contact details are identified from a variety of methods for patients lacking capacity (admission paperwork, provided to ambulance staff, previous hospital admissions etc.) and stored on electronic health records or similar. If patients are admitted lacking capacity and without the usual methods for identifying and logging contact details for a personal legal representative, then, in the rare event that no contact has been identified by the clinical care team (this will be checked with the clinical care team and on electronic health record (or similar) at site) the research team may consider seeking consent from a professional legal representative.

- At the first opportunity, the trial will be discussed with the personal legal representative if identified. We will ask the clinical team to update the research team on contact with a personal legal representative and check electronic health records or similar on a regular basis.
- Once contacted, the personal legal representative can opt for the participant to remain in the trial (and receive an information sheet and consent form) or decide to withdraw the participant from the trial.

If a personal legal representative cannot be identified after 24 hours, a professional legal representative can be approached. In keeping with the Medicines for Human Use (Clinical Trials) Regulations, 2004, a professional legal representative will be a clinician responsible for the medical treatment of the patient if they are independent of the study or a person nominated by the healthcare provider. A professional legal representative will only be approached if a personal legal representative is not available for the reasons outlined above. A Professional legal represenative will have 24 hours (or less if appropriate) to decide whether to take part.

Where it is not feasible to obtain an ink signature from the professional legal representative this will be overcome using electronic methods.

5.5.1.4 Participants Regaining Capacity

If a participant regains capacity during the course of the study then the participant will be given the opportunity to be re-consented. The personal legal representative (or professional) will be informed that this will be the case when consenting for the participant to take part in the study. The participant will be provided with an appropriate PIS/consent form and asked if they wish to continue with the study.

If a participant regains capacity and is discharged before we can consent them we will seek to contact them at home. To do this we will request a suitable phone number and email address for the participant and will attempt to contact them twice through both means. If we fail to establish contact we will assume their ongoing consent and continue to include them in applicable aspects of the study.

5.5.1.5 Electronic and Witnessed Methods of Obtaining Signatures

Consent will normally be recorded in writing, dated and signed or otherwise marked by the participant or their legal representative. In most instances this will take the form of a face to face consent process with a wet ink signature.

If face to face consent is not possible or feasible, verbal consent over the phone or video-call will be utilised, this will be witnessed and recorded in writing. If a verbal witnessed consent procedure is utilised we will also attempt to obtain a written signature from the participant or their legal representative. Electronic signature software will be offered in the first instance as an Advanced Electronic Signature (AES).

The preferred method will always be face to face and telephone communication and this will be utilised wherever possible. If electronic signatures are used, patient information will be stored on a secure cloud.

5.6 SCREENING FOR ELIGIBILITY

Participant eligibility will be verified by a clinical trial physician after written informed consent has been obtained. Confirmation of eligibility will be recorded within the participants' medical records. Women who are of child bearing potential will be required to undergo a urine pregnancy test to confirm eligibility. Only those patients who are confirmed SARS-CoV2positive will be randomised to a treatment arm, however, it is permissible for patients who are highly suspected of having COVID-19 to be screened and consented. SARS-CoV tests can often take up to 72 hours to provide a result, although most are returned within 24-36 hours, in which time the research team could approach the patient, provide them with time to consider their participation and consent. The patient would only be randomised if they were COVID positive. This will allow the team to streamline identification and approach and would enable treatment to be initiated earlier. In the small number of cases where reporting of results are delayed this may result in some screening activities becoming invalid (exceeding the acceptable time window) as detailed in the table of assessments within this protocol or the relevant treatment appendix. If this situation arises the relevant screening assessments will be repeated prior to randomisation.

Contraceptive Requirements

Pregnant and breastfeeing women are excluded from participation in the trial. Women of child bearing potential and men with female partners of child bearing potential are eligible to participate in the trial provided that they agree to use effective contraception throughout the

trial period and for 90 days post study completion. In the event of any use of medications eg monoclonal antibodies this period may be extended and will be addressed within the specific agent appendix.

Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
    - Oral
    - Injectable
    - Implantable
- Intraurine device (IUD)
- Intraurine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised partner
- Condoms
- Sexual abstinence

5.7 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

If participants are not entered into the study, for whatever reason, another participant can take their place. All ineligible participants will continue to receive standard care.

5.8 RANDOMISATION

Randomisation will be performed using a web-based randomisation system (built in REDCap) hosted at the Edinburgh Clinical Trials Unit (ECTU) at the University of Edinburgh (a fully registered UKCRC CTU (registration #15)). Since these studies are designed to be small, this study will balance underlying risk across the allocations using the method of minimisation.

There are multiple interventions across several cohorts on the COVID-19 pathway (from community based, to breathless in hospital to ventilation with different intensities), with potentially different primary outcomes. Hence, it is not practicable to produce bespoke minimisation algorithms for every possibility, so instead we will base the minimisation algorithm on what is currently known about risk factors associated with admission to ICU or death. The minimisation will include a random element (set at 20%) to increase unpredictability of allocation.

5.8.1 Supply of study treatments

More details regarding packaging and labelling can be found in the relevant appendix.

5.8.2 Emergency Unblinding Procedures

This trial will not be blinded.

5.9 WITHDRAWAL OF TRIAL PARTICIPANTS

Participants are free to withdraw from the study at any point, without giving a reason. This will not affect the patients ongoing care in the institution. A participant can be withdrawn by the Investigator if the investigator believes that continued participation is against the best interests of the patient. If withdrawal occurs, the primary reason for withdrawal, if available,

will be documented in the participant's case record form. The participant will have the option of withdrawal from:

(i) trial medication with continued trial procedures and/or collection of clinical and/or safety data. The participant can withdraw from any further procedures/interventions but remain on trial, complete follow-up visits and/or allow medical record review for relevant trial data, e.g. results of clinical blood results, ECG recordings and physical examinations;
(ii) all aspects of the trial but continued use of data collected up to that point. To safeguard rights, the minimum personally-identifiable information possible will be collected.

Randomised patients who wish to be withdrawn from the trial before they have received first dose of trial medication will be withdrawn from the trial and another participant will be recruited to replace them.

6. SAFETY ASSESSMENTS AND TREATMENT ARMS

6.1 Safety Assessments

The safety assessments listed below will be carried out on all treatment and control participants. Additional assessments specific to the treatment are listed in the relevant appendix.

6.1.1 Physical Examinations

A general physical examination will be performed at screening, including assessment of presenting symptoms. At subsequent assessments, a symptom-directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint.

6.1.2 Vital Signs

Temperature, pulse rate, blood pressure, and respiratory rate will be assessed. Blood pressure and pulse measurements will be assessed with a completely automated device. SpO2 will also be assessed. Manual techniques will be used only if an automated device is not available.

Vital signs measurements will contribute to the NEWS2 score.

Respiratory support will be recorded. FiO2, the assumed percentage of oxygen concentration participating in gas exchange in the alveoli, will also be recorded. Measurements will be taken in line with standard practices for the study centre.

6.1.3 Clinical Safety Laboratory Assessments

Fasting is not required before collection of laboratory samples.

Screening bloods
The following are the minimum blood results required for screening:
Biochemistry – Urea, Sodium, Potassium Creatinine and Alkaline Phosphatase (Alk Phos), LFTs - Total Bilirubin and ALT
Haematology - FBC (haemoglobin, haematocrit, differential WCC (excluding eosinophils) and platelets).
Coagulation screen – International Normalised Ratio (INR), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

If these have been taken in the past 48 hours and satisfy eligibility criteria, no further bloods are required.

For daily assessments post randomisation, the following tests wil be performed:

Haematology: Full blood count and differential white cell count

Coagulation: D-dimer, Fibrinogen,Activated partial thromboplastin time (aPTT), Prothrombin time (PT), International Normalised Ratio (INR), Cd39, ecto-ADPase, nitrous oxide, PGI2 Thrombomodlin,EPCR, kallikrein.

Biochemistry: Urea and electrolytes (urea, sodium, potassium, chloride, magnesium, bicarbonate, creatinine); liver function tests (Total protein, albumin, globulin, total bilirubin, SGOT(AST), SGPT(ALT), GGT, LDH, Alkaline Phosphatase); C-reactive protein (CRP); ferritin; Triglycerides; Troponin; Creatine kinase.

BMs will be taken to assess blood glucose levels on a daily basis. Antithrombin and protein c will be collected via safety bloods however, they will be batch analysed as these are not considered safety measurements.

6.1.4 Cardiac Evaluations

12 lead ECGs or telemetry where available, will be conducted as per the relevant table of assessments.

6.1.5 Adverse Events

The definition and reporting requirements for Adverse events are described in Section 10.

6.2 Schedule of Assssments

The schedule of assessments in Table 1 defines the collection of information by timepoint throughout the trial. All patients receiving standard care will be assessed as indicated below. Those assessments marked with a * will be optional for certain study centres due to access to specialist equipment or tests or tolerbality of patients to the procedure.

TABLE 1: Assessments for participants randomised to the standard care arm						
Activities	**Screening and Enrolment (day -1 or day 1)**	**Baseline (day 1)**	**Daily until hospital discharge or hospital day 16, whichever comes first**	**Day 30 (±6 days) ward, home visit or telephone call**	**Day 60 (±6 days) ward, home visit or telephone call**	**Day 90 (±6 days) ward, home visit or telephone call**
Eligibility						
Informed consent	✓					
Review and confirm eligibility	✓					
Urine pregnancy test	✓[a]					
Review of SARS-CoV-2 diagnostic tests	✓					
Medical history	✓[b]					
12-lead Electrocardiogram	✓ [d]	✓ [d f]	✓ [e]			
Study intervention						
Randomisation (day -1 or day 1)	✓	✓				
Administation of treatment in addition to SoC	**Defined in each protocol appendix**					
SoC treatment	✓	✓	✓			
Clinical study procedures and assessments						
NEWS2 Score and WHO ordinal scale		✓	✓			
Vital signs including SpO2, FiO2, RR, PR		✓ [e]	✓ [e]			
Physical assessment (including presenting symptoms, height, weight)	✓ [e]					
Targeted physical examination (focused on lung auscultation)			✓ [g]			
Chest X Ray and CT Perfusion/CTPA		✓	✓			

		(both a CXR and CTPA will take place prior to treatment [c] *	**(both a CXR and CT Perfusion/CTPA will take place post treatment or as close to discharge as possible [c] ***			
Safety assessments						
Laboratory Safety Assessments, routine bloods and BMs	✓[d]	✓[f]	✓[e]			
AE/SAE recording and assessment		✓	✓	✓	✓	✓
Survival status		✓	✓	✓	✓	✓
Research laboratory sampling						
Biomarker blood sampling (10ml) and Thromboelastography		✓ *	✓ [h]*			
Throat and upper airway nasal absorption samples	✓*	✓*	**Days 1, 3, 5, 8, 11, 15 (all ±1 day) while hospitalised***			
Viral Load (saliva)	✓		**Days 1, 3, 5, 8, 11, 15 (all ±1 day) while hospitalised**			

a **only women of child bearing potential**
b **Medical history includes estimated date of first symptoms and number of co-morbidities (eg, respiratory, cardiovascular, metabolic, malignancy, endocrine, gastrointestinal, immunologic, renal).**
c **Due to logistics during this COVID pandemic, it may not be possible to complete these scans. Wherever possible, imaging will take place but if it cannot take place, the patient will remain in the study. A baseline CXR and CT Perfusion/CTPA scan (pre-treatment) and a post treatment CT Perfusion/CTPA and CXR scan will be taken. Post treatment scans will take place as close to discharge as possible. If CXR or CTPA scans have been taken as part of clinical care within the past 48 hours, results can be recorded and no additional scans are required.**

d **If these assessments have been done as part of routine clinical care within the last 48 hours, results can be recorded and no additional assessment required. For bloods please refer to section 6.1.3 for a list of the blood tests required.**

e **If these assessments have been taken as part of routine clinical care on the same calendar day as the research assessment is to be conducted, results can be recorded and no additional assessments conducted.**

f **If these assessments were done as part of screening they do not require to be repeated.**

g **A symptom-directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint.**

h **Biomarkers will be collected on Day1 (baseline) and then at D4 and D7/or discharge if before this.**

*** This assessment will be optional if the study team cannot access the specialist equipment or testing facility to complete this assessment or if the participant declines.**

6.3 IMAGING

Ideally, up to two Chest X Rays and up to two CT Perfusion/CT Perfusion Pulmonary Angiogram scans (iodine will be the contrast agent) will be performed. Imaging will determine the extent of microthrombus in the pulmonary vasucular circulation. Due to logistics and capacity during this COVID pandemic, it may not be possible to complete these scans. Imaging will be scheduled to take place but if for whatever reason it does not go ahead, the patient will remain in the study. A baseline scan (pre-treatment) and a post treatment scan will be taken. A post treatment scan will take place as close to discharge as possible. If these scans have been conducted for clinical reasons within the past 48 hours, results can be reviewed and recorded.

6.4 LONG TERM FOLLOW UP ASSESSMENTS

For those hospitalised, participants will be followed up for 90 days and any adverse events will be recorded post discharge. These follow-ups may be completed in person (e.g. on the ward or at home) or by telephone.

6.5 STORAGE AND ANALYSIS OF SAMPLES

Full details of the storage and analysis of samples, including the laboratories involved, can be found in the separate clinical samples working instruction document (DEFINE – WI001 Clinical Samples).

As samples are infectious and taken from COVID-19 patients, handling, storage and analysis will be conducted in accordance with the appropriate requirements in line with UK Health and Safety Executive and local Health and Safety committee review and guidance.

Biomarker blood samples will be processed in a University of Edinburgh CL2 lab at the QMRI, if deemed appropriate in accordance with updated HSE/PHE guidance on research blood samples from COVID-19 patients. Materials with high levels of virus e.g. BAL or samples where cells will be cultured after isolation will be processed in a University CL3 with full HSE approval and notification. If taken outside of Edinburgh, biomarker bloods will be processed, stored frozen and shipped to Edinburgh for analysis when appropriate.

PK/PD bloods will either be analysed in the Centre for Inflammation Research or externally. A Material Transfer Agreement will be in place between the University and an external lab prior to any sample transfer.

Saliva samples and nose/throat swabs will be sent for analysis to either NHS/Academic labs or external labs.

Blood plasma and processed respiratory samples will be stored for a period of 10 years in the Centre for Inflammation Research.

Rationale and justification for Biomarker analysis:

Cytokine release syndrome is associated with severe pathology in a subset of COVID-19 patients. This phenomenon occurs 7-10 days after initial symptoms and is associated with the need for ventilatory support, a hypercoagulable state and high mortality. The trigger for this state is uncertain. Superficially the lung injury seems similar to acute respiratory distress syndrome (ARDS). However COVID-induced lung inflammation does not behave like ARDS physiologically, and the limited post mortem data indicates a preponderance of monocytes/macrophages in the injured lung as opposed to the neutrophil-dominant diffuse alveolar damage associated with ARDS.

Significantly higher levels of IL-1β, IL-6, IL-8 and TNF-α have been identified in the serum of patients requiring intensive care, leading scientists at Mount Sinai Hospital (New York) and the Spallanzani Hospital and National Institute for Infectious Disease in Rome to propose this as a core signature indicative of a cytokine storm. These four cytokines have been incorporated into a multiplexed assay allowing rapid quantitation of serum cytokines on an automated ELISA platform with rapid turnaround. Broader analyses using highly multiplexed assays have identified a few analytes, including CXCL-10 and IL-1ra, as factors significantly elevated in patients with severe COVID-19. We will measure these and other cytokines using multiplexed assays.

Participant samples may be analysed for biological indices detailed below – however, this is not an exhaustive list. Due to the nature of this research additional analytical tests may be developed or required in order to profile COVID-19 and develop therapies. All tests will be defined in relevant Standard Operating Procedures / work instructions. Analyses of samples may include DNA or genome wide analysis. Explicit consent for such analyses will be sought from participants.

- Development of **standardised assays** to characterise inflammatory changes or immune responses in patients presenting with COVID-19, especially, but not limited to, those who develop severe lung injury.

- **Flow cytometry** to phenotype cellular subsets including neutrophils, monocytes and T cell subsets, assaying markers of activation, exhaustion or cell death and regulatory T cells as well as cytokine production.

- **Cytokine quantification** to measure plasma cytokines for a broad spectrum of targets upregulated during COVID-19 infection.

- Isolation of protein and RNA from peripheral blood mononuclear cells, neutrophils, adherent respiratory cells (alveolar macrophages) or other BAL cell populations if present.

- Isolate identified cells from blood and respiratory samples to perform functional assays of isolated alone or with epithelial cells in the presence or absence of target drugs, to develop a simple screen for the effectiveness of experimental drugs.

7. DATA COLLECTION

Screening, baseline, study treatment period and follow up data is outlined in the table of assessments in each appendix.

SOURCE DATA DOCUMENTATION

Source data is defined as all information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents.

Source documents are original documents, data and records where source data are recorded for the first time.

7.1 CASE REPORT FORMS

In the first instance, this study will use a paper case report form and these will be specific for the various treatment arms. When an electronic CRF has been designed, the research team will upload previous data entered onto a paper CRF.

7.2 TRIAL DATABASE

The electronic data will be stored securely and confidentially on a Master Study database in REDCap at the Data & Statistics Centre at the Edinburgh CTU (ECTU, University of Edinburgh). Data entry will be done remotely via a dedicated Master Study web portal, with separate web pages and functionality for each qualifying study. There will be a library of common elements to build the core e-CRF for each study, supplemented by bespoke e-CRF for individual studies according to their requirements. Access will be role-based, with remote data entry at the clinical site by authorised users, trained in their data entry tasks.

Query sets will be agreed by researchers and ECTU data managers, and the resultant queries resolved at the site and uploaded. There will be improbable/impossible value checks hardcoded into the eCRF to minimise rogue data being entered at point of entry.

Authorised clients (such as the study statistician needing access to produce progress reports to the independent Data Monitoring Committee and/or the interim or final statistical analyses; and the Trial Manager to produce blinded logistics reports; and the QA manager for audit and ongoing quality assurance reports) will be given protected access to relevant data.

The pseudo-anonymised electronic data will be retained for a period of 25 years, or according to the Funders requirements. The study data will be made available after an appropriate delay for sharing for the purpose of legitimate research projects, with a protocol submitted for approval to the investigators.

7.3 ARCHIVING OF THE TRIAL DATABASE

Following the end of the trial, electronic data will be archived by ECTU.

8. DATA MANAGEMENT

8.1.1 Personal Data

The following personal data will be collected as part of the research:

- Patient details (e.g. name, CHI number, date of birth, sex, ethnicity, email address, telephone number, health information, smoking status, residence in care home status).

No personal data will be held electronically.

All paper files containing personal data will be held in site files. These files will be held securely in a locked filing cabinet in a key card restricted area of the clinical site. Access to the research documents will be by the research team only.

CHI numbers (obtained from the paper files) will be used for checking medical records on an ongoing basis as part of the study.

8.1.2 Transfer of Data

De-identified data collected or generated by the study will be transferred to external individuals or organisations outside of the Sponsoring organisation(s). No personal data will be shared. This is vital to ensure that any findings can be fed into national trials and consortia involved in COVID-19 treatments.

8.1.3 Data Controller

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

8.1.4 Data Breaches

Any data breaches will be reported to the University of Edinburgh and NHS Lothian Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

9. STATISTICS AND DATA ANALYSIS

9.1 SAMPLE SIZE CALCULATION

The proposed 'hybrid' platform (multiple interventions) and basket (multiple phenotypes) type randomised trial is early phase to investigate biomarker response relevant to demonstrating COVID-19 clinical activity in re-purposed drugs. As such, formal sample size calculations which would be mandatory for a confirmatory phase III randomised trial are neither feasible nor appropriate.

However, an indicative sample size of 20 per group, considering the comparison of one active drug against control, assuming 5% missing data, would give 80% power at a 1-sided 10% level of significance, using a two sample t-test, to detect an effect size of 0.7 in the difference of means in the biomarker between active and control.

That is, the study would be able to detect a mean difference of 0.7 standard deviations for the biomarker. So if biomarker was diastolic blood pressure, and the standard deviation was 10 mmHg, the study could detect a mean difference of 7 mmHg.

9.2 PROPOSED ANALYSES

The statistical analyses for each study will be comprehensively specified in a Study Specific Appendix for each separate study, in a study specific Statistical Analysis Plan (SAP) pre specified before data lock.

If there are multiple comparisons then these will be appropriately adjusted for to control the experiment-wise false positive rate. Usually the biomarker will be a continuous measure and the treatment effect estimated via a linear model which will adjust for baseline covariates highly correlated with the primary outcome, including possibly the baseline measurement of the primary outcome biomarker.

The study will not be powered for subgroup analyses and these will be exploratory, on a limited number of subgroups pre-specified in the study specific SAP.

Due to the small sample sizes there will be no formal adjustment for missing data, and the primary analysis set – appropriate for early phase proof of signal studies – could be a suitably defined per-protocol set (e.g. those that were compliant with their randomised medications).

Safety data will be analysed on the as-treated data-set (anyone who initiated on randomised treatment) and will be presented descriptively.

The independent DMC will scrutinise accumulating data, unblinded to the randomised groups. Their first and foremost responsibility will be the safety of the participants, and the committee may terminate the study at any time on the grounds of safety. They will also inspect the emerging data to see whether the study can be adapted (an arm dropped, for example; or the randomisation allocation altered by adaptive randomisation) or stopped early for either overwhelming evidence of efficacy, or on the other hand, for futility. If a sequential interim analysis is requested, this will be designed with a Bayesian approach to ensure maximal flexibility and ease of implementation and interpretation

10. PHARMACOVIGILANCE

The clinical research team are responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

Full details of contraindications and side effects that have been reported following patient consent to participation to the end of the study can be found in the relevant addendum for each therapeutic intervention.

Participants in the interventional arms will be instructed to contact the research team at any time after consent to study participation if any symptoms develop. For all participants, all adverse events (AE) (except for those listed in Section 10.3.2) that occur from the time of consent until 90 days after the final dose of investigational medication must be recorded in the Case Report Form (CRF) or AE log. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment.

Adverse event reporting will be undertaken for the control (standard care) arm. Clinical data and disease progression will be documented via linkage to control participants' medical records.

All adverse events (AE) that are not related to the patient's underlying condition or clinical interventions will be recorded following consent. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment.

10.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical occurrence in a clinical trial participant which does not necessarily have a causal relationship with an investigational medicinal product (IMP).

An **adverse reaction** (AR) is any untoward and unintended response to an IMP which is related to any dose administered to that participant.

A **serious adverse event** (SAE), **serious adverse reaction** (SAR). Any AE or AR that at any dose:

- results in death of the clinical trial participant;

- is life threatening*;
- requires in-patient hospitalisation^ or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect;
- results in any other significant medical event not meeting the criteria above.

*Life-threatening in the definition of an SAE or SAR refers to an event where 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.

^Any hospitalisation that was planned prior to enrolment will not meet SAE criteria. Any hospitalisation that is planned post enrolment will meet the SAE criteria.

A suspected unexpected serious adverse reaction (SUSAR) is any AR that is classified as serious and is suspected to be related to the IMP, that it is not consistent with the information about the IMP in the Summary of Product Characteristics (SPC) booklet, Investigator's Brochure or product specific protocol addendum.

10.2 IDENTIFYING AEs AND SAEs

If there is any doubt as to whether a clinical observation is an AE, the event will be recorded.

AEs and SAEs may also be identified via information from support departments e.g. laboratories.

10.3 RECORDING AEs AND SAEs

When an AE/SAE occurs, it is the responsibility of the Chief Investigator, or another suitably qualified physician in the research team who is delegated to record and report AEs/SAEs, to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the CRF/AE log and on the SAE form (if the AE meets the criteria of serious).

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

10.3.1 Pre-existing Medical Conditions

Pre-existing medical conditions (i.e. existed prior to informed consent) should be recorded as medical history and only recorded as adverse events if medically judged to have worsened during the study.

10.3.2 Worsening of the Underlying Condition during the Trial

Medical occurrences or symptoms of deterioration that are expected due to the participant's underlying condition should be recorded in the patient's medical notes and only be recorded as AEs on the AE log if medically judged to have unexpectedly worsened during the study. ***Events that are consistent with the expected progression of the underlying disease should not be recorded as AEs. These may include:***

- ***Respiratory failure requiring ventilator support***
- ***Specific organ function support (heart failure, myocardial infarction, stroke, pulmonary embolism, liver or renal failure requiring dialysis or haemofiltration)***
- ***Death due to COVID-19 unless administration of investigational medication is considered to have contributed to these events.***

MedDRA terms for the adverse events that will not be reported have been included in the table below:

• **Respiratory failure requiring ventilator support**	Respiratory Failure	Respiratory Failure
	Ventilatory Failure	Respiratory Failure
	Dependence on Ventilator	Dependence on respirator
• **Specific organ function support (heart failure, myocardial infarction, stroke, pulmonary embolism, liver or renal failure requiring dialysis or haemofiltration)**	Heart Failure	Cardiac Failure
	Myocardial Infarction	Myocardial Infarction
	Pulmonary embolism	Pulmonary embolism
	Stroke	Cerebrovascular accident
	Liver failure	Hepatic Failure
	Renal failure	Renal Failure
	Dialysis	Dialysis
	Haemofiltration	Haemofiltration
• **Death due to COVID-19 unless administration of investigational medication is considered to have contributed to these events.**	Death	Death

10.4 ASSESSMENT OF AEs AND SAEs

Each AE must be assessed for seriousness, causality, severity and ARs must be assessed for expectedness by the Principal Investigator or another suitably qualified physician in the research team who has been delegated this role.

The Chief Investigator (CI) may not downgrade an event that has been assessed by an Investigator as an SAE or SUSAR, but can upgrade an AE to an SAE, SAR or SUSAR if appropriate.

10.4.1 Assessment of Seriousness

The Investigator will make an assessment of seriousness as defined in Section 10.1.

10.4.2 Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to the IMP according to the definitions below.

- Unrelated: where an event is not considered to be related to the IMP.

- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the study drug.

Where non Investigational Medicinal Products (NIMPs) e.g. rescue/escape drugs are given: if the AE is considered to be related to an interaction between the IMP and the NIMP, or where the AE might be linked to either the IMP or the NIMP but cannot be clearly attributed to either one of these, the event will be considered as an AR. Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated. The blind should not be broken for the purpose of making this assessment.

10.4.3 Assessment of Expectedness

If the event is an AR the evaluation of expectedness will be made based on the Reference Safety Information as defined or cited in the relevant protocol appendix.

The event may be classed as either:

Expected: the AR is consistent with the toxicity of the IMP listed in the Refernce Safety Information.

Unexpected: the AR is not consistent with the toxicity in the Reference Safety Information.

Fatal and life threatening SARs should usually be considered unexpected. Fatal SARs can only be expected for IMPs with an MA in the EU, when it is clearly stated in the Reference Safety Infromation that the IMP causes fatal SARs.

10.4.4 Assessment of Severity

The Investigator will make an assessment of severity for each AE/SAE/SAR/SUSAR and record this on the CRF/AE log or SAE form according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.
Severe: an event that prevents normal everyday activities.

Note: the term ‘severe’, used to describe the intensity, should not be confused with ‘serious’ which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

10.5 RECORDING OF AEs

All adverse events for each participant will be recorded on the AE log and will be assigned the appropriate MedDRA Systems Organ Class (SOC) code.

10.6 REPORTING OF SAEs/SARs/SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance **within 24 hours**. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying ACCORD. The SAE report form can be updated when the additional information is received.

The SAE report will provide an assessment of causality and expectedness at the time of the initial report to ACCORD according to Sections 10.4.2, Assessment of Causality and 10.4.3, Assessment of Expectedness.

The SAE form will be transmitted via email to safety@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

Any therapy-specific onward reporting safety requirements are detailed in the appropriate appendices. All reports sent to ACCORD and any follow up information will be retained by the Investigator in the Investigator Site File (ISF).

10.7 REGULATORY REPORTING REQUIREMENTS

ACCORD is responsible for pharmacovigilance reporting on behalf of the co-sponsors (The University of Edinburgh and NHS Lothian).

ACCORD has a legal responsibility to notify the regulatory competent authority and relevant ethics committee (Research Ethics Committee (REC) that approved the trial). Fatal or life threatening SUSARs will be reported no later than 7 calendar days and all other SUSARs will be reported no later than 15 calendar days after ACCORD is first aware of the reaction.

ACCORD (or delegate) will inform Investigators at participating sites of all SUSARs and any other arising safety information.

ACCORD will be responsible for providing safety line listings and assistance; however, it is the responsibility of the Investigator to prepare the Development Safety Update Report. This annual report lists all SARs and SUSARs reported during that time period. The responsibility of submitting the Development Safety Update Report to the regulatory authority and RECs, lies with ACCORD.

10.8 PREGNANCY REPORTING

All pregnancies that occur within the active trial period (either the trial participant or the participant's partner) will be reported to the CI and sponsor using the relevant Pregnancy Notification Form within 24 hours of notification. The pregnancy will be followed up until the end of pregnancy. If the trial participant is a male, informed consent will be sought from his female partner.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, thus would be considered an SAE.

10.9 FOLLOW UP PROCEDURES

After initially recording an AE or recording and reporting an SAE, the Investigator should make every effort to follow each event until a final outcome can be recorded or reported as necessary. Follow up information on an SAE will be reported to the ACCORD office.

If, after follow up, resolution of an event cannot be established, an explanation should be recorded on the CRF or AE log or additional information section of SAE form.

11. TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

11.1 TRIAL MANAGEMENT GROUP

The trial will be coordinated by a Project Management Group, consisting of the grant holders, the Chief Investigator, Project Managers and members of the clinical research team.

A Delegation Log will be prepared for each site, detailing the responsibilities of each member of staff working on the trial.

11.2 TRIAL STEERING COMMITTEE (TSC)

A TSC will be convened for this study. The TSC will provide oversight with independent input and will work in collaboration with the research team to decide on which therapies will be tested as part of this platform. A stand alone document details the therapy selection process.

11.2 DATA MONITORING COMMITTEE

An independent DMC will be established to oversee the safety of participants.

During the study, the DMC will review any safety assessments that the research team present to them if there are any concerns raised.

The DMC will continually review any SUSARS or other safety signals that are reported. The DMC will also have scheduled meetings to review the safety data at intervals of every 5 patients who have completed the dosing regime for each IMP. The precise workings of the DMC is detailed in the DMC charter.

11.4 SUB STUDIES

Proposals for sub-studies must be approved by the Trial Management Group and by the relevant ethics committee and competent authorities (where required) as a substantial amendment or separate study before they begin.

11.5 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of an audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

11.6 STUDY MONITORING AND AUDIT

ACCORD clinical trial monitors, or designees, will perform monitoring activities in accordance with the study monitoring plan. This will involve on-site visits and remote monitoring activities as necessary. ACCORD QA personnel, or designees, will perform study audits in accordance with the study audit plan. This will involve investigator site audits, study management audits and facility (including 3rd parties) audits as necessary (delete where not required).

12. GOOD CLINICAL PRACTICE

12.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).
Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met. Required approvals will be expedited via the NIHR COVID-19 research portal.

12.2 REGULATORY COMPLIANCE

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, as amended.

12.3 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

Delegated tasks must be documented on a Delegation Log and signed by all those named on the list prior to undertaking applicable study-related procedures.

12.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any study specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants or personal legal representatives must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant or personal legal representatives must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant or personal legal representatives must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant or personal legal representatives will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s).

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The original will be signed in the Investigator Site File (ISF). The participant will receive a copy of the signed consent form and a copy will be filed in the participant's medical notes.

12.3.2 Study Site Staff

The Investigator must be familiar with the IMP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties.

12.3.3 Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

12.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the ACCORD Research Governance & QA Office, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.
- ACCORD will ensure all other documents required by ICH GCP are retained in a Trial Master File (TMF) or Sponsor File, where required. The Principal Investigator will ensure that the required documentation is available in local Investigator Site files ISFs. Under certain circumstances the TMF responsibilities may be delegated to the research team by ACCORD.

12.3.5 GCP Training

All study staff must hold evidence of appropriate GCP training.

12.3.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

12.3.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including where applicable the General Data Protection Regulation with regard to the collection, storage, processing and disclosure of personal information. Access to personal information will be restricted to individuals from the research team treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

13. STUDY CONDUCT RESPONSIBILITIES

13.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Proposed amendments will be submitted to the Sponsor for classification and authorisation.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to implementation.

13.2 PROTOCOL NON COMPLIANCE

13.2.1 Definitions

Deviation - Any change, divergence, or departure from the study design, procedures defined in the protocol or GCP that does not significantly affect a subjects rights, safety, or well-being, or study outcomes.
Violation - A deviation that may potentially significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being

13.2.2 Protocol Waivers

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

13.2.3 Management of Deviations and Violations

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. Deviation logs / violation forms will be transmitted via email to QA@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

13.3 URGENT SAFETY MEASURES

The Investigator may implement a deviation from or change to the protocol to eliminate an **immediate hazard** to trial participants without prior approval from the REC and the MHRA. This is defined as an urgent safety measure and the investigator must contact the Clinical Trial Unit at the MHRA and discuss the issue with a medical assessor immediately (+44 (0) 20 3080 6456).

The Investigator will then notify the MHRA (clintrialhelpline@mhra.gsi.gov.uk), the REC and ACCORD, in writing of the measures taken and the reason for the measures within 3 days by submitting a substantial amendment.

13.4 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree: (a)

the safety or physical or mental integrity of the participants of the trial; or (b)

the scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (QA@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to regulatory authorities and research ethics committees as necessary.

13.5 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 15 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

13.6 END OF TRIAL

The end of the scheduled treatment phase is defined as the date of the last treatment day of the last participant. The end of the trial is defined as the last data entry for the last participant completing the scheduled treatment phase.

The Investigators or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.
The Investigators and/or the trial management group and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, Regulatory Authority, R&D Office(s) and cosponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the cosponsors via email to resgov@accord.scot.

In accordance with ACCORD SOP CR011, a Clinical Study Report (CSR) will be provided to the Sponsor (QA@accord.scot) and REC within 1 year of the end of the study.

Upon completion of the study, the Investigator will upload clinical trial results onto the EudraCT database on behalf of the Sponsor.

The Investigator will submit a short confirmatory e-mail to the MHRA (CT.Submission@mhra.gsi.gov.uk) once the result-related information has been uploaded to EudraCT, with 'End of trial: result-related information: EudraCT XXXX-XXXXXX-XX' as the subject line. The Sponsor(s) will be copied in this e-mail (QA@accord.scot). It should be noted that you will not get an acknowledgment e-mail or letter from the MHRA.

13.7 CONTINUATION OF DRUG FOLLOWING THE END OF STUDY

Access to the experimental therapies will be not be permitted following the end of the participant's treatment period.

13.8 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been authored by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities. Sites which are part of the United Kingdom's National Health Service have the benefit of NHS Indemnity.

- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.
- The manufacturer supplying IMP has accepted limited liability related to the manufacturing and original packaging of the study drug and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown Adverse Events which arise out of the manufacturing and original packaging of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

14. REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

14.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with ICH guidelines.

14.2 PUBLICATION

Scientific publications and the sharing of clinical data generated as part of this trial is crucial to better understanding COVID-19 and developing new treatments. As such, the results of each nested study detailed in the relevant appendices will be published as soon as the treatment arm has finished recruitment, data has been cleaned and any outstanding patient safety follow-ups completed.

The Clinical Study Report (CSR) will be submitted to the Sponsor and REC within 1 year of the end of the study. Where acceptable, a published journal article may be submitted as the CSR. The Chief Investigator will provide the CSR to ACCORD, for review, prior to finalization. The clinical study report may be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study. The results of the study, together with other mandated information, will be uploaded to the European clinical trials database within 1 year of the end of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion).

14.3 DATA SHARING

Consent will be sought from participants to permit sharing of anonymised data with funders, commercial and non-commercial collaborators or published on publically available resources as appropriate.

14.4 PEER REVIEW

This protocol has been reviewed by the University of Edinburgh emergency COVID-19 College of Medicine and Veterinary Medicine committee.

Academic and Clinical Central Office for Research and Development

DEFINE

Evaluating therapies for COVID 19

PROTOCOL APPENDIX 1

Investigational Medicinal Product:	Galactin-3 Inhibitor (TD139)
Route of administration:	Dry powder for inhalation
Manufacturer:	Galecto Incorporated
DEFINE COVID-19 Cohort(s):	**Cohort 2A and 2B**

APPENDIX APPROVAL SIGNATURE PAGE

DEFINE - Evaluating therapies for COVID 19

Signature details removed for registry upload

CONTENTS

INTRODUCTION

1.1 BACKGROUND

Galectin-3 is a pro-fibrotic, mammalian ß-galactoside binding lectin found to be highly upregulated in the injured lung, particularly in patients with idiopathic pulmonary fibrosis (IPF) and in those suffering acute exacerbations (1). It is also an important regulator of immune homeostasis and response to infections. Global deletion of Gal-3 reduces collagen deposition in both bleomycin and TGFβ adenovirus-induced pulmonary fibrosis models (1). Gal-3 has been shown to stimulate migration and collagen synthesis in fibroblasts (2) whilst regulating alternative, pro-fibrotic and macrophage activation (3). Galectin-1 and -3, can affect various aspects of viral infections, including viral binding, replication, budding, transmission, and infection associated inflammation.

COVID-19 is a viral disease with no immediate remedies. Galecto, Inc. have developed a galectin-3 inhibitor (Gal-3i) which has been tested in man and shown tolerability and effects on biomarkers suggesting it may be useful in COVID-19 related pneumonitis, subsequent morbidity and remaining lung tissue destruction post-infection. Galecto's lead inhaled small molecule Gal-3i (TD139) works by binding to gal-3 and preventing the pro-fibrotic and pro-inflammatory activities of gal-3. The primary cellular targets include macrophages and neutrophils.

It is the purpose of this investigation to examine the potential for delivery of this inhibitor in pre-ventilator patients hospitalised with COVID-19 to examine whether this may lead to detectable changes in blood biomarkers, reduce viral load and also reduce disease severity such as time to ventilation. The control arm will be patients receiving standard clinical care. Galecto have shown in sterile models of acute lung injury that Gal-3 is pro-inflammatory and that selective inhibition with TD139 reduces injury via a reduction in neutrophil recruitment and activation.

TD139 has been shown to:

- Reduce pro-inflammatory and pro-fibrotic cytokines/chemokines in the bronchoalveolar lavage fluid
- Reduce histology inflammation score in pulmonary injury models
- Accelerate neutrophil apoptosis and neutrophil activation
- Reduce interstitial neutrophil recruitment and activation levels in vivo
- Shift M2 macrophage phenotype in vivo

1.2 Safety Of TD139

1.2.1 Preclinical Safety Profile

TD139 is non genotoxic and has been shown to be well tolerated when administered via inhalation in repeat dose toxicology studies of 2-39 weeks duration in rodents and dogs. There is no evidence of clinical significant potential for drug-drug interactions. Although systemic concentrations are achieved in all animal species following pulmonary delivery, systemic exposure is low. No impact was observed on measures of cardiac, respiratory or CNS function during in vitro/ in vivo preclinical studies. A beneficial effect of treatment was observed in animal models of lung inflammation. Based on preclinical information, TD139 is assessed as being of low risk and potential benefit in humans with lung inflammation.

1.2.2 Previous Human Experience

A first-in-human clinical study (GB-HV-01) of TD139 has been completed in healthy volunteers and in patients with IPF. In healthy volunteers, TD139 was administered as a dry powder via

inhalation using a Plastiape™ device at the following doses; 0.15mg, 1.5mg, 3mg, 10mg, 20mg and 50mg. TD139 demonstrated predictable PK characteristics with dose proportionality observed throughout the tested dosing range. No serious AEs were observed. Treatment emergent adverse events (TEAEs) were reported by ~42% (15/36) subjects; the observed AEs were mild in severity and resolved without intervention. The most commonly occurring TEAE associated with TD139 was mild dysgeusia (36.1% of subjects) that tended to be reported more frequently at the higher dose levels (10 mg, 20 mg and 50 mg) when compared to placebo, 0.15 mg, 1.5 mg and 3 mg of TD139. The dysgeusia was considered to be of almost definite relationship to the study medication, occurring at/immediately (0 to 1 minute) post dose and was generally transient in nature lasting between 1 and 59 minutes in all but 2 subjects. TD139 was administered as a neat blend at the three highest doses, and it is likely taste is directly related to TD139 or the masking effect of lactose. Cough was also reported in the 3 mg, 10 mg and 50 mg TD139 dose groups, although the incidence was low (8.3% of subjects overall). There were no other TEAEs of note and in general apart from the dysgeusia at the higher dose levels, there did not appear to be any dose related trends observed in any TEAE profile. TD139 had no impact on biochemistry, hematology, vital signs or 12 lead ECG data.

In Part 2 of this study, TD139 doses of up to 10mg were administered daily for 14 days to patients with IPF. TD139 was considered to be well tolerated in this part of the study. One patient developed a community acquired pneumonia symptoms of which began 2 days after completion of study medication following a protocol required bronchoscopy. Despite hospitalization, antibiotic treatment and ventilatory support the patient developed multiorgan failure and died one month after study completion. This event was not considered to be due to study medication. A total of 54 mild and moderate TEAEs were reported by 11 (45.8%) and 8 (33.3%) of patients, respectively, during the study. The most commonly occurring TEAE was cough (mild-moderate severity) reported by 1 patient in the placebo group, 1 patient in the 0.3mg daily group and 2 patients in the 10mg daily cohort. With the exception of one patient receiving 10mg daily, all these events were single events, were mild and self-limiting. One patient in the 10mg dose group reported cough for 3 consecutive days toward the end of their treatment period (day 10 to 13). One patient had reported pyrexia on two occasions in association with an upper respiratory tract infection which was treated with doxycycline. There were no other TEAEs of note and there did not appear to be any dose-related trends observed in any TEAE profile. In addition, there were no clinically significant changes observed in biochemistry, haematology, or 12-lead ECGs following treatment with placebo, 0.3 mg, 3 mg or 10 mg.

An ongoing study is investigating the efficacy of 10mg daily TD139 to prevent deterioration of airways function in patients with IPF. The study will recruit 450 subjects.

1.3 Benefit-Risk Statement

TD139 is a specific inhibitor of galectin-3 which has been investigated in healthy volunteers and patients with IPF. No serious drug related serious adverse events have been reported to date. TD139 had no impact on cardiac, haematological or biochemical measures of safety during trials in humans to date. Beneficial effects on biomarker measures of lung inflammation were observed in patients with IPF. Systemic exposure to the drug occurs following pulmonary delivery, but concentrations reached are well within the no overall adverse effect level noted in animal toxicology studies. Patients enrolled into the trial will be under direct observation in hospital and thereby will be kept under close observation throughout the treatment period. Patients will undergo regular assessment of clinical status, including respiratory function measures (SpO_2, RR), cardiac monitoring and assessment of laboratory safety measures. In the event of any perceived adverse effect of treatment -which is being given open label – TD139 treatment can be immediately withdrawn and patients given relevant supportive treatment for any adverse effect observed. Based on these considerations, the potential for benefit in COVID-

19 disease exceeds the known and potential risks of TD139 therapy in the proposed clinical study.

1.4 ADAPTIVE PROTOCOL DESIGN

This appendix has been written to be used in conjunction with the main DEFINE COVID-19 trial protocol which describes an overarching and adaptive trial design to test candidate therapies for COVID-19 positive patients.

The information detailed within this appendix applies only to Galactin-3 Inhibitor (TD139).

2. INTERVENTION-SPECIFIC OBJECTIVES

2.1 OBJECTIVES AND ENDPOINTS

In addition to the master protocol objectives and endpoints, treatment specific objectives and endpoints are highlighted below.

Objectives	Endpoints
Primary	
To evaluate the safety of candidate agents as add-on therapy to SoC in patients with COVID-19.	Safety will be assessed using: • Haematological and biochemical safety laboratory investigations. • Physical examination • Vital signs (blood pressure/heart rate/temperature and respiratory rate) • Daily electrocardiogram (ECG) readings • Adverse events
Secondary	
To explore the PK or appropriate surrogate of bioavailability of the proposed trial treatments in COVID-19 patients.	To explore the PK/PD of the proposed trial treatments
Assess the response of key exploratory biomarkers during treatment period.	Evaluate the change from baseline values for key exploratory biomarkers of target engagement for each treatment. See below for selected biomarkers.
To evaluate the improvement or deteroriation of patients in each treatment arm.	Record changes to WHO ordinal scale and NEWS2 score
To evaluate the number of oxygen-free days.	Duration (days) of oxygen use and oxygen-free days.
To evaluate ventilator-free days and incidence and duration of any form of new ventilation use.	• Duration (days) of ventilation and ventilation-free days. • Incidence of any form of new ventilation use and duration (days) of new ventilation use.
Change in the ratio of the oxygen saturation to fraction of inspired oxygen concentration (SpO2/FiO2)	• SpO2/FiO2, measured daily from randomisation to Day 15, hospital discharge, or death

To evaluate SARS-CoV-2 viral load.	Qualitative and quantitative polymerase chain reaction (PCR) determination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva samples while hospitalised on Days 1, 3, 5, 8, 11, 15, and oropharyngeal/nasal swab on the same days if tolerated.
To evaluate time to discharge	• Duration of total hospital stay • Duration to discharge following treatment
To evaluate the use of renal dialysis or haemofiltration for each treatment arm.	Record requirement for renal dialysis or haemofiltration
Determine the effect of TD 139 on peripheral blood inflammatory cell mobilisation and phenotype as a surrogate of viral-induced inflammation.	**Daily measurement of:** • **Analysis of cytokine levels in blood as surrogates of viral induced inflammation** • **Perpiheral blood myeloid cell function and exhaustion.**
Determine respiratory tract inflammatory and viral parameters in Bronchoalveolar Lavage Fluid (if undertaken for clinical reasons) and throat and nasal absorption samples (if possible)	**ELISA/LC-MS** • **BALF Gal-3 levels** • **BALF Cytokine levels** • **BALF total protein and IgM for vascular leak assessment** • **BALF PK** • **Urea concentration for epithelial lining fluid calculation** **qPCR** • **Viral load** **Flow Cytometry** • **Full BAL cell and Gal-3 characterisation** **AnnV/PI staining of neutrophils – does Gal-3 inhibition accelerate neutrophil apoptosis.**
Determine the extent of Gal-3 inhibition on the coagulation pathway.	**Measure daily plasma levels of CD40 ligand, von Willebrand Factor (vWF), platelet factor-4 (PF-4), Cd39, ecto-ADPase, nitrous oxide, PGI2, antithrombin, Thrombomodlin, protein c, EPCR, kallikrein and microparticles (MP) using commercial ELISA kits.**
Determine systemic levels of Galectin-3	**Daily measurement of:** • **Plasma Galectin-3**
Determine the effect on certain markers of fibrosis	**Measurement of:** **YKL-40 (Chi3L1), PAI-1, PDGF-AA and -BB, HGF, MMP-8, Osteopontin**

3. DESIGN

This is an open label randomised experimental phase Ib/IIa interventional clinical trial with TD139.

4. STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

It is anticipated that 20 participants from Cohort 2 of the DEFINE COVID-19 trial will be recruited to this arm of the trial. These will be compared to the control arm of standard care within the same target cohorts.

4.2 TARGET POPULATION

Participants from Cohort 2 will be recruited to this arm.

Cohort 2 (Both 2A and 2B)	Hospitalised confirmed COVID positive patients with: new changes on CXR or new changes on CT compatible with COVID-19 with or without supplemental oxygen

4.3 INCLUSION & EXCLUSION CRITERIA

Eligibility criteria for participants randomised to this treatment arm are outlined below.

Inclusion criteria:

- Provision of informed consent from the patient or representative
- Aged at least 16 years
- COVID-19 positive test result within last 14 days
- Hospitalised patients with confirmed COVID-19 with new changes on CXR or new changes on CT compatible with COVID-19 with or without the requirement for supplemental oxygen (Cohort 2).
- If the patient is a woman or a male partner of a woman of child bearing potential*, the patient, and their partner(s), agree to use medically-accepted double-barrier methods of contraception (eg, barrier methods, including male condom, female condom or diaphragm with spermicidal gel) during the study and for at least 90 days after termination of study therapy. A vasectomised partner would be considered an appropriate birth control method provided that the partner is the sole male sexual partner and the absence of sperm has been confirmed.

Exclusion criteria:

- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1.73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Women who are pregnant or breastfeeding.
- Participation in another clinical trial of an investigational medicinal product (CTIMP)
- Known hypersensitivity to the IMP or excipients (e.g. lactose intolerance).
- Concomittant use of treatments for COVID-19 that are not recognised as locally approved standard care.

- Significant electrolyte disturbance (hyperkalaemia K+ >5.0 mmol/L or hyponatraemia Na+ < 120mmol/L)
- Patient currently receiving potassium sparing diuretics that cannot be reasonably withheld
- Patient currently receving prophylactic or therapeutic anticoagulants or antiplatelet agents that cannot be reasonably withheld if randomised to Nafamostat
- Patients (or their partners) planning on donating sperm/eggs during the trial period
- Ongoing dialysis
- History of serious liver disease (Child Pugh score > 10)
- Severe uncontrolled diabetes mellitus

* A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1 STUDY DRUG

5.1.1 Study Drug Identification

Galectin-3 inhibitor – TD139

5.1.2 Study Drug Owner

Galecto Incorporated

5.1.3 Marketing Authorisation Holder

Not applicable. TD139 is not a licensed medication.

5.1.4 Labelling and Packaging

Galecto Incorporated will be responsible for the providing packaged TD139. TD139 is currently stored in Catalent, Bathgate and will be transported to The Investigational Supplies Group (ISG) for labelling.

Medication labels will be in the local language and comply with the legal requirements of Annex 13 of the European Union's Good Manufacturing Practice (GMP). They will include storage conditions for the drug, but no information about the patient.

5.1.5 Storage

TD139 will be stored in the site pharmacy at room temperature (between 15-25°C) with appropriate temperature monitoring in place. Transport between the manufacturing sites and the clinical site will be temperature monitored.

No temperature monitoring will take place once the IMP has been dispensed from pharmacy.

5.1.6 Regulatory Release to Site

TD139 will be certified by a Qualified Person (QP) at ISG before inviting the Sponsor to release to the clinical trial.

5.1.7 Destruction of Trial Drug

Any IMP remaining at the end of the trial will be returned to the manufacturer.

5.1.8 Investigators Brochure

Please refer to the currently approved TD139 Investigator's Brochure.

5.2 DOSING REGIME

Patients will inhale 5mg x 2 (10 mg) twice daily for the first 48 hrs and then subsequently 5mg x 2 (10 mg) once daily for the remaining 12 days. Unless a participant is discharged from hospital or can no longer use an inhaler – in which case treatment will be stopped at such time.

- **Day 1 - TD139 is administered twice, with the second dose administered 3-4 hours post first dose.**
- **Day 2 - TD139 is administered twice, with the second dose administered 8-10 hours post first dose.**
- **Days 3-14 – TD139 is administered once before midday.**

CE marked inhalers will be provided by the Manufacturer. All patients will receive guidance on how to use the inhaler by an appropriately trained member of the research team. Two individual inhalers will be used by each patient over the course of the 14 day study period (each inhaler will be used by one patient for 7 days) and will be thoroughly cleaned with an antiseptic wipe before and after each use.

5.3 PARTICIPANT COMPLIANCE

TD139 will be administered in the clinical setting under supervision. No compliance issues are anticipated.

5.4 OVERDOSE

There are no current data for overdose with TD139. Overdose should be managed symptomatically.

5.5 OTHER MEDICATIONS

5.5.1 Non-Investigational Medicinal Products

Not applicable.

5.5.2 Permitted Medications

Any drug required for the normal clinical care of these patients will be permitted.

5.5.3 Prohibited Medications

TD139 did not have any dose-limiting toxicity in pre-clinical toxicology studies with no risk for drug-drug interaction. There are no prohibited drugs based on either PK or PD interaction.

6. STUDY ASSESSMENTS

Data collection for the trial is detailed within the main DEFINE COVID-19 trial protocol. Clinical and safety assessments highlighted in blue are in addition to the SoC arm.

A table of assessments for this nested study is provided below. Those assessments marked with a * will be optional for certain study centres due to access to specialist equipment or tests or tolerbality of patients to the procedure.

TABLE 1: Assessments for participants randomised to TD139						
Activities	**Screening and Enrolment (day -1 or day 1)**	**Baseline (day 1)**	**Daily until hospital discharge or hospital day 16, whichever comes first**	**Day 30 (±6 days) ward, home visit or telephone call**	**Day 60 (±6 days) ward, home visit or telephone call**	**Day 90 (±6 days) ward, home visit or telephone call**
Eligibility						
Informed consent	✓					
Review and confirm eligibility	✓					
Urine pregnancy test	✓[a]					
Review of SARS-CoV-2 diagnostic tests	✓					
Medical history	✓[b]					
12-lead Electrocardiogram	✓ [d]	✓ [d f]	✓ [e]			
Study intervention						
Randomisation (day -1 or day 1)	✓					
Administation of treatment in addition to SoC	**On completion of ALL screening and baseline assessments the treatment can be initiated and will continue for 14 days or until hospital discharge, whichever comes first. This means that treatment can be initiated on the same day as Screening and Enrolment and Baseline Visits. If this happens all will be completed on Day1 (there will be no Day -1).**					
SoC treatment	✓	✓	✓			

Clinical study procedures and assessments						
NEWS2 Score and WHO ordinal scale		✓	✓			
Vital signs including SpO2, FiO2, RR, PR		✓e	✓e			
Physical assessment (including presenting symptoms, height, weight)	✓e					
Targeted physical examination (focused on lung auscultation)			✓g			
Cough symptom score		✓	**Daily until 48 hours (± 4 hours) following end of treatment period**			
Safety assessments						
PK/PD		✓c	**Days 3, 5, 8, 11 (all ±1 day) while hospitalised**			
Laboratory Safety Assessments	✓d	✓f	✓e			
AE/SAE recording and assessment		✓	✓	✓	✓	✓
Survival status		✓	✓	✓	✓	✓
Research laboratory sampling						
Biomarker blood sampling (10ml)		✓*	✓*			
Throat and upper airway nasal absorption samples	✓*	✓*	**Days 1, 3, 5, 8, 11, 15 (all ±1 day) while hospitalised***			

Viral Load (saliva)	✓		**Days 1, 3, 5, 8, 11, 15 (all ±1 day) while hospitalised**			

a **only women of child bearing potential**
b **Medical history includes estimated date and time of first symptoms and number of co-morbidities (eg, respiratory, cardiovascular, metabolic, malignancy, endocrine, gastrointestinal, immunologic, renal).**
c **These samples must be taken pre-dose**
d **If these assessments have been done as part of routine clinical care within the last 48 hours, results can be recorded and no additional assessments required. For bloods please refer to section 6.1.3 of the master protocol for a list of the blood tests required.**
e **If these assessments have been taken as part of routine clinical care on the same calendar day as the research assessment is to be conducted, results can be recorded and no additional assessments conducted.**
f **If these assessments were done as part of screening they do not require to be repeated**
g **A symptom-directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint.**

* **This assessment will be optional if the study team cannot access the specialist equipment or testing facility to complete this assessment or if the participant declines.**

6.1 SAMPLING

Existing pharmacokinetic (PK) and pharmacodynamic (PD) data have been obtained from a number of pulmonary fibrosis trials, to date. To provide confidence that this data also reflects COVID-19 positive population, serial blood samples to analyse PK/PD properties will be taken.

Blood samples (8 ml) will be collected at the following intervals to measure the concentration of TD139:

- Prior to TD139 treatment starting (post randomisation, pre first dose of TD139)

PK/PD samples will also be taken on the following study days (+/- 1 day) whilst the participant remains in hospital and prior to TD139 dosing on the day:

- Day 3, 5, 8 and 11

A daily 10 mL blood sample will be obtained from patients to assess for changes in relevant inflammatory biomarkers. These samples will enable the pilot study to investigate if TD139 is affecting key pathways in the inflammatory cascade and will provide valuable mechanistic outcome data.

Saliva and throat/upper airway samples will also be taken as per the table above.

No more than 400 mL of blood will be taken from each patient as part of this treatment arm.

6.2 SAFETY ASSESSMENTS

The key safety assessments for this treatment arm are:

- Daily haematology, biochemistry, liver function tests, coagulation
- Adverse event recording
- Daily ECG

6.3 STUDY ASSESSMENTS

Study assessment are outlined in the table of assessments. In addition, baseline, ongoing and follow up information will be collected as per the main protocol.

6.4 STORAGE AND ANALYSIS OF SAMPLES

Storage of biomarker samples is outlined in the main protocol. PKPD blood samples will be sent to the Centre for inflammation Research or an external lab for analysis. Haematolgical and biochemical bloods taken as part of safety assessments will be analysed in hospital laboratories and then destroyed.

Justification and the rationale for particular biomarkers is included in Section 6.4 of the master protocol.

7. STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE CALCULATION

An indicative sample size, considering the comparison of one active drug against control, each with n=20 per group, and assuming 5% missing data, would give 80% power at a 1-sided 10% level of significance, using a two sample t-test, to detect an effect size of 0.7 in the difference of means in the biomarker between active and control.

That is, the study would be able to detect a mean difference of 0.7 standard deviations for the biomarker. So if biomarker was diastolic blood pressure, and the standard deviation was 10 mmHg, the study could detect a mean difference of 7 mmHg.

7.2 PROPOSED ANALYSES

The statistical analyses for each study will be comprehensively specified in a study specific Statistical Analysis Plan (SAP).

8. PHARMACOVIGILANCE

Pharmacovigilance procedures are detailed within the main trial protocol.

8.1 ADDITIONAL REPORTING REQUIREMENTS

The assessment of expectedness will be made against the reference safety information found in the Investigator's Brochure (section 5.5). No Serious Adverse Reactions (SARs) are considered expected by Galecto for the purpose of expedited reporting of Suspected Unexpected Serious Adverse Reaction (SUSARs) and identification of SUSARs in the "Cumulative summary tabulation of serious adverse reactions" in the Development Safety Update Report (DSUR) for the IMP.

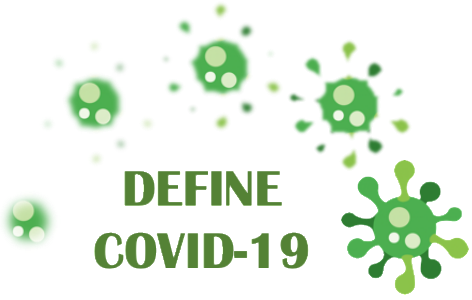
All SAEs that occur in participants dosed with TD139 will be reported to the Sponsor as specified in the main protocol (i.e. within 24 hours of identification of the event). The Sponosr will undertake to report the event onward to Galecto's representatives (listed below) within one working day of notification by site.

Anders Petersen (AP@galecto.com)
Bertil Lindmark (bertil.lindmark@galecto.com)
Hans Schambye (hs@galecto.com)

Additionally, all SAEs considered to be possibly or probably serious reactions to TD139 will be reported according to Section 10.6 of the Master Protocol.

9. REFERENCES

1. Mackinnon AC, Gibbons MA, Farnworth SL, et al. Regulation of transforming growth factor-β1-driven lung fibrosis by galectin-3. Am J Respir Crit Care Med. 2012;185(5):537–546.
2. Nishi Y, Sano H, Kawashima T, et al. Role of galectin-3 in human pulmonary fibrosis. Allergol Int. 2007;56(1):57–65.
3. MacKinnon AC, Farnworth SL, Hodkinson PS, et al. Regulation of alternative macrophage activation by galectin-3. J Immunol. 2008;180(4):2650–2658.
4. Slack RJ, Hirani N Gibons MA et al Translational pharmacology of TD139, an inhaled small molecule galectin-3 (Gal-3) inhibitor for the treatment of idiopathic pulmonary fibrosis (IPF) 21 April 2020 https://doi.org/10.1096/fasebj.2020.34.s1.02311



DEFINE

Evaluating therapies for COVID 19

PROTOCOL APPENDIX 2

Investigational Medicinal Product:	Nafamostat
Route of administration:	Intravenous administration
Manufacturer:	Torii Pharmaceutical Co., Ltd.
DEFINE COVID-19 Cohort(s):	**Cohort 2A and 2B**
This nested study represents a collaboration between the University of Oxford, University of Edinburgh and Nichi-Iko Pharmaceutical Co., Ltd (MA holder).	

APPENDIX APPROVAL SIGNATURE PAGE

DEFINE - Evaluating therapies for COVID 19

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CONTENTS

INTRODUCTION

1.1 BACKGROUND

There are currently only limited therapies for SARS-CoV-2 (COVID-19) (Liu et al. 2020). For COVID-19 to infect human cells, after binding to its receptor (ACE2) on the cell surface, a serine protease that is also expressed at the cell surface called TMPRSS2 (Shirato, Kawase, and Matsuyama 2013) must then 'prime' the S protein on the virus to facilitate its activation and enable the fusion (Chhikara et al. 2020). Two therapies, Camostat (Otsuki et al. 1990) and Nafamostat (Yamamoto et al. 2016), which have been in routine clinical use since 1985 (Liu et al. 2020), are known inhibit this enzymatic priming activity directly (Hoffmann et al. 2020; Kawase et al. 2012; Yamaya and Shimotai 2016; Zhou et al. 2015). Camostat has been shown to inhibit SARS-CoV-2 entry into epithelial cells (Kawase et al. 2012) and improved outcome in vivo in a mouse model of MERS (Zhou et al. 2015), and Nafamostat effectively inhibits MERS-CoV S protein-initiated membrane fusion.

Nafamostat was identified as a potential therapy using a Dual Split Protein (DSP) reporter fusion assay to screen a library consisting of 1,017 FDA-approved drugs. This screening result, together with experimental data from MERS-CoV infection of cultured airway epithelial cell-derived Calu-3 cells, highlighted that Nafamostat, over all other screened compounds, could be effective at inhibiting viral entry. Further trials are ongoing in Japan (Harrison 2020). Thus, these drugs may present an immediate solution to treat COVID-19 infected patients. Camostat is available as an oral formulation, but Nafamostat is administered intravenously and thereby more suitable for in-patient use (Liu et al. 2020; Yamaya and Shimotai 2016).

In addition to its putative antiviral effect, Nafamostat is an inactivator of coagulation fibrinolysis, and platelet aggregation with potent inhibitory activity against thrombin, coagulation factors in active form (XIIa, Xa), kallikrein, plasmin, and complement factors (Clr,Cls). For this reason, Nafamostat is used to treat disseminated intravascular coagulation (DIC) routinely in Japan. In severe cases of COVID-19 patients can progress rapidly and develop acute respiratory distress syndrome, septic shock, metabolic acidosis and coagulopathy. In 183 consecutive patients in Wuhan the 11.5% non-survivors had marked derangements in haemostatic measures at the time of admission with prolongation of APTT, PT, elevated D-dimers and fibrin degradation products (FDP) (Tang et a. 2020) indicating disseminated intravascular coagulopathy (DIC) associated with enhanced fibronolysis. Nafamostat may therefore be effective against COVID-19 from both "anti-viral" and "anti-DIC with enhanced fibrinolysis" perspectives (Asakura and Ogawa 2020).

Nafamostat mesilate was first reported by Fujii in 1981 (Fujii and Hitomi 1981) as a synthetic serine protease inhibitor. Nafamostat is used for the treatment of DIC, acute pancreatitis and as an anticoagulant in extracorporeal haemofiltration/dialysis. Nafamostat has also been investigated for prevention postreperfusion syndrome following solid organ transplantation. Nafamostat is approved in Japan and in several Asian countries as an anticoagulant therapy for patients undergoing continuous renal replacement therapy due to acute kidney injury.

Nafamostat mesilate inhibits various enzymes including thrombin, Xa, and XIIa, the kallikrein–kinin system, the complement system, pancreatic proteases and activation of protease-activated receptors. Nafamostat inhibits lipopolysaccharide-induced nitric oxide production, apoptosis, and interleukin (IL)-6 and IL-8 levels in cultured human trophoblasts (Fujii and Hitomi 1981; Tsukagoshi 2001). It has been shown to act as an antioxidant in TNF-α-induced ROS production (Yamaya and Shimotai 2016). Nafamostat inhibits the activity of pancreatic trypsin, kallikrein, enterokinase, phospholipase A2, C1, and C1 esterase (Fujii and Hitomi 1981; Tsukagoshi 2001).

PHARMACOLOGY AND PK

The approved dosing regimen for the treatment of pancreatitis and DIC is a 24 hour continuous infusion. Intravenous infusion of 0.16 mg/mL in humans results in a plateau plasma concentration of Nafamostat of 0.27 μM, which achieves the range of inhibition demonstrated for the in vitro antiviral effects with IC50s in the range of 0.0001 μM to 0.1 μM.

ANTI-VIRAL PROPERTIES
In a cell-based membrane fusion assay investigating MERS, the IC50 was IC50 = 0.001 to 0.1 μM in a viral fusion assay, depending on the cell type IC50 = 0.0001 μM in an internalized RNA assay and IC50 = 0.01 μM in a viral replication assay (Yamamoto et al. 2016).

Nafamastat inhibited influenza virus A and B replication in MDCK cells, which do not rely on TMPRSSw, with an IC50 of 0.4 (1.2 μM) and 1.5 μg/mL (4.3 μM), respectively (Hosoya et al. 1992).

Both MERS and SARS viruses are thought to have evolved to enter a cell directly across the plasma membrane to bypass the endolysosomal system and evade innate immunity (Shirato et al. 2017; Hoffmann et al. 2020). Viral entry is mediated by the cell surface protease transmembrane protease serine 2 (TMPRSS2), which processes the spike protein. Inhibitors of serine proteases are effective treatments for viral entry (Yamaya and Shimotai 2016; Hosoya et al. 1992; Hoffmann et al. 2020).

The potency of Nafamostat for inhibition of TMPRSS2 has not been reported, but it is reasonable to think it compares to the values reported for similar serine proteases. Specifically, the IC50s in micromolar are: trypsin 0.05; thrombin 30-100; kallikrein 2; plasmin 3-12; and chymotrypsin, cathepsin G sputum elastase pancreatic elastase are all > 100 (Senokuchi et al. 1995). Nafamostat has recently been reported as the most potent inhibitor of SARS-CoV2 viral entry into lung epithelial cells with a greater than 600 fold potency over other antivirals (Ko et al 2020).

PHARMACOKINETIC STUDIES IN HUMANS
In a study with 30 healthy Chinese volunteers, Nafamostat was dissolved in 250 mL glucose solution and was given via intravenous-drip infusion over 120 min. The three doses were 10, 20 and 40 mg (Y. Cao et al. 2008). These concentrations are 40, 80 and 160 μg/mL. The 10, 20 and 40 mg doses resulted in Tmax of 1-2 hours, and Cmax of 14, 40 and 60 ng/mL. These Cmaxs are 0.04 μM, 0.12 μM and 0.17 μM. In a Japanese study by Abe et al. 1984 which also dosed 10, 20 and 40 mg resulting in a Tmax of 30, 120 and 60 min, and Cmax of 17, 62 and 93 ng/mL , equivalent to 0.05 μM, 0.18 μM and 0.27 μM. In humans, half-life was 23 min after continuous intravenous infusion (*Abe1984; *Tsukagoshi2000)(Tsukagoshi 2001; Y. Cao et al. 2008). The volume of distribution is unknown.

METABOLISM
Nafamostat is an ester conjugate of 6-amidino-2-naphthol (AN) and p- guanidinobenzoic acid (p-GBA), and is hydrolyzed by hepatic carboxyesterase and long-chain acyl-CoA hydrolase in human liver cytosol. In vivo in humans about 20% of the nafamostat administrated was hydrolyzed in blood and nearly 80% in tissues (Y.-G. Cao et al. 2008). The specific enzymes are arylesterases in blood and carboxylesterase 2 in tissues (Yamaori et al. 2006). The resulting metabolites are p-guanidinobenzoic acid (PGBA) and 6-amidino-2-naphthol (AN) which are both inactive as protease inhibitors. The parent drug was the only active form (Y. Cao et al. 2008; Yang et al. 1990). The metabolites (Yamaori et al. 2006) are renally excreted.

TOXICITY
Preclinical studies have shown no evidence of genotoxicity, no effects on fertility or reproduction, no adverse effect on foetal growth and no post natal effects in rodent studies. No significant toxicological effects were noted in repeat dose studies of up to 20 mg/kg/day given IP or IV in rodents or dogs for up to 6 months. The reported acute LD50 value following intravenous administration in rats is 16.4 mg/kg, vastly in excess of the proposed human dose to be used in this study.

Serious Adverse Reactions reported in clinical trials:
Nafamostat has been generally well tolerated in human studies supporting approval of Nafamostat in Japan. Serious drug reactions were uncommon, and include: infusion reactions including, rarely, anaphylactic shock (0.16%) and electrolyte disturbance includinghyperkalaemia (0.2-4.5%) and hyponatraemia (0.5%). These effects were considered to be secondary to the effect of parent drug and metabolites on the amiloride-sensitive sodium

(Na) conductance at the collecting ducts, resulting in inhibition of potassium secretion and hyperkalemia (Muto, Imai, and Asano 1994). Thrombocytopenia was reported by <0.1% of recipients and leukopenia in a similar proportion (around 0.1%). Abnormal liver function tests occur in less than 1% of treated patients, but jaundice is rare (less than 0.5% of treated subjects).

Reported incidences of agranulocytosis, hyperkalemia, and anaphylaxis are rare. The use of nafamostat has been reported to cause cardiac arrest in patients receiving dialysis due to a sudden change in the patient's condition such as dyspnea (Kim et al. 2016).

Non Serious AEs reported in Clinical Use:
Nafamostat has been used to treat pancreatitis since 1986 and for DIC and extracorporeal circulation since 1989. The drug showed approximately 56% efficacy in a multi-centre phase II clinical trial for the treatment of 108 DIC cases (Shibata et al. 1987). The adverse effects of Nafamostat were investigated with 6,732 cases of acute pancreatitis, 3,602 cases of DIC, and 4,053 cases of extracorporeal circulation, with the administration of drug doses of 10-40 mg/day or more, 0.05-0.26 mg/kg/h or more and 20-60 mg/h, respectively. AEs werereported in 117 (1.74%), 241 (6.69%) and 48 cases (1.18%), respectively. The most frequently observed abnormalities among these cases were hypercalcemia which occurred in 0.19% of the patients with acute pancreatitis; hyperkalaemia and hyponatremia, 4.35% and 0.47%, respectively, of the patients with DIC and nausea, 1.01% of the extra- corporeal circulation cases (Japanese Society of Hospital Pharmacists 2015).

Rationale for endpoints:

<u>Blood Clotting parameters:</u>

- **D-Dimer** : A potential link between mortality, D-dimer values and a prothrombotic syndrome has been reported in patients with COVID-19 infection. D-dimer is a degradation product of cross-linked fibrin and reflects blood clot formation and its subsequent fibrinolysis. Testing uses an enzyme-linked immunoabsorbent assay (ELISA) or microlatex agglutination assay. D-dimer has a very high sensitivity for thrombotic disease, but its specificity is poor. Various studies in patients with COVID-19 have consistently shown a very strong association between increased D-dimer levels and severe disease/poor prognosis.

  While many pro-inflammatory cytokines trigger the coagulation system, it has been shown that IL-6 levels appeared to increase only 13 days after disease onset, whereas D-dimer levels were already 10-fold increased by that time. This observation suggests that the very high D-dimer levels observed in COVID-19 patients are not only secondary to systemic inflammation, but also reflect true thrombotic disease, possibly induced by cellular activation that is triggered by the virus.

- **FDPs, PT, APTT :** In 183 consecutive patients in Wuhan the non-survivors (11.5%) had marked derangements in haemostatic measures at the time of admission with prolongation of APTT, PT, elevated D-dimers and fibrin degradation products (FDPs) (Tang et a. 2020) indicating disseminated intravascular coagulopathy (DIC) associated with enhanced fibronolysis.

Benefit Risk Assessment
Nafamostat has been shown to have potential antiviral effects against MERS CoV and is thought to possibly inhibit SARS CoV2 infection via inhibition of viral entry due to inhibition of TMPRSS2. In addition, nafamostat has potent anticoagulant properties which may provide benefit in patients with DIC, a common finding in serious cases of COVID-19. As a result of the anti-cogulant effect of Nafamastat there is an increased risk of beeding. Nafamostat has been broadly well tolerated in clinical trials in patients with DIC and acute pancreatitis. ***Serious adverse reactions are rare, may be monitored simply by regular clinical observation and periodic assessment of blood biochemistry and are likely to revert following drug***

withdrawal. In the conditions of use by hospitalised COVID-19 patients these events can be rapidly identified and effectively managed in practice. Given these considerations the benefit risk balance of this product in COVID-19 is likely to be positive.

1.2 ADAPTIVE PROTOCOL DESIGN

This appendix has been written to be used in conjunction with the main DEFINE COVID-19 trial protocol which describes an overarching and adaptive trial design to test candidate therapies for COVID-19 positive patients.

The information detailed within this appendix applies only to Nafamostat.

2. INTERVENTION-SPECIFIC OBJECTIVES

2.1 ENDPOINTS

In addition to the master protocol objectives and endpoints, treatment specific objectives and endpoints are highlighted below.

Objectives	Endpoints
Primary	
To evaluate the safety of candidate agents as add-on therapy to SoC in patients with COVID-19.	Safety will be assessed using: • Haematological and biochemical safety laboratory investigations. • Physical examination • Vital signs (blood pressure/heart rate/temperature and respiratory rate) • Daily electrocardiogram (ECG) readings • Adverse events
Evaluate the effect of Nafamostat on routinely measured blood clotting parameters	**Daily measurement of :** • **D-dimer** • **Fibrin degradation products (FDPs)** • **APTT, PT**
Secondary	
To explore the PK/PD or appropriate surrogate of bioavailability of the proposed trial treatments in COVID-19 patients.	To explore the PK/PD of the proposed trial treatments.
Assess the response of key exploratory biomarkers during treatment period.	Evaluate the change from baseline values for key exploratory biomarkers of target engagement for each treatment. See below for selected biomarkers.
To evaluate the improvement or deteroriation of patients in each treatment arm.	Record changes to WHO ordinal scale and NEWS2 score
To evaluate the number of oxygen-free days.	Duration (days) of oxygen use and oxygen-free days.

To evaluate ventilator-free days and incidence and duration of any form of new ventilation use.	• Duration (days) of ventilation and ventilation-free days. • Incidence of any form of new ventilation use and duration (days) of new ventilation use.
Change in the ratio of the oxygen saturation to fraction of inspired oxygen concentration (SpO2/FiO2)	• SpO2/FiO2, measured daily from randomisation to Day 15, hospital discharge, or death
To evaluate SARS-CoV-2 viral load.	Qualitative and quantitative polymerase chain reaction (PCR) determination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva samples while hospitalised on Days 1, 3, 5, 8, 11, 15, and oropharyngeal/nasal if tolerated
To evaluate time to discharge	• Duration of total hospital stay • Duration to discharge following treatment
To evaluate the use of renal dialysis or haemofiltration for each treatment arm.	Record requirement for renal dialysis or haemofiltration
Evaluate the effect of Nafamostat on *exploratory/experimental* blood clotting parameters	**Measurement of :** • **Rotational Thromboelastography will be performed to analyse whole blood clotting properties to determine a patient's overall coagulation status.** • **Cd39, ecto-ADPase, nitrous oxide, PGI2, antithrombin, Thrombomodlin, protein c, EPCR, kallikrein.**
Determine the effect of Nafamostat on peripheral blood inflammatory cell mobilisation and phenotype as a surrogate of viral-induced inflammation.	**Measurement of:** • **Analysis of cytokine levels in blood as surrogates of viral induced inflammation** • **Perpiheral blood myeloid cell function and exhaustion.**
Investigate the change in clotting parameters following Nafamostat infusion in regional 4D dynamic contrast enhanced perfusion CT.	**Conduct a CT Perfusion Scan on all patients before and after Nafamostat infusion to:** - **Calculate thrombus load and ventricular diameter will be determined.** - **Correlate perfusion maps with residual endoluminal filling defects.**

3. DESIGN

This is an open label open label randomised experimental phase Ib/IIa interventional clinical trial with Nafamostat.

4. STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

It is anticipated that a total of 20 participants from Cohort 2 of the DEFINE COVID-19 trial will be recruited to this treatment arm. These will be compared to the control arm of standard care within the same target cohorts.

4.2 TARGET POPULATION

A total of 20 participants from the patient cohort below will be recruited to this arm.

Cohort 2 (Both Cohort 2A and 2B	Hospitalised confirmed COVID positive patients with: new changes on CXR or new changes on CT compatible with COVID-19 with or without need for supplemental oxygen

4.3 INCLUSION & EXCLUSION CRITERIA

Eligibility criteria for participants randomised to this treatment arm are outlined below.

Inclusion criteria:

- Provision of informed consent from the patient or representative
- Aged at least 16 years
- COVID-19 positive test result within the last 14 days
- Hospitalised patients with confirmed COVID-19 with new changes on CXR or new changes on CT compatible with COVID or with or without the requirement for supplemental oxygen (Cohort 2)
- If the patient is a woman or a male partner of a woman of child bearing potential*, the patient, and their partner(s), agree to use medically-accepted double-barrier methods of contraception (eg, barrier methods, including male condom, female condom or diaphragm with spermicidal gel) during the study and for at least 90 days after termination of study therapy. A vasectomised partner would be considered an appropriate birth control method provided that the partner is the sole male sexual partner and the absence of sperm has been confirmed.

Exclusion criteria:

- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1.73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Women who are pregnant or breastfeeding.
- Participation in another clinical trial of an investigational medicinal product (CTIMP)
- Known hypersensitivity to the IMP or excipients.
- Concomittant use of treatments for COVID-19 that are not recognised as locally approved standard care.
- Significant electrolyte disturbance (hyperkalaemia K+ >5.0 mmol/L or hyponatraemia Na+ < 120mmol/L)
- Patient currently receiving potassium sparing diuretics that cannot be reasonably withheld
- Patient currently receving prophylactic or therapeutic anticoagulants or antiplatelet agents that cannot be reasonably withheld if randomised to Nafamostat
- Patients (or their partners) planning on donating sperm/eggs during the trial period
- Ongoing dialysis
- History of serious liver disease (Child Pugh score > 10)
- Severe uncontrolled diabetes mellitus
- In the Investigator's opinion, patient is unwilling or unable to comply with drug administration plan, laboratory tests or other study procedures.

* A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical

cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1 STUDY DRUG

5.1.1 Study Drug Identification

Nafamostat

5.1.2 Study Drug Manufacturer

Torii Pharmaceutical Co., Ltd., Sakura Plant, 2183-1, Teranosaku, Oota, Sakura-shi, Chiba, Japan.

5.1.3 Marketing Authorisation Holder

Nichi-Iko Pharmaceutical Co., Ltd.

5.1.4 Labelling and Packaging

Nichi-Iko Pharmaceutical Co., Ltd. will be responsible for the providing packaged Nafamostat.

The Investigational Supplies Group (ISG) at the University of Edinburgh will be responsible for the importation of Nafamostat and subsequent labelling. Medication labels will be in the local language and comply with the legal requirements of Annex 13 of the European Union's Good Manufacturing Practice (GMP). They will include storage conditions for the drug, but no information about the patient.

5.1.5 Storage

Prior to the trial, Nafamostat will be stored in ISG under appropriate, monitored temperature conditions. Following release by ISG, Nafamostat will be stored in the site pharmacy. Nafamostat will be stored at room temperature (between 15-25°C) with appropriate temperature monitoring in place.

No temperature monitoring will take place once the IMP has been dispensed from pharmacy.

5.1.6 Regulatory Release to Site

Nafamostat will be certified by a Qualified Person (QP) at ISG before the Sponsor will be invited to release to the trial.

5.1.7 Destruction of Trial Drug

Any Nafamostat remaining at the end of the trial will be returned to the manufacturer.

5.1.8 REFERENCE SAFETY INFORMATION

There is no IB or SmPC relating to Nafamostat as marketed in the UK/EU, however a Pharmaceutical Interview Form (PIF) for Nafamostat has been published in Japan by the manufacturer. For the location of the currently approved RSI, please refer to section 8.1 of this appendix.

5.2 DOSING REGIME

It is intended that the licensed dose (0.2mg/kg/hr) in Japan will be used. Patients randomised to Nafamostat will receive a continuous intravenous infusion at 0.2 mg/kg/hr for 7 days.

If a participant is discharged from hospital or can no longer receive this treatment, the treatment will be stopped.

5.3 NAFAMOSTAT RECONSTITUTION

1. The Nafamostat dose will be rounded up to the nearest 5mg over a 24 hour period
2. For a 50 mg vial, add 5 mL of 5% glucose injection or water for injection.
3. Completely dissolve.
4. Mix the dissolved solution with 5% glucose injection solution. Normally, one day dosage is dissolved into 5% glucose injection 1,000 mL, and as Nafamostat Mesilate, continuously infuse [intravenously] 0.20 mg/kg over the time of 24 hours.
5. **Do not add physiological saline or a solution containing inorganic salts directly to the vial, as this may cause cloudiness or precipitation of crystals.**

Once the infusion has been made up, the infusion has been shown to be stable for 24 hours. No compatibility issues have been noted for any infusion bags or the chemical components of infusion bags.

The reconstitution of Nafamostat must be checked and signed off by two registered nurses/ medical staff. This includes confirming the correct dose calculation, correct drug, correct patient and expiry date and is standard practice for all IV infusions.

If Remdesivir is licensed for the treatment of COVID-19 during the course of this study, Nafamostat must be administered via a separate IV line to avoid any possible drug-drug interactions.

5.4 PARTICIPANT COMPLIANCE

Nafamostat will be administered in the clinical setting under supervision. No compliance issues are anticipated.

5.5 OVERDOSE

The drug will be administered via an infusion pump by appropriately trained health care professionals according to local clinical protocols. Therefore, the risk of overdose is low. The half-life of Nafamostat is very short (8 minutes). Thus, in the case of an accidental dosing error, or any adverse event the pump should be stopped.

5.6 OTHER MEDICATIONS

5.6.1 Non-Investigational Medicinal Products

Not applicable.

5.6.2 Permitted Medications

Any drug required for the normal clinical care of these patients will be permitted.

5.6.3 Prohibited Medications

The risk or severity of bleeding and haemorrhage can be increased when Nafamostat is combined with other anticoagulants. Clotting parameters will be assessed prior to the start of the infusion (as part of eligibility criteria) and then assessed on a daily basis. For this reason, patients currently receving prophylactic or therapeutic anticoagulants or antiplatelet agents will be excluded.

As nafamostat may cause constitutive hyperkalaemia, potassium sparing diuretics should be avoided during use. Therefore, patients receiving these will also be excluded.

5.6.4 Imaging

Ideally, up to two Chest X Rays and up to two CT Perfusion/CT Perfusion Pulmonary Angiogram scans (iodine will be the contrast agent) will be performed. Scans will be

performed prior to the Nafamostat infusion and after the infusion has finished. Imaging will determine the extent of microthrombus in the pulmonary vasucular circulation during/after Nafamostat treatment. A baseline scan (pre-treatment) and a post treatment scan will be taken. A post treatment scan will take place as close to discharge as possible. If these chest x-rays/ scans have been conducted for clinical reasons within the past 48 hours, results can be reviewed and recorded.

6. STUDY ASSESSMENTS

Data collection for the trial is detailed within the main DEFINE COVID-19 trial protocol. Clinical and safety assessments highlighted in blue are in addition to the SoC arm.

A table of assessments for this nested study is provided below. Those assessments marked with a * will be optional for certain study centres due to access to specialist equipment or tests or tolerbality of patients to the procedure.

TABLE 1: Assessments for participants randomised to Nafamostat						
Activities	**Screening and Enrolment (day -1 or day 1)**	**Baseline (day 1)**	**Daily until hospital discharge or hospital day 16, whichever comes first**	**Day 30 (±6 days) ward, home visit or telephone call**	**Day 60 (±6 days) ward, home visit or telephone call**	**Day 90 (±6 days) ward, home visit or telephone call**
Eligibility						
Informed consent	✓					
Review inclusion/exclusion criteria and confirm eligibility	✓					
Urine pregnancy test	✓[a]					
Review of SARS-CoV-2 diagnostic tests	✓					
Medical history	✓[b]					
12-lead Electrocardiogram	✓ [d]	✓ [d f]	✓ [e]			
Study intervention						
Randomisation (day -1 or day 1)	✓					
Administation of treatment in addition to SoC	**On completion of ALL screening and baseline assessments the treatment can be initiated and will continue for 7 days or until hospital discharge, whichever comes first. This means that treatment can be initiated on the same day as Screening and Enrolment and Baseline Visits. If this happens all will be completed on Day1 (there will be no Day -1).**					
SoC treatment	✓	✓	✓			
Clinical study procedures and assessments						
NEWS2 Score and WHO ordinal scale		✓	✓			
Vital signs including SpO2, FiO2, RR, PR		✓ [e]	✓ [eg]			

Physical assessment (including presenting symptoms, height, weight)	✓[e]					
Targeted physical examination (focused on lung auscultation)			✓[g]			
Chest X Ray and CT Perfusion/CTPA scan		✓(both a CXR and CT Perfusion/CTPA will take place prior to treatment [c] *	✓(both a CXR and CT Perfusion/CTPA will take place post treatment or as close to discharge as possible [c] *			
Safety assessments						
Laboratory Safety Assessments & routine bloods[d]	✓[d]	✓[f]	✓[e]			
PK/PD		✓[h]				
Glucose monitoring		✓[e]	Daily until 48 hours (± 4 hours) following end of infusion[i]			
Cardiac monitoring (ECG/continuous telemetry)	✓	✓	Until 48 hours (± 4 hours) following end of infusion			
AE/SAE recording and assessment	✓	✓	✓	✓	✓	✓
Survival status		✓	✓	✓	✓	✓
Research laboratory sampling						
Biomarker blood sampling (10ml) and Thromboelastography		✓*	✓* [j]			

Throat and upper airway nasal absorption samples	✓*	✓*	**Days 1, 3, 5, 8, 11, 15(all ±1 day) while hospitalised***			
Viral Load (saliva)	✓		**Days 1, 3, 5, 8, 11, 15 (all ±1 day) while hospitalised**			

a **only women of child bearing potential**
b **Medical history includes estimated date and time of first symptoms and number of co-morbidities (eg, respiratory, cardiovascular, metabolic, malignancy, endocrine, gastrointestinal, immunologic, renal).**
c **Due to logistics during this COVID pandemic, it may not be possible to complete these scans. Wherever possible, imaging will take place but if it cannot take place, the patient will remain in the study. A baseline CXR and CT Perfusion/CTPA scan (pre-treatment) and a post treatment CT Perfusion/CTPA and CXR scan will be taken. Post treatment scans will take place between days 7-10. If scans have been taken as part of clinical care within the past 48 hours, results can be recorded and no additional scans are required.**
d **If these assessments have been taken as part of routine clinical care within the last 48 hours, results can be recorded and no additional assessments are required. For bloods please refer to section 6.1.3 of the master protocol for a list of the blood tests required.**

e **If these assessments have been taken as part of routine clinical care on the same calendar day as the research assessment is to be conducted, results can be recorded and no additional assessments conducted.**
f **If these assessments were done as part of screening they do not require to be repeated.**
g **A symptom-directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint.**
h **Blood samples (8 ml) will be collected at the following intervals to measure the concentration of Nafamostat:**
Pre-infusion
6 - 36 hours post infusion starting (not to be taken any less than 6 hours post commencement of infusion)
i **If a patient is diabetic, glucose meter readings will be analysed at least twice daily, ideally one am and one pm. If testing has been performed for clinical reasons on the same calendar day as the research visit, results can be reviewed and recorded.**
j **Biomarkers will be collected on Day1 (baseline) and then at D4 and D7/or discharge if before this.**

* **This assessment will be optional if the study team cannot access the specialist equipment or testing facility to complete this assessment or participant refuses.**

6.1 SAMPLING

Existing pharmacokinetic (PK) and pharmacodynamic (PD) data have been obtained from Japanese and Asian population, to date. To provide confidence that this data also reflects a Caucasian population, serial blood samples to analyse PK/PD properties will be taken.

Blood samples (8 ml) will be collected at the following intervals to measure the concentration of Nafamostat:

- Prior to the infusion starting (post randomisation, pre commencement of infusion)
- 6 - 36 hours post infusion starting (not to be taken any less than 6 hours post commencement of infusion)

In addition, a daily 10 mL blood sample will be obtained to assess for changes in relevant inflammatory biomarkers. These samples will enable the pilot study to investigate if Nafamostat is affecting key pathways in the coagulation/inflammatory cascade and will provide valuable mechanistic outcome data.

Saliva and throat/upper airway samples will also be taken as per the table above.

No more than 400 mL of blood will be taken from each patient as part of this treatment arm.

6.2 SAFETY ASSESSMENTS

The key safety assessments for this treatment arm are:

- Daily haematology, biochemistry, liver function tests, U&Es, D Dimer, fibrinogen, coagulation
- Daily ECGs or continuous telemetry where available
- Glucose monitoring (BMs)
- Adverse event recording

6.3 STUDY ASSESSMENTS

Study assessment are outlined in the table of assessments. In addition, baseline, ongoing and follow up information will be collected as per the main protocol.

6.4 STORAGE AND ANALYSIS OF SAMPLES

Storage and analysis of biomarker samples is outlined in the main protocol. PKPD blood samples will be sent to the University of Oxford for analysis. Haematolgical and biochemical bloods taken as part of safety assessments will be analysed in hospital laboratories and then destroyed.

7. STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE CALCULATION

An indicative sample size, considering the comparison of one active drug against control, each with n=20 per group, and assuming 5% missing data, would give 80% power at a 1-sided 10%

level of significance, using a two sample t-test, to detect an effect size of 0.7 in the difference of means in the biomarker between active and control.

That is, the study would be able to detect a mean difference of 0.7 standard deviations for the biomarker. So if biomarker was diastolic blood pressure, and the standard deviation was 10 mmHg, the study could detect a mean difference of 7 mmHg.

7.2 PROPOSED ANALYSES

The statistical analyses for each study will be comprehensively specified in a study specific Statistical Analysis Plan (SAP). Additional safety measurements are outlined in Section 6.2.

8. PHARMACOVIGILANCE

Pharmacovigilance procedures are detailed within the main trial protocol.

8.1 ADDITIONAL REPORTING REQUIREMENTS

For this arm, all SAEs occurring in the cohort will be assessed for potential of the event to be a Serious Adverse Reaction (SAR) to nafamostat: if the event is considered to be a potential SAR to nafamostat, it will be assessed for expectedness and reported using the applicable reporting requirements for serious unexpected serious adverse reactions (SUSAR) described in the master protocol.
The assessment of expectedness will be made against the reference safety information, which is considered to be comprised of section VIII, part 8, of the Pharmaceutical Interview Form.

All SAE/SARs that occur in participants dosed with Nafamostat will be reported to the Sponsor as specified in the main protocol (i.e. within 24 hours of identification of the event). The Sponsor will undertake to report the event onward to Nichi-Iko's representatives (listed below) within one working day of notification by site.

Kenta Hatatani k.hatatani@nichiiko.co.jp

Additionally, all SAEs considered to be possibly or probably serious reactions to nafamostat will be reported according to Section 10.6 of the Master Protocol.

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Academic and Clinical Central Office for Research and Development

DEFINE

Evaluating therapies for COVID 19

Co-sponsors	The University of Edinburgh & Lothian Health Board ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Funder	Life Arc
Chief Investigator	Professor Kev Dhaliwal Professor of Molecular Imaging & Healthcare Technology Consultant in Respiratory Medicine, Tuberculosis /Infection and Interventional Medicine Centre for Inflammation Research University of Edinburgh
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PROTOCOL APPROVAL SIGNATURE PAGE

DEFINE - Evaluating therapies for COVID 19

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CONTENTS

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development
AE	Adverse Event
AES	Advanced Electronic Signature
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
CI	Chief Investigator
CRF	Case Report Form
CSR	Clinical Study Report
CT	Computed tomography
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CXR	Chest X ray
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator Brochure
ISF	Investigator Site File
MHRA	Medicines and Healthcare products Regulatory Agency
MV	Mechanical Ventilation
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic

QA	Quality Assurance
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
WOCBP	Woman of childbearing potential

INTRODUCTION

1.1 BACKGROUND AND SYNOPSIS

COVID-19 is a community acquired pneumonia caused by infection with a novel coronavirus, SARS CoV2 and is a serious condition with high mortality in hospitalised patients. Urgent investigation of potential treatments for this condition is required.

This protocol describes an overarching and adaptive trial designed to provide safety, pharmacokinetic (PK)/ pharmacodynamic (PD) information and exploratory biological surrogates of efficacy which may support further development and deployment of candidate therapies in larger scale trials of SARS-CoV2 positive patients receiving normal standard of care.

Given the spectrum of clinical disease, community based infected patients or hospitalised patients can be included. SARS-CoV2 positive patients will be divided into cohorts, a) community b) hospitalised patients requiring or not requiring supplemental oxygen and c) hospitalised patients requiring assisted ventilation. Participants may be recruited from all three of these cohorts, depending on the experimental therapy, its route of administration and mechanism of action. The relevant cohort(s) for any given therapy will be detailed in the therapy-specific appendix.

Candidate therapies can be added to the protocol and previous candidates removed from further investigation as evidence emerges. Candidate assets will be novel or repurposed products that are first-in-COVID-19. The trial will be monitored by an independent Data Monitoring Committee (DMC) to ensure patient safety.

This is an experimental medicine platform trial encompassing early phase studies and as such formal sample size calculations are not appropriate.

Each candidate therapy will include a small cohort of patients, either randomised or allocated to candidate therapy or existing standard of care management dependent on disease stage at entry. Cohort numbers will be defined in the protocol appendices and can be changed by the trial management group as the study progreses. To enable additional sites to participate in this study, certain assessments that require specialised equipment or techniques to address secondary endpoints will be optional.

1.2 RATIONALE FOR STUDY

This early phase trial platform aims to support promising novel and repurposed therapeutic assets/therapies but without prior information on use in COVID-19, to determine safety, PK-PD profile and determine exploratory biological markers of efficacy in small cohorts of COVID-19 patients. The results of these studies are intended to provide initial safety, pharmacokinetic and pharmacodynamic data (or equivalent surrogates) and experimental medicine data to support further development in follow on clinical studies in COVID-19 patients.

A major limitation in the design of many early clinical trials is the limited amount of mechanistic data from patients with COVID-19. Mechanisms have been inferred from animal models, related infections or clinical syndromes.

There is a clear and urgent need to pursue experimental medicine studies in humans to establish a solid mechanistic basis for rapid evaluation, including in existing clinical trial platforms against COVID-19 (e.g DoH RECOVERY).

The trial platform will be as flexible as possible to ensure a broad range of patients can be recruited and candidate therapies can be added or removed as evidence emerges. The interim trial results will be monitored by an independent DMC to evaluate any patient safety signals.

As COVID-19 follows a variable clinical path in individual patients, the protocol is designed to enable inclusion of patients across the disease stages. The trial is intended to provide mechanistic data from patients receiving standard of care therapy and from patients treated with the therapy candidates. The study will enable delivery of pharmacokinetic information and effects of standard of care and candidate agents on surrogate biomarkers of the disease process and the specific drug or therapy target.

2. STUDY OBJECTIVES

2.1 OBJECTIVES AND ENDPOINTS

The main study objectives and endpoints are listed below. These, along with treatment specific endpoints will be detailed in the relevant appendix, Any changes to the main study objectives and endpoints will be detailed in the relevant appendicies.

Objectives	Endpoints
Primary	
To evaluate the safety and tolerability of candidate agents as add-on therapy to SoC in patients with COVID-19.	Safety will be assessed using: • Haematological and biochemical safety laboratory investigations. • Physical examination (directed cardio-respiratory physical examination) • Vital signs (blood pressure/heart rate/temperature and respiratory rate) • Daily electrocardiogram (ECG) readings (if applicable for the specific appendix) • Adverse events
Secondary	
To evaluate the improvement or deteroriation of patients in each treatment arm.	Record changes to WHO ordinal scale and NEWS2 score
To evaluate the number of oxygen-free days.	Duration (days) of oxygen use and oxygen-free days.
Where oxygen is required, change in the ratio of the oxygen saturation to fraction of inspired oxygen concentration (SpO2/FiO2)	• SpO2/FiO2, measured daily and for duration detailed in the relevant appendix
To evaluate time to discharge	• Duration of total hospital stay • Duration to discharge following treatment
Secondary Non Essential – these data will be collected if available however, they are not essential to the core analysis.	
To evaluate SARS-CoV-2 viral load/status.	Qualitative and quantitative polymerase chain reaction (PCR) determination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in saliva and/or blood samples . Oropharyngeal/nasal swab will be collected on the same days if tolerated.

3. STUDY DESIGN

All research staff involved in any aspect of the study will be required to adhere to local practice regarding use of personal protective equipment and other applicable safety protocols and guidelines relating to contact with COVID-19 patients and sample collection.

DEFINE is an open label exploratory early interventional clinical experimental medicine platform trial. Candidate assets will be included in the appropriate phase (described in the treatment appendix) according to prior knowledge, with a view to progressing promising assets along the development pathway. Depending on the nature of the treatment arms and whether they need to be directly compared to the standard of care arm, arms may or may not be randomised. If certain therapies are tested in small numbers of patients and do not need a control arm, patients will be allocated to that treatment arm by the research team. If patients are directly allocated to a study arm and other arms requiring randomisation are open to recruitment at the same time, patient recruitment will be equitable across all arms and no treatment or therapy will be prioritised.

4. TARGET POPULATION

Patients with confirmed SARS CoV2 infection within the cohorts as described will be recruited into this trial. As SARS CoV2 has a range of clinical manifestations, representatives of three target patient cohorts will be included in this trial. Participants may be recruited from one or more of these cohorts, depending on the experimental therapy under investigation. The relevant cohort(s) for any given therapy will be detailed in the drug-specific appendix.

While the study will approach patients with suspected COVID-19 and conduct screening assessments whilst the test results are outstanding, only patients who are confirmed COVID-19 positive will included in a treatment arm.

Cohort 1A	Community (primary care) patients with confirmed COVID-19
Cohort 1B	Community (primary care) patients with confirmed COVID-19 with new changes on CXR or CT scan compatible with COVID-19 and deemed 'high-risk' of hospitalisation or death*
Cohort 2A	Hospitalised confirmed COVID positive patients with new changes on CXR or new changes on CT compatible with COVID-19 but not requiring supplemental oxygen,
Cohort 2B	Hospitalised confirmed COVID positive patients with: new changes on CXR or new changes on CT compatible with COVID-19 and requiring supplemental oxygen ,
Cohort 2C	Confirmed COVID-19 positive patients with ≥92% O_2 saturations on air
Cohort 2D	Confirmed COVID-19 positive patients with ≥92% O_2 saturations on supplemental oxygen (maximum FiO_2 of 28% (2 – 6 L/min depending on delivery device used))
Cohort 3	Hospitalised patients with confirmed COVID-19 requiring assisted ventilation (including non-invasive and mechanical ventilation)

* High risk is defined as over 50 years of age with comorbidies.

Where required or appropriate for the treatment being investigated, all recruited participants who go on to receive an investigational product will be provided with a study contact card. This card will contain details of the trial (including title, investigator and treatment received) and guidance for the participant along with emergencey contact details. The card will also contain instructions and contact details should a female participant, or the female partner of a male participant become pregnant in the 90 days (or as detailed in the relevant appendix) following administration of the investigational product. Any pregnancy notification will be reported and followed up in accordance with the relevant sponsor SOP applicable at the time of the notification. Where it is required that a study card is issued this will be detailed in the relevant appendix.

4.3 INCLUSION CRITERIA

Inclusion criteria:

- Provision of informed consent (*refer to relevant appendix for type of consent permitted – patient, representative or both*)
- Aged at least 16 years
- COVID-19 positive test (lateral flow followed by confirmatory PCR or PCR only) result within last 14 days
- If the patient is of child bearing potential*, or is a male with a female partner with child bearing potential, the patient, and their partner(s), agree to use medically-accepted contraception. *(Refer to relevant appendix for details of acceptable contraception and duration.)*

Exclusion criteria:

- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1.73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Women who are pregnant or breastfeeding.
- Participation in another clinical trial of an investigational medicinal product (CTIMP)
- Known hypersensitivity to the therapy or excipients (e.g. lactose)
- Patients (or their partners) planning on donating sperm/eggs during the trial period
- Ongoing dialysis
- History of serious liver disease (Child Pugh score > 10)
- Severe uncontrolled diabetes mellitus
- Concomittant use of treatments for COVID-19 that are not recognised as locally approved standard care.
- In the Investigator's opinion, patient is unwilling or unable to comply with trial intervention (e.g. IMP/ATIMP administration plan), laboratory tests or other study procedures.

* A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

4.4 TREATMENT ARMS

There is no restriction on the number of treatment arms that will be recruiting at any one time. It may be the case, where multiple treatment arms are open simultaneously, that one (or more) arm may be single treatment (non-randomised) with other arms being randomised (either between multiple interventions, plus or minus standard of care (SoC) or between a single intervention and SoC. Each arm of the trial will have its own Participant Information Sheet and Consent Form so that participants have clarity on which arm of the trial they are being asked to consider.

Treatment arm 1	Standard care relevant to the disease stage at entry (e.g. antipyretic/analgesics, cough linctus, oxygen, CPAP, intubation and ventilation with/without prone positioning, ECMO, cardiac support including ionotropes/intra-aortic balloon pump).
Treatment arm 2	Standard care and TD139 (**Appendix 1**)
Treatment arm 3	Standard care and Nafamostat (**Appendix 2**)
Treatment arm 4	Standard care and SARS-CoV-2 VSTs (**Appendix 3**)

Further details on each of these treatment options can be found in the relevant appendix.

Patient numbers are defined in each appendix. It will be possible to increase these numbers following advice from the Trial Management Group, Data Monitoring Group and Statistician. It will be possible to affect a minor increase to the total patient number of one or two arms, without seeking the aforementioned advice, solely to ensure all active arms recruit the minimum number of participants. This option is necessary because of the database design.

Other arms will be added when/if evidence emerges of a mechanistic basis for proceeding to experimental studies for specific candidate agents. Conversely, in some patient populations, not all study arms are appropriate (e.g. due to contraindications based on comorbid conditions or concomitant medication).

4.4.1 Concomitant Medications

Routinely used medications for the alleviation/treatment of symptoms of COVID-19 are permitted. Only locally and nationally approved therapies to treat SARS-CoV-2/COVID-19 will be permitted. Concomittant use of treatments for SARS-CoV-2/COVID-19 that are not recognised as locally approved standard care will not be permitted.

Medicines specifically contraindicated if used in association with specific IMPs or therapies are listed in the candidate specific appendix.

Any other medication (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrolment (including screening) or receives during the study must be recorded in the eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

4.4.2 Risk/Benefit Assessment

This study is designed to evaluate preliminary safety, pharmacokinetic and pharmacodynamic features of agents which have a likelihood of potential beneficial effects on either viral replication, immune cell functionand/or elaboration of an inflammatory response which characterises this disease. All patients in this study will receive standard of care management relevant to their stage of COVID-19 disease at trial entry and may progress to higher level intervention including ventilatory support, if required during the trial period. Patients will be kept under review with regular assessments of vital signs, oxygenation status, laboratory markers of organ system function and electrocardiogram (ECG) parameters enabling relevant intervention and step up care where required. Detailed information about the known and expected risks and reasonably expected adverse events (AEs) of each candidate agent may be found in the corresponding appendix for that agent. Given the urgent need for therapy for this condition, the potential benefit to public health from identification of agents which may ameliorate COVID-19 progression which may accrue from the early study of their biological effects in this study outweigh the potential risks of these interventions in the conditions applied during the study.

4.5 CO-ENROLMENT

Co-enrolment with a Clinical Trial of an Investigational Medicinal Product (CTIMP) will not be permitted. Co-enrolment with a clinical investigation of a Medical Device or a non-interventional clinical study will be considered on a study-by-study basis and in discussion with the relevant Chief Investigators and Sponsors and industrial collaborators. Such co-enrolment will be undertaken in line with the Sponsor co-enrolment policy (POL008).

Co-enrolment involving non-interventional research (including questionnaire or tissue only studies) will be allowed provided this is not expected to affect the outcomes of both studies or place undue burden upon participants and their families.

5. PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

The first approach will always be done by member of the clinical care team (including members of the research team embedded within the clinical team) who is a qualified nurse or doctor.

Clinical members of the research team will liaise with members of the clinical care team to identify potential participants. A member of the clinical care team (which may include embedded research nurses or clinicians), will make the first approach regarding screening for eligibility.

It is permissible for patients who are highly suspected of having COVID-19 to be screened and consented. If the patient tests SARS-CoV2 negative during or after screening/consent etc, they will be withdrawn from the study. **Only those patients who are confirmed SARS-CoV2 positive will be randomised or allocated to a treatment arm.** This strategy will help to expedite recruitment and therapy administration.

5.2 CONSENTING PARTICIPANTS

Due to the nature of this study and the progression of the disease under investigation, it is likely that participants will fall into one of three following categories:

- Capacity to provide ongoing consent for the duration of the study
- Lose capacity during the course of the study (following consent)
- Incapacity to provide consent from the outset of the study

Consent procedures for each of these categories is described below.

5.3 CONSENTING PARTICIPANTS WITH CAPACITY

After being given the participant information sheet, potential participants will have 24 hours (or less if appropriate) to decide whether to take part. All willing and potentially eligible participants will be asked to provide written informed consent which will be taken by an appropriately qualified member of the research team who is delegated to do so. The original will be retained by the research team, one copy will be filed in the medical notes and one copy will be provided to the participant. Where it is not feasible to obtain an ink signature from the participant this will be overcome using electronic methods. **It will be made very clear to the patients at consent that, if tests are outstanding, that patients will only be allocated to a treatment arm if they are confirmed SARS-COV2 positive.**

5.4 PARTICIPANTS WHO LOSE CAPACITY DURING THE TRIAL

There may be participants who have capacity to consent at the beginning of the trial, who then go on to become incapacitated over the course of the trial (i.e. due to progression of the infection). It will be explained to all participants at the time of consent that informed consent will be respected following loss of capacity and will not affect their ongoing participation in the study but may result in them being unable to continue with the study drug.

5.5 CONSENTING PARTICIPANTS WITH INCAPACITY

It is anticipated that some critically ill incapacitated patients will be included in this trial. The selection and enrolment of adults with incapacity will take place within the legal framework described in Adults with Incapacity (Scotland) Act 2000 and Medicines for Human Use (Clinical

Trials) Regulations, 2004. As some of these patients will be ventilated via an endotracheal tube it is very unlikely that they will have the capacity to consent for themselves due to the use of iatrogenic sedation and/or the presence of incapacitating illness.

Therefore, typically, a personal legal representative (also known as guardian/welfare attorney/nearest relative) for each potential participant will be identified and approached by a member of the clinical care team. Options for consent are outlined below. Personal legal represenatives will have 24 hours (or less if appropriate) to decide whether to take part.

5.5.1.1 Consent in person

An appropriate member of the research team, who has been delegated the duty of obtaining consent, will approach the relevant individual to discuss the study. The appropriate participant information sheet (PIS) will be given to the personal legal representative. Sufficient time will be given to consider participation in the trial. The research team will be available to answer any questions or discuss any aspect of the trial.

Details of who the personal legal representative may be obtained from information on the Electronic Patient Record system used at site. . These details will not be recorded. The personal legal representative is identified routinely in the intensive care nursing record.

If the patient/personal legal representative chooses to proceed, written, informed consent will be obtained. The original consent form will be signed and two copies made. One copy will be given to the personal legal representative and one stored in the participant's medical notes along with a copy of the information sheet.

Where it is not feasible to obtain an ink signature from the personal legal representative this will be overcome using electronic methods.

5.5.1.2 Consent via Telephone

Every effort will be made to approach and consent the personal legal representative in person. If the personal legal representative is only contactable by telephone then the informed consent process is permitted via telephone, provided the following:

- The representative who is being telephoned has previously had the opportunity to discuss the clinical aspects of the patient's care with the clinical team.
- A member of the clinical team has sought permission for the personal legal representative to be contacted via telephone regarding potential involvement in the study
- The approach to discuss consent is clearly separate to any discussions made between the representative and the clinical team

A member of the research team will contact the personal legal representative by telephone to explain what the study entails and answer any questions they may have. The personal legal representative will be given ample time to read and consider the information sheet. If the personal legal representative chooses to enrol the patient onto the study, verbal consent will be obtained by a member of the research team who will sign the consent form. This will be witnessed. All efforts will be made to obtain a signature electronically or on a paper copy of the consent form mailed out. However, in the absence of this signature, a consent form completed by a member of the research team and witnessed by an independent member of staff will be acceptable. A copy of the information sheet and consent form will be sent to the personal legal representative.

5.5.1.3 Consent via Professional Legal Representative

A personal legal representative should be identified and consulted unless it is not reasonably practicable to contact a personal legal representative before the decision to enter the adult into the trial is made.

Every attempt will first of all be made to identify the personal legal representative of the patient, this will include searching electronic health records or similar and speaking to the clinical team. In non Covid-19 circumstances contact details are identified from a variety of methods for patients lacking capacity (admission paperwork, provided to ambulance staff, previous hospital admissions etc.) and stored on electronic health records or similar. If patients are admitted lacking capacity and without the usual methods for identifying and logging contact details for a personal legal representative, then, in the rare event that no contact has been identified by the clinical care team (this will be checked with the clinical care team and on electronic health record (or similar) at site) the research team may consider seeking consent from a professional legal representative.

- At the first opportunity, the trial will be discussed with the personal legal representative if identified. We will ask the clinical team to update the research team on contact with a personal legal representative and check electronic health records or similar on a regular basis.
- Once contacted, the personal legal representative can opt for the participant to remain in the trial (and receive an information sheet and consent form) or decide to withdraw the participant from the trial.

If a personal legal representative cannot be identified after 24 hours, a professional legal representative can be approached. In keeping with the Medicines for Human Use (Clinical Trials) Regulations, 2004, a professional legal representative will be a clinician responsible for the medical treatment of the patient if they are independent of the study or a person nominated by the healthcare provider. A professional legal representative will only be approached if a personal legal representative is not available for the reasons outlined above. A Professional legal represenative will have 24 hours (or less if appropriate) to decide whether to take part.

Where it is not feasible to obtain an ink signature from the professional legal representative this will be overcome using electronic methods.

5.5.1.4 Participants Regaining Capacity

If a participant regains capacity during the course of the study then the participant will be given the opportunity to be re-consented. The personal legal representative (or professional) will be informed that this will be the case when consenting for the participant to take part in the study. The participant will be provided with an appropriate PIS/consent form and asked if they wish to continue with the study.

If a participant regains capacity and is discharged before we can consent them we will seek to contact them at home. To do this we will request a suitable phone number and email address for the participant and will attempt to contact them twice through both means. If we fail to establish contact we will assume their ongoing consent and continue to include them in applicable aspects of the study.

5.5.1.5 Electronic and Witnessed Methods of Obtaining Signatures

Consent will normally be recorded in writing, dated and signed or otherwise marked by the participant or their legal representative. In most instances this will take the form of a face to face consent process with a wet ink signature.

If face to face consent is not possible or feasible, verbal consent over the phone or video-call will be utilised, this will be witnessed and recorded in writing. If a verbal witnessed consent procedure is utilised we will also attempt to obtain a written signature from the participant or their legal representative. Electronic signature software will be offered in the first instance as an Advanced Electronic Signature (AES).

The preferred method will always be face to face and telephone communication and this will be utilised wherever possible. If electronic signatures are used, patient information will be stored on a secure cloud.

5.6 SCREENING FOR ELIGIBILITY

Participant eligibility will be verified by a clinical trial physician after written informed consent has been obtained. Confirmation of eligibility will be recorded within the participants' medical records. Women who are of child bearing potential will be required to undergo a urine pregnancy test to confirm eligibility. Only those patients who are confirmed SARS-CoV2 positive will be randomised or allocated to a treatment arm, however, it is permissible for patients who are highly suspected of having COVID-19 to be screened and consented. SARS-CoV tests can often take up to 72 hours to provide a result, although most are returned within 24-36 hours, in which time the research team could approach the patient, provide them with time to consider their participation and consent. The patient would only be randomised or allocated if they were COVID-19 positive. This will allow the team to streamline identification and approach and would enable treatment to be initiated earlier. In the small number of cases where reporting of results are delayed this may result in some screening activities becoming invalid (exceeding the acceptable time window) as detailed in the table of assessments within this protocol or the relevant treatment appendix. If this situation arises the relevant screening assessments will be repeated prior to randomisation.

Contraceptive Requirements

Pregnant and breastfeeing women are excluded from participation in the trial. Women of child bearing potential and men with female partners of child bearing potential are eligible to participate in the trial provided that they agree to use medically accepted contraception. Full details of the acceptable level and duration of contraception can be found in the appendix specific inclusion criteria within the relevant protocol appendix.

5.7 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

If participants are not entered into the study, for whatever reason, another participant can take their place. All ineligible participants will continue to receive standard care.

5.8 RANDOMISATION AND ALLOCATION

If applicable, randomisation will be performed using a web-based randomisation system (built in REDCap) hosted at the Edinburgh Clinical Trials Unit (ECTU) at the University of Edinburgh (a fully registered UKCRC CTU (registration #15)). Since these studies are designed to be small, this study will balance underlying risk across the allocations using the method of minimisation.

For appendicies where there is no randomisation required, the research team will allocate treatment according to the relevant appendix and enter details onto the trial database for a participant ID number to be allocated.

There are multiple interventions across several cohorts on the COVID-19 pathway (from community based, to breathless in hospital to ventilation with different intensities), with potentially different primary outcomes. Hence, it is not practicable to produce bespoke minimisation algorithms for every possibility, so instead we will base the minimisation algorithm on what is currently known about risk factors associated with admission to ICU or death. The minimisation will include a random element (set at 20%) to increase unpredictability of allocation.

5.8.1 Supply of study treatments

More details regarding packaging and labelling can be found in the relevant appendix.

5.8.2 Emergency Unblinding Procedures

This trial will not be blinded.

5.9 WITHDRAWAL OF TRIAL PARTICIPANTS

Participants are free to withdraw from the study at any point, without giving a reason. This will not affect the patients ongoing care in the institution. A participant can be withdrawn by the Investigator if the investigator believes that continued participation is against the best interests of the patient. If withdrawal occurs, the primary reason for withdrawal, if available, will be documented in the participant's case record form. The participant will have the option of withdrawal from:

(i) trial medication or therapy with continued trial procedures and/or collection of clinical and/or safety data. The participant can withdraw from any further procedures/interventions but remain on trial, complete follow-up visits and/or allow medical record review for relevant trial data, e.g. results of clinical blood results, ECG recordings and physical examinations;
(ii) all aspects of the trial but continued use of data collected up to that point. To safeguard rights, the minimum personally-identifiable information possible will be collected.

Patients who wish to be withdrawn from the trial before they have received any amount of trial medication or therapy will be withdrawn from the trial and another participant will be recruited to replace them.

6. SAFETY ASSESSMENTS AND TREATMENT ARMS

6.1 Safety Assessments

The safety assessments listed below will be carried out on all treatment and control participants. Additional assessments specific to the treatment are listed in the relevant appendix.

6.1.1 **Physical Examinations**

A general physical examination will be performed at screening, including assessment of presenting symptoms. At subsequent assessments, a symptom-directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint.

6.1.2 **Vital Signs**

Temperature, pulse rate, blood pressure, and respiratory rate will be assessed. Blood pressure and pulse measurements will be assessed with a completely automated device. SpO2 will also be assessed. Manual techniques will be used only if an automated device is not available.

Vital signs measurements will contribute to the NEWS2 score.

Respiratory support will be recorded. FiO2, the assumed percentage of oxygen concentration participating in gas exchange in the alveoli, will also be recorded. Measurements will be taken in line with standard practices for the study centre.

6.1.3 **Clinical Safety Laboratory Assessments**

Clinical Safety Laboratory Assessments specific to the treatment are listed in the relevant appendix.

SCREENING BLOODS
For most appendicies biochemistry and haematology samples will be collected as part of the screening blood sampling. Where applicable to the asset under investigation, other screening assessments such as LFTs and coagulation will be added. Specific and minimal results will be detailed within the relevant appendix. Please refer to the appropriate appendix for full details of the screening bloods required and also the duration in which these remain applicable for this time point.

DAILY ASSESSMENTS
Blood sampling that is to be undertaken as part of the daily assessments, post randomisation/allocation or treatment will be fully detailed in the treatment specific appendix. In most cases, daily assessments will include biochemistry and haematology samples, with other specific sampling detailed. A list of the required results will be detailed in the appropriate section of the appendix for each asset.

In addition to sampling details, the appendix will also include the appropriate time frame in which, where permitted, clinical blood results can be used and reorded in the pCRF/database, along with the frequency and duration of the daily assessments.

6.1.4 **Cardiac Evaluations**

12 lead ECGs or telemetry where available, will be conducted as per the relevant table of assessments.

6.1.5 **Adverse Events**

The definition and reporting requirements for Adverse events are described in Section 10.

6.2 Schedule of Assessments

The table below details the generic assessments that will be carried out for all assets added to the DEFINE platform trial. A schedule(table) of all assessments for each asset can be found in the relevant appendix. The timelines detailed in the table below are subject to change and will be appropriate for the particular asset (treatment. being investigated. Please refer to the relevant appendix for assessments and timepoints specific to a particular asset.

TABLE 1: Table of Assessments				
Activities	**Screening and Enrolment**	**Baseline**	**Daily until hospital discharge or as per the relevant appendix**	**Follow-up***
Eligibility				
Informed consent	✓			
Review and confirm eligibility	✓			
Urine pregnancy test	✓[a]			
Review of SARS-CoV-2 diagnostic tests	✓			
Medical history	✓[b]			
12-lead Electrocardiogram	✓[c]			
Study intervention				
Randomisation or treatment allocation	✓	✓		
Administation of treatment in addition to SoC	**Defined in each protocol appendix**			
SoC treatment	✓	✓	✓	
Clinical study procedures and assessments				
NEWS2 Score and WHO ordinal scale		✓	✓	
Vital signs including SpO2, FiO2, RR, PR		✓[c]	✓[c]	
Physical assessment (including baseline medical examination, height, weight)	✓[c]			
Directed cardio-respiratory physical examination		✓[d, e]	✓[e]	
Cough Symptom Score		✓	✓	✓
Safety assessments				
Laboratory Safety Assessments, routine bloods	✓[c, e]	✓[c,d, f]	✓[c, f]	
AE/SAE recording and assessment		✓	✓	✓
Survival status		✓	✓	✓
Research laboratory sampling				
SARS-CoV-2 Blood sample	✓[h]	✓[h]	✓[h]	
Throat and upper airway nasal absorption samples	✓[h]	✓[h]	✓[h]	
Viral Load (saliva)	✓[h]		✓[h]	
Research blood sampling (inc. plasma and biomarker testing as relevant to the specific appendix)	✓[g, h]	✓[g, h]	✓[g, h]	

Footnotes to table of assessments (above)	
a	**Only women of child bearing potential**
b	**Medical History includes estimated date of first symptoms and co-morbidity data**
c	**If these assessments have been done as part of routine clinical care within the time period stated in the relevant appendix, results can be recorded and no additional assessments required**
d	**If these assessments were done as part of screening they may not require to be repeated at baseline, please refer to relevant appendix to confirm**
e	**A symptom directed (targeted) physical examination will be performed as required by the condition of the patient and the presenting complaint**
f	**Refer to the relevant appendix for full details of the laboratory safety tests required, including frequency of sampling**
g	**Refer to appendix for specific details of what research blood samples will be collected and frequency of sampling**
h	**These assessments are optional if the study team cannot access the specialist equipment or testing facilities to complete, or the participant may refuse to provide the sample (will not be considered a deviaition)**
*	**Each appendix will detail the specific time points required as part of follow-up.Frequency and duration of follow-up will be appropriate for the specific asset being investigated.**

6.3 IMAGING

If applicable, up to two Chest X Rays and up to two CT Perfusion/CT Perfusion Pulmonary Angiogram scans (iodine will be the contrast agent) will be performed. Imaging will determine the extent of microthrombus in the pulmonary vasucular circulation. Due to logistics and capacity during this COVID pandemic, it may not be possible to complete these scans. Imaging will be scheduled to take place but if for whatever reason it does not go ahead, the patient will remain in the study. A baseline scan (pre-treatment) and a post treatment scan will be taken. A post treatment scan will take place as close to discharge as possible. If these scans have been conducted for clinical reasons within the past 48 hours, results can be reviewed and recorded.

6.4 LONG TERM FOLLOW UP ASSESSMENTS

For those hospitalised, participants will be followed up for either 90 days or the length of time stipulated in the appendix. Any adverse events will be recorded post discharge. These follow-ups may be completed in person (e.g. on the ward or at home) or by telephone.

6.5 STORAGE AND ANALYSIS OF SAMPLES

Full details of the storage and analysis of samples, including the laboratories involved, can be found in the separate clinical samples working instruction document (DEFINE – WI001 Clinical Samples).

As samples are infectious and taken from COVID-19 patients, handling, storage and analysis will be conducted in accordance with the appropriate requirements in line with UK Health and Safety Executive and local Health and Safety committee review and guidance.

Blood will be collected for measurement of biomarkers (including pharmacodynamics (PD) and safety biomarker subtypes as per FDA classification).

Biomarker blood samples (including PD analysis) will be processed in a University of Edinburgh CL2 lab at the QMRI, or at nominated laboratories if deemed appropriate in accordance with updated HSE/PHE guidance on research blood samples from COVID-19 patients. Materials with high levels of virus e.g. BAL or samples where cells will be cultured after isolation will be processed

in a University CL3 with full HSE approval and notification. If taken outside of Edinburgh, biomarker bloods will be processed, stored frozen and shipped to Edinburgh for analysis when appropriate.

PK samples will be analysed externally (e.g Oxford University or Galecto). A Material Transfer Agreement will be in place between the University and an external lab prior to any sample transfer.

Saliva samples and nose/throat swabs will be sent for analysis to either NHS/Academic labs or external labs.

Blood plasma and processed respiratory samples will be stored for a period of 10 years in the Centre for Inflammation Research.

Rationale and justification for Biomarker analysis:
Cytokine release syndrome is associated with severe pathology in a subset of COVID-19 patients. This phenomenon occurs 7-10 days after initial symptoms and is associated with the need for ventilatory support, a hypercoagulable state and high mortality. The trigger for this state is uncertain. Superficially the lung injury seems similar to acute respiratory distress syndrome (ARDS). However COVID-induced lung inflammation does not behave like ARDS physiologically, and the limited post mortem data indicates a preponderance of monocytes/macrophages in the injured lung as opposed to the neutrophil-dominant diffuse alveolar damage associated with ARDS.

Significantly higher levels of IL-1β, IL-6, IL-8 and TNF-α have been identified in the serum of patients requiring intensive care, leading scientists at Mount Sinai Hospital (New York) and the Spallanzani Hospital and National Institute for Infectious Disease in Rome to propose this as a core signature indicative of a cytokine storm. These four cytokines have been incorporated into a multiplexed assay allowing rapid quantitation of serum cytokines on an automated ELISA platform with rapid turnaround. Broader analyses using highly multiplexed assays have identified a few analytes, including CXCL-10 and IL-1ra, as factors significantly elevated in patients with severe COVID-19. We will measure these and other cytokines using multiplexed assays.
Participant samples may be analysed for biological indices detailed below – however, this is not an exhaustive list. Due to the nature of this research additional analytical tests may be developed or required in order to profile COVID-19 and develop therapies. All tests will be defined in relevant Standard Operating Procedures / work instructions. Analyses of samples may include DNA or genome wide analysis. Explicit consent for such analyses will be sought from participants.

- Development of **standardised assays** to characterise inflammatory changes or immune responses in patients presenting with COVID-19, especially, but not limited to, those who develop severe lung injury.

- **Flow cytometry** to phenotype cellular subsets including neutrophils, monocytes and T cell subsets, assaying markers of activation, exhaustion or cell death and regulatory T cells as well as cytokine production.

- **Cytokine quantification** to measure plasma cytokines for a broad spectrum of targets upregulated during COVID-19 infection.

- Isolation of protein and RNA from peripheral blood mononuclear cells, neutrophils, adherent respiratory cells (alveolar macrophages) or other BAL cell populations if present.

- Isolate identified cells from blood and respiratory samples to perform functional assays of isolated alone or with epithelial cells in the presence or absence of target drugs, to develop a simple screen for the effectiveness of experimental drugs.

7. DATA COLLECTION

Screening, baseline, study treatment period and follow up data is outlined in the table of assessments in each appendix.

SOURCE DATA DOCUMENTATION

Source data is defined as all information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents.

Source documents are original documents, data and records where source data are recorded for the first time.

7.1 CASE REPORT FORMS

In the first instance, this study will use a paper case report form and these will be specific for the various treatment arms. When an electronic CRF has been designed, the research team will upload previous data entered onto a paper CRF. It is also permissible for the research team to enter data directly onto the electronic CRF, rather than recording all data on a paper CRF beforehand.

7.2 TRIAL DATABASE

The electronic data will be stored securely and confidentially on a Master Study database in REDCap at the Data & Statistics Centre at the Edinburgh CTU (ECTU, University of Edinburgh). Data entry will be done remotely via a dedicated Master Study web portal, with separate web pages and functionality for each qualifying study. There will be a library of common elements to build the core e-CRF for each study, supplemented by bespoke e-CRF for individual studies according to their requirements. Access will be role-based, with remote data entry at the clinical site by authorised users, trained in their data entry tasks.

Query sets will be agreed by researchers and ECTU data managers, and the resultant queries resolved at the site and uploaded. There will be improbable/impossible value checks hardcoded into the eCRF to minimise rogue data being entered at point of entry.

Authorised clients (such as the study statistician needing access to produce progress reports to the independent Data Monitoring Committee and/or the interim or final statistical analyses; and the Trial Manager to produce blinded logistics reports; and the QA manager for audit and ongoing quality assurance reports) will be given protected access to relevant data.

The pseudo-anonymised electronic data will be retained for a period of 25 years, or according to alternative requirements stipulated in the relevant appendix. The study data will be made available after an appropriate delay for sharing for the purpose of legitimate research projects, with a protocol submitted for approval to the investigators.

7.3 ARCHIVING OF THE TRIAL DATABASE

Following the end of the trial, electronic data will be archived by ECTU.

8. DATA MANAGEMENT

8.1.1 Personal Data

The following personal data will be collected as part of the research:

- Patient details (e.g. name, CHI number, date of birth, sex, ethnicity, email address, telephone number, health information, smoking status, residence in care home status).

No personal data will be held electronically.

All paper files containing personal data will be held in site files. These files will be held securely in a locked filing cabinet in a key card restricted area of the clinical site. Access to the research documents will be by the research team only.

CHI numbers (obtained from the paper files) will be used for checking medical records on an ongoing basis as part of the study.

8.1.2 Transfer of Data

De-identified data collected or generated by the study will be transferred to external individuals or organisations outside of the Sponsoring organisation(s). No personal data will be shared. This is vital to ensure that any findings can be fed into national trials and consortia involved in COVID-19 treatments.

8.1.3 Data Controller

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

8.1.4 Data Breaches

Any data breaches will be reported to the University of Edinburgh and NHS Lothian Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

9. STATISTICS AND DATA ANALYSIS

9.1 SAMPLE SIZE CALCULATION

The proposed 'hybrid' platform (multiple interventions) and basket (multiple phenotypes) type randomised trial is early phase to investigate biomarker response relevant to demonstrating COVID-19 clinical activity in re-purposed drugs. As such, formal sample size calculations which would be mandatory for a confirmatory phase III randomised trial are neither feasible nor appropriate.

However, an indicative sample size of 20 per group for the randomised therapies, considering the comparison of one active drug against control, assuming 5% missing data, would give 80% power at a 1-sided 10% level of significance, using a two sample t-test, to detect an effect size of 0.7 in the difference of means in the biomarker between active and control.

That is, the study would be able to detect a mean difference of 0.7 standard deviations for the biomarker. So if biomarker was diastolic blood pressure, and the standard deviation was 10 mmHg, the study could detect a mean difference of 7 mmHg.

The sample sizes are not applicable in allocated groups.

9.2 PROPOSED ANALYSES

The statistical analyses for each study will be comprehensively specified in a Study Specific Appendix for each separate study, in a study specific Statistical Analysis Plan (SAP) pre specified before data lock.

If there are multiple comparisons then these will be appropriately adjusted for to control the experiment-wise false positive rate. Usually the biomarker will be a continuous measure and the treatment effect estimated via a linear model which will adjust for baseline covariates highly correlated with the primary outcome, including possibly the baseline measurement of the primary outcome biomarker.

The study will not be powered for subgroup analyses and these will be exploratory, on a limited number of subgroups pre-specified in the study specific SAP.
Due to the small sample sizes there will be no formal adjustment for missing data, and the primary analysis set – appropriate for early phase proof of signal studies – could be a suitably defined per-protocol set (e.g. those that were compliant with their randomised medications).

Safety data will be analysed on the as-treated data-set (anyone who initiated on randomised treatment) and will be presented descriptively.

The independent DMC will scrutinise accumulating data, unblinded to the randomised groups. Their first and foremost responsibility will be the safety of the participants, and the committee may terminate the study at any time on the grounds of safety. They will also inspect the emerging data to see whether the study can be adapted (an arm dropped, for example; or the randomisation allocation altered by adaptive randomisation) or stopped early for either overwhelming evidence of efficacy, or on the other hand, for futility. If a sequential interim analysis is requested, this will be designed with a Bayesian approach to ensure maximal flexibility and ease of implementation and interpretation.

10. PHARMACOVIGILANCE

The clinical research team are responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

Full details of contraindications and side effects that have been reported following patient consent to participation to the end of the study can be found in the relevant addendum for each therapeutic intervention.

Participants in the interventional arms will be instructed to contact the research team at any time after consent to study participation if any symptoms develop. For all participants, all adverse events (AE) (except for those listed in Section 10.3.2) that occur from the time of consent until the relevant follow up period after the final dose of investigational medication must be recorded in the Case Report Form (CRF) or AE log. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment.

Adverse event reporting will be undertaken for the control (standard care) arm, as well a treatment arms. Clinical data and disease progression will be documented via linkage to control participants' medical records.

All adverse events (AE) that are not related to the patient's underlying condition or clinical interventions will be recorded following consent. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment.

10.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical occurrence in a clinical trial participant which does not necessarily have a causal relationship with an investigational medicinal product (IMP).

An **adverse reaction** (AR) is any untoward and unintended response to an IMP which is related to any dose administered to that participant.

A **serious adverse event** (SAE), **serious adverse reaction** (SAR). Any AE or AR that at any dose:

- results in death of the clinical trial participant;
- is life threatening*;
- requires in-patient hospitalisation^ or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect;
- results in any other significant medical event not meeting the criteria above.

*Life-threatening in the definition of an SAE or SAR refers to an event where 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.

^Any hospitalisation that was planned prior to enrolment will not meet SAE criteria. Any hospitalisation that is planned post enrolment will meet the SAE criteria.

A suspected unexpected serious adverse reaction (SUSAR) is any AR that is classified as serious and is suspected to be related to the IMP, that it is not consistent with the information about the IMP in the Summary of Product Characteristics (SPC) booklet, Investigator's Brochure or product specific protocol addendum.

10.2 IDENTIFYING AEs AND SAEs

If there is any doubt as to whether a clinical observation is an AE, the event will be recorded.

AEs and SAEs may also be identified via information from support departments e.g. laboratories.

10.3 RECORDING AEs AND SAEs

When an AE/SAE occurs, it is the responsibility of the Chief Investigator, or another suitably qualified physician in the research team who is delegated to record and report AEs/SAEs, to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the CRF/AE log and on the SAE form (if the AE meets the criteria of serious).

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

10.3.1 Pre-existing Medical Conditions

Pre-existing medical conditions (i.e. existed prior to informed consent) should be recorded as medical history and only recorded as adverse events if medically judged to have worsened during the study.

10.3.2 Worsening of the Underlying Condition during the Trial

Medical occurrences or symptoms of deterioration that are expected due to the participant's underlying condition should be recorded in the patient's medical notes and only be recorded as AEs on the AE log if medically judged to have unexpectedly worsened during the study. ***Events that are consistent with the expected progression of the underlying disease should not be recorded as AEs. These may include:***

- ***Respiratory failure requiring ventilator support***
- ***Specific organ function support (heart failure, myocardial infarction, stroke, pulmonary embolism, liver or renal failure requiring dialysis or haemofiltration)***
- ***Death due to COVID-19 unless administration of investigational medication is considered to have contributed to these events.***

MedDRA terms for the adverse events that will not be reported have been included in the table below:

• **Respiratory failure requiring ventilator support**	Respiratory Failure	Respiratory Failure
	Ventilatory Failure	Respiratory Failure
	Dependence on Ventilator	Dependence on respirator
• **Specific organ function support (heart failure, myocardial infarction, stroke, pulmonary embolism, liver or renal failure requiring dialysis or haemofiltration)**	Heart Failure	Cardiac Failure
	Myocardial Infarction	Myocardial Infarction
	Pulmonary embolism	Pulmonary embolism
	Stroke	Cerebrovascular accident
	Liver failure	Hepatic Failure
	Renal failure	Renal Failure
	Dialysis	Dialysis
	Haemofiltration	Haemofiltration
• **Death due to COVID-19 unless administration of investigational medication is considered to have contributed to these events.**	Death	Death

10.4 ASSESSMENT OF AEs AND SAEs

Each AE must be assessed for seriousness, causality, severity and ARs must be assessed for expectedness by the Principal Investigator or another suitably qualified physician in the research team who has been delegated this role.

The Chief Investigator (CI) may not downgrade an event that has been assessed by an Investigator as an SAE or SUSAR, but can upgrade an AE to an SAE, SAR or SUSAR if appropriate.

10.4.1 Assessment of Seriousness

The Investigator will make an assessment of seriousness as defined in Section 10.1.

10.4.2 Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to the IMP according to the definitions below.

- Unrelated: where an event is not considered to be related to the IMP.
- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the study drug.

Where non Investigational Medicinal Products (NIMPs) e.g. rescue/escape drugs are given: if the AE is considered to be related to an interaction between the IMP and the NIMP, or where the AE might be linked to either the IMP or the NIMP but cannot be clearly attributed to either one of these, the event will be considered as an AR. Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated. The blind should not be broken for the purpose of making this assessment.

10.4.3 Assessment of Expectedness

If the event is an AR the evaluation of expectedness will be made based on the Reference Safety Information as defined or cited in the relevant protocol appendix.

The event may be classed as either:

Expected: the AR is consistent with the toxicity of the IMP listed in the Refernce Safety Information.

Unexpected: the AR is not consistent with the toxicity in the Reference Safety Information.

Fatal and life threatening SARs should usually be considered unexpected. Fatal SARs can only be expected for IMPs with an MA in the EU, when it is clearly stated in the Reference Safety Infromation that the IMP causes fatal SARs.

10.4.4 Assessment of Severity

The Investigator will make an assessment of severity for each AE/SAE/SAR/SUSAR and record this on the CRF/AE log or SAE form according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.
Severe: an event that prevents normal everyday activities.

Note: the term ‘severe’, used to describe the intensity, should not be confused with ‘serious’ which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

10.5 RECORDING OF AEs

All adverse events for each participant will be recorded on the AE log and will be assigned the appropriate MedDRA Systems Organ Class (SOC) code.

10.6 REPORTING OF SAEs/SARs/SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance **within 24 hours**. If the Investigator does not have all information regarding an SAE, they should not wait for this additional

information before notifying ACCORD. The SAE report form can be updated when the additional information is received.

The SAE report will provide an assessment of causality and expectedness at the time of the initial report to ACCORD according to Sections 10.4.2, Assessment of Causality and 10.4.3, Assessment of Expectedness.

The SAE form will be transmitted via email to safety@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

Any therapy-specific onward reporting safety requirements are detailed in the appropriate appendices. All reports sent to ACCORD and any follow up information will be retained by the Investigator in the Investigator Site File (ISF).

10.7 REGULATORY REPORTING REQUIREMENTS

ACCORD is responsible for pharmacovigilance reporting on behalf of the co-sponsors (The University of Edinburgh and NHS Lothian).

ACCORD has a legal responsibility to notify the regulatory competent authority and relevant ethics committee (Research Ethics Committee (REC) that approved the trial). Fatal or life threatening SUSARs will be reported no later than 7 calendar days and all other SUSARs will be reported no later than 15 calendar days after ACCORD is first aware of the reaction.

ACCORD (or delegate) will inform Investigators at participating sites of all SUSARs and any other arising safety information.

ACCORD will be responsible for providing safety line listings and assistance; however, it is the responsibility of the Investigator to prepare the Development Safety Update Report. This annual report lists all SARs and SUSARs reported during that time period. The responsibility of submitting the Development Safety Update Report to the regulatory authority and RECs, lies with ACCORD.

10.8 PREGNANCY REPORTING

All pregnancies that occur within the active trial period (either the trial participant or the participant's partner) will be reported to the CI and sponsor using the relevant Pregnancy Notification Form within 24 hours of notification. The pregnancy will be followed up until the end of pregnancy. If the trial participant is a male, informed consent will be sought from his female partner.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, thus would be considered an SAE.

10.9 FOLLOW UP PROCEDURES

After initially recording an AE or recording and reporting an SAE, the Investigator should make every effort to follow each event until a final outcome can be recorded or reported as necessary. Follow up information on an SAE will be reported to the ACCORD office.

If, after follow up, resolution of an event cannot be established, an explanation should be recorded on the CRF or AE log or additional information section of SAE form.

11. TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

11.1 TRIAL MANAGEMENT GROUP

The trial will be coordinated by a Project Management Group, consisting of the grant holders, the Chief Investigator, Project Managers and members of the clinical research team.

A Delegation Log will be prepared for each site, detailing the responsibilities of each member of staff working on the trial.

11.2 TRIAL STEERING COMMITTEE (TSC)

A TSC will be convened for this study. The TSC will provide oversight with independent input and will work in collaboration with the research team to decide on which therapies will be tested as part of this platform. A stand alone document details the therapy selection process.

11.2 DATA MONITORING COMMITTEE

An independent DMC will be established to oversee the safety of participants.

During the study, the DMC will review any safety assessments that the research team present to them if there are any concerns raised.

The DMC will continually review any SUSARS or other safety signals that are reported. The DMC will also have scheduled meetings to review the safety data at intervals as defined in the DMC charter applicable for a specific appendix. The precise workings of the DMC is detailed in the DMC charter.

11.4 SUB STUDIES

Proposals for sub-studies must be approved by the Trial Management Group and by the relevant ethics committee and competent authorities (where required) as a substantial amendment or separate study before they begin.

11.5 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of an audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

11.6 STUDY MONITORING AND AUDIT

ACCORD clinical trial monitors, or designees, will perform monitoring activities in accordance with the study monitoring plan. This will involve on-site visits and remote monitoring activities as necessary. ACCORD QA personnel, or designees, will perform study audits in accordance with the study audit plan. This will involve investigator site audits, study management audits and facility (including 3rd parties) audits as necessary (delete where not required).

12. GOOD CLINICAL PRACTICE

12.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).
Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met. Required approvals will be expedited via the NIHR COVID-19 research portal.

12.2 REGULATORY COMPLIANCE

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, as amended.

12.3 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

Delegated tasks must be documented on a Delegation Log and signed by all those named on the list prior to undertaking applicable study-related procedures.

12.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any study specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants or personal legal representatives must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant or personal legal representatives must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant or personal legal representatives must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant or personal legal representatives will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s).

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The original will be signed in the Investigator Site File (ISF). The participant will receive a copy of the signed consent form and a copy will be filed in the participant's medical notes.

12.3.2 Study Site Staff

The Investigator must be familiar with the IMP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties.

12.3.3 Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

12.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the ACCORD Research Governance & QA Office, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.
- ACCORD will ensure all other documents required by ICH GCP are retained in a Trial Master File (TMF) or Sponsor File, where required. The Principal Investigator will ensure that the required documentation is available in local Investigator Site files ISFs. Under certain circumstances the TMF responsibilities may be delegated to the research team by ACCORD.

12.3.5 GCP Training

All study staff must hold evidence of appropriate GCP training.

12.3.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

12.3.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including where applicable the General Data Protection Regulation with regard to the collection, storage, processing and disclosure of personal information. Access to personal information will be restricted to individuals from the research team treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

13. STUDY CONDUCT RESPONSIBILITIES

13.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Proposed amendments will be submitted to the Sponsor for classification and authorisation.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to implementation.

13.2 PROTOCOL NON COMPLIANCE

13.2.1 Definitions

Deviation - Any change, divergence, or departure from the study design, procedures defined in the protocol or GCP that does not significantly affect a subjects rights, safety, or well-being, or study outcomes.
Violation - A deviation that may potentially significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being

13.2.2 Protocol Waivers

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

13.2.3 Management of Deviations and Violations

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. Deviation logs / violation forms will be transmitted via email to QA@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner.

13.3 URGENT SAFETY MEASURES

The Investigator may implement a deviation from or change to the protocol to eliminate an **immediate hazard** to trial participants without prior approval from the REC and the MHRA. This is defined as an urgent safety measure and the investigator must contact the Clinical Trial Unit at the MHRA and discuss the issue with a medical assessor immediately (+44 (0) 20 3080 6456).

The Investigator will then notify the MHRA (clintrialhelpline@mhra.gsi.gov.uk), the REC and ACCORD, in writing of the measures taken and the reason for the measures within 3 days by submitting a substantial amendment.

13.4 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree: (a) the

safety or physical or mental integrity of the participants of the trial; or (b) the

scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (QA@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to regulatory authorities and research ethics committees as necessary.

13.5 STUDY RECORD RETENTION

For Appendix 1 and 2 all study documentation will be kept for a minimum of 15 years from the protocol defined end of study point. For all other appendices, study documentation will be kept for the minimum period defined in the relevant protocol appendix, which will take into account any specific local or regulatory requirements for that particular asset and trial phase.

When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

13.6 END OF TRIAL

The end of the scheduled treatment phase is defined as the date of the last treatment day of the last participant. The end of the trial is defined as the last data entry for the last participant completing the scheduled treatment phase.

The Investigators or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.
The Investigators and/or the trial management group and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, Regulatory Authority, R&D Office(s) and cosponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the cosponsors via email to resgov@accord.scot.

In accordance with ACCORD SOP CR011, a Clinical Study Report (CSR) will be provided to the Sponsor (QA@accord.scot) and REC within 1 year of the end of the study.

Upon completion of the study, the Investigator will upload clinical trial results onto the EudraCT database on behalf of the Sponsor.

The Investigator will submit a short confirmatory e-mail to the MHRA (CT.Submission@mhra.gsi.gov.uk) once the result-related information has been uploaded to EudraCT, with 'End of trial: result-related information: EudraCT XXXX-XXXXXX-XX' as the subject line. The Sponsor(s) will be copied in this e-mail (QA@accord.scot). It should be noted that you will not get an acknowledgment e-mail or letter from the MHRA.

13.7 CONTINUATION OF DRUG FOLLOWING THE END OF STUDY

Access to the experimental therapies will be not be permitted following the end of the participant's treatment period.

13.8 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been authored by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities. Sites which are part of the United Kingdom's National Health Service have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.
- The manufacturer supplying IMP has accepted limited liability related to the manufacturing and original packaging of the study drug and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown Adverse Events which arise out of the manufacturing and original packaging of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

14. REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

14.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with ICH guidelines.

14.2 PUBLICATION

Scientific publications and the sharing of clinical data generated as part of this trial is crucial to better understanding COVID-19 and developing new treatments. As such, the results of each nested study detailed in the relevant appendices will be published as soon as the treatment arm has finished recruitment, data has been cleaned and any outstanding patient safety follow-ups completed.

The Clinical Study Report (CSR) will be submitted to the Sponsor and REC within 1 year of the end of the study. Where acceptable, a published journal article may be submitted as the CSR. The Chief Investigator will provide the CSR to ACCORD, for review, prior to finalization. The clinical study report may be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study. The results of the study, together with other mandated information, will be uploaded to the European clinical trials database within 1 year of the end of the study.

Results for each protocol appendix will be made publically available via the Translational Healthcare Technologies Research Group website following completion of the appendix and analysis of the data. Results for each protocol appendix will also be published in peer reiew journals. Each of these will be done within 1 year of the appendix being completed.

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion).

14.3 DATA SHARING

Consent will be sought from participants to permit sharing of anonymised data with funders, commercial and non-commercial collaborators or published on publically available resources as appropriate.

14.4 PEER REVIEW

This protocol has been reviewed by the University of Edinburgh emergency COVID-19 College of Medicine and Veterinary Medicine committee.

Academic and Clinical Central Office for Research and Development

DEFINE

Evaluating therapies for COVID 19

PROTOCOL APPENDIX 3

Therapy:	**Allogeneic SARS-CoV-2 VSTs**
Route of administration:	Intraveneous infusion
Manufacturer:	NHS NSS Scottish National Blood Transfusion Service
DEFINE COVID-19 Cohort(s):	2C - Confirmed COVID-19 positive patients with ≥92% O_2 saturations on air. 2D - Confirmed COVID-19 positive patients with ≥92% O_2 saturations on supplemental oxygen (maximum FiO_2 of 28% (2 – 6 L/min depending on delivery device used))

APPENDIX APPROVAL SIGNATURE PAGE

DEFINE - Evaluating therapies for COVID 19

Signature details removed for registry upload

Version No	Changes	Date Approved by MHRA/Regulatory Authority
1.0	Original submission	N/A
2.0	Response to GfNA (09/02/2022) Sections 1.2.3, 4.3, 5.2	04 Apr 2022
3	Update to statistical analysis section (section 7.2) – removal of reference to Bayesian analysis	N/A
4	**Page 2** – Site sign off section for protocol Appendix 3 added (due to addition of second site – WGH). **Section 5.2** – Named senior cover for WGH added. **Section 6** – Table of assessments and relevant footnotes updated/amended. **Section 6.2** – updated to reflect changes made to table **Section 6.3** – removal of reference to RIE, made more general to cover activities being carried out at RIE and/or WGH. Change to pulse oximetry readings in participant diary as source for oxygenation levels from day 3, (even if participant remains in hospital) to ensure participant is comfortable with process of measuring and recording prior to discharge.	07Sep2023
5	**Section 6 – Table of Assessments** ● Administrative correction to table (footnotes) ● Addition of oxygen therapy data collection, via diary, up to day 21.	01Nov2023
6	**Section 1.2.3 - Benefit-Risk Statement,** **Section 2.1 – Objectives and Endpoints** **Section 4 - Study population** (including appendix specific inclusion and exclusion criteria) • Addition of new cohort (2D) **Section 4.3 – Inclusion and Exclusion Criteria** • Addition of corticosteroid exclusion (App3 specific exclusion)	DDMMMYYYY

CONTENTS

LIST OF ABBREVIATIONS

ATIMP	Advanced Therapy Investigational Medicinal Product
ATMP	Advanced Therapy Medicinal Product
CCD	COVID-19 Convalescent Donors
CCS	Cytokine Capture System
COVID-19	Coronavirus Disease-2019
CRF	Case Report Form • eCRF - electronic CRF (database) • pCRF – paper CRF
CTIMP	Clinical Trial of an Investigational Medicinal Product
DMC	Data Monitoring Committee
DMSO	Dimethyl Sulfoxide
EBV-CTL	Epstein Barr Virus – Cytotoxic T Lymphocytes
ECCRG	Edinburgh Critical Care Research Group
ECTU	Edinburgh Clinical Trials Unit
HLA	Human Leukocyte Antigen
HSCT	Haematopoietic stem cell transplantation
IL-2	Interleukin-2
IMP	Investigational Medicinal Product
LCL	Lymphoblastoid Cell Line
MHC	Major Histocompatibility Complex
MNC	Mononuclear Cells
PHE	Public Health England
PTLD	Post-Transplant Lymphoproliferative Disorder
PRNT-50	Plaque Reduction Neutralisation Test
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome - Corona Virus - 2
SNBTS	Scottish National Blood Transfusion Service
T-Liven PR™	T-Cell validated pooled Human Platelet Lysate (pathogen reduced)
TA-GvHD	Transfusion Associated Graft Versus Host Disease
TSC	Trial Steering Committee
VST	Virus Specific T Cells
WOCBP	Women of Childbearing Potential

1. INTRODUCTION

1.1 BACKGROUND

Coronavirus infection (COVID-19) caused by the SARS-CoV-2 virus is characterised by dysregulation of effector T cells and accumulation of exhausted T cells. Ineffective or absent T cell responses to viruses can be temporarily corrected by adoptive cellular therapy using donor-derived Virus-Specific T cells (VSTs). The Scottish National Blood Transfusion Service (SNBTS) has more than 15 years' experience in the manufacture and clinical use of donor derived VSTs through the allogeneic anti-Epstein Barr Virus Cytotoxic T Lymphocyte (EBV-CTL) products which have been used clinically for treatment of EBV driven malignancies, in particular Post-Transplant Lymphoproliferative Disorder (PTLD). This clinical experience includes a multicentred phase II clinical trial using SNBTS's first generation stock of partially HLA-matched allogeneic anti-EBV CTLs which demonstrated this treatment to be safe and effective. In addition, a second generation stock from 25 HLA-typed apheresis donors has been issued on a named patient basis under SNBTS's Manufacturers' Specials (MS) licence 3473 which also demonstrated good efficacy with a strong safety profile. A third generation stock is under development using pooled EBV peptides to isolate EBV-specific T cells.

The SARS-CoV-2 VST product has been developed based on experience with the SNBTS new third generation Epstein Barr Virus (EBV) - Virus Specific T Cell therapy. We have shown that blood donations from COVID-19 Convalescent Donors (CCD) contain CD4 and CD8 T cells specific for SARS-CoV-2 antigens. The SARS-CoV-2 VST product is manufactured from allogeneic human donor leukapheresis collections, which are stimulated using a pool of synthetic SARS-CoV-2 peptides, then selected using the IFN-gamma Cytokine Capture System (CCS) (Miltenyi Biotec) and expanded in culture media containing IL-2 and pathogen-reduced platelet lysate over 14 days. The resultant expanded T cell population of allogeneic SARS-CoV-2 VSTs is harvested, washed and cryopreserved into units each with a target of $1x10^7$ cells/mL in cryoprotectant.

A stock of several HLA-typed donor T cell products is in the process of manufacture. The HLA typed SARS-CoV-2 VSTs will be selected from the stock, matched appropriately and issued to consented participants on the trial.

1.2 SAFETY of SARS-CoV-2 VSTs

The SNBTS SARS-CoV-2 VSTs have not previously been administered/infused into humans. The manufacture of this product has been developed based on the development and use of the SNBTS allogeneic EBV-CTL product, which has been administered clinically (33 from the first generation stock and 88 patients from the second generation stock, at time of writing). In terms of safety, no adverse effects were reported following infusion of the first generation EBV-CTL products (Haque *et al.* 2007), and similarly the second generation products have a good safety record with no serious adverse reactions (SAR) directly attributed to the product. Further details regarding product safety and previous human experience are given in section 1.2.2.

The manufacturing process of the first and second generation EBV-CTL products differs to that for the SARS CoV-2 VSTs, with the former using repeated stimulation of Mononuclear cells (MNCs) with autologous gamma irradiated EBV infected Lymphoblastoid Cell Line (LCL) cells in order to amplify EBV specific CTLs (Wilkie *et al.* 2004). The peptide stimulation method used to manufacture the SARS-CoV-2 VSTs is based on that developed for the third generation EBV-CTL product and offers several

advantages including a significantly shorter manufacturing process (2 weeks compared to 8-12 weeks (inclusive of LCL generation time) for the first/second generation EBV-CTL process), it is predominantly a closed process therefore reducing the risk of introducing contamination, and does not use live virus for generation of the antigen stimulus. The third generation EBV-CTL stock has not been administered clinically so far. However, despite the above mentioned differences in manufacture, comparison of the composition and surface phenotype of the second and third generation products show them to be similar suggesting these two manufacturing processes generate similar products, albeit with potentially-differing EBV virus antigen specificities.

1.2.1 Preclinical Safety Profile

For convalescent plasma and therapeutic antibodies, virus neutralisation titre ($PRNT_{50}$) is considered the gold standard *in vitro* model of efficacy; Receptor Binding Domain (RBD)-ACE2 assays have been used as a partial surrogate for a $PRNT_{50}$ assay. These assay formats are however not relevant for VST products as they are designed for antibodies not T cells.

There are several *in vivo* animal models of COVID-19 infection available. However, none of these models allow for the assessment of efficacy, safety, biodistribution or tumorigenicity in respect of SARS-CoV-2 VSTs.

These *in vivo* models include the murine, ferret and Syrian hamster models, which can both assess aspects of virus replication and virus pathology. The non-human primate model is also capable of assessing virus replication and pathology as well as cellular immune response pharmacology. However, these models would not yield translatable data on human SARS-CoV-2 VSTs because of lack of recognition of animal MHC and xenogeneic rejection. Therefore no suitable *in vivo* non-clinical studies are available prior to the commencement of this dose escalation study.

The SNBTS allogeneic T cell therapy product (1^{st} and 2^{nd} generation EBV-CTL stocks) provides evidence for the general safety of this type of cell therapy product through results from clinical administration in over 100 patients.

Additionally the likelihood of alloreactivity is considered very low as blood components in the UK currently contain up to 10^6 leukocytes/unit (up to $5x10^6$ leukocytes/unit between 1999 and 2004). Prior to the introduction of routine leukodepletion in 1999 blood components contained between 10^8 and 10^9 leukocytes / unit with very high safety level as demonstrated by SHOT reporting (launched in 1996).

1.2.2 Previous Human Experience

The allogeneic SARS-CoV-2 VSTs have not been administered in humans before. Likewise the third generation EBV-CTL product manufactured using the Cytokine Capture System has also not been administered to humans before. However SNBTS has manufactured allogeneic EBV-CTLs for several years (first and second generation EBV-CTL products) which have been used clinically for treatment of EBV driven malignancies, in particular Post-Transplant Lymphoproliferative Disorder (PTLD). This clinical experience includes a multicentred phase II clinical trial (Haque et al. 2007) using SNBTS's first generation stock of partially HLA-matched allogeneic EBV-CTLs which demonstrated this treatment to be safe and effective. In addition, a second generation stock from 25 HLA-typed apheresis donors has been issued on a named patient basis under the SNBTS Manufacturers' Specials (MS) licence 3473. A census of those patients treated with the second generation stock was performed in 2017 (59

patients). The patient group was mainly post-transplant patients (solid organ or haematopoietic stem cell transplantation) who had failed conventional treatments or who were too ill to tolerate chemotherapy. This census demonstrated some form of clinical response in 35 (59%) patients, 23 (39%) complete and 12 (20%) partial (Kazi et al 2019). No clinical responses were apparent for 24 (41%) patients, none of whom survived for more than three months. Note, the condition of these patients was very poor, with most dying before the completion of treatment (the EBV-CTL treatment schedule is 4 doses ($2x10^6$cells/kg/dose) at one dose per week), suggesting that they had presented very late in the course of their disease. These failures of the EBV-CTL treatment may therefore be related to the extent of the illness by the time this product was administered.

In terms of safety, no adverse effects with infusion of the first generation EBV-CTLs were reported (Haque *et al.* 2007), and similarly the second generation EBV-CTLs have a good safety record with no serious adverse reactions (SARs) directly attributed to the product. Specifically, there have been two cases of mild, transient skin graft-versus-host disease (GvHD) both treated with topical agents and one case of temporary exacerbation of neurological symptoms, thought to be caused by a tumour flare in a patient who responded (there has been one possibly related Serious Adverse Event (SAE) since the census reported in Kazi et al 2019, where an abnormal liver enzyme level was detected post infusion with first dose which then normalised and was not detected on subsequent infusions). There has been no evidence of cytokine release syndrome or neurological syndromes seen with CAR-T cell administration.

In relation to immunogenicity of the allogeneic EBV-CTLs, for the first and second generation stocks, patients were screened for evidence of an antibody response to mismatched HLA molecules. For the first generation EBV-CTLs, testing of pre- and post-CTL infusion sera from all trial participants (33 participants) revealed that only one patient had developed an antibody response against a mismatched donor HLA antigen (Haque *et al.* 2007). For the second generation EBV-CTLs, in those patients where twelve-week post-final infusion samples were available (20/59), none were positive for HLA alloantibodies directed against CTL mismatched antigens.

1.2.3 Benefit-Risk Statement

The SARS-CoV-2 VSTs are an allogeneic T cell therapy to be administered to patients with a recent (within the preceding 14 days) diagnosis of SARS-CoV-2 who are confirmed to be COVID-19 positive, with ≥92% O_2 saturations on air or a maximum supplemental FiO_2 of 28%. These patients may or may not have evidence of pneumonitis on chest radiology.

As the SARS-CoV-2 VSTs have not been administered in humans before, a cautious dose escalation strategy is being applied from $2x10^4$ cells/kg to $2x10^6$ cells/kg (based on standard 75kg weight; target dose of $1.5x10^6$ cells to $150x10^6$ cells) to ensure recipient safety. Initially, there will be a 3 week gap between each patient within the first Dose Group (cross refer to section 5.2 (Fig. 1) dosing schedule). Data Monitoring Committee (DMC) review and approval will be required prior to progressing to the next Dose Group. Thereafter, the dosing schedule and decisions to proceed will be determined by the DMC. Adverse reactions, vital signs (including SpO2, FiO2, RR, PR, temperature, blood pressure) and laboratory safety assessments (as defined in the Table 1 below) will submitted to the DMC for review at each stage.

Note, the dosing stratey and the above mentioned cautious approach and inclusion of flexibility regarding dosing interval is based on clinical experience (see paragraph below) and was discussed with the MHRA at a Scientific Advice meeting held on 23rd Setpember 2020, and follows the European Medicines Agency First in Human

Guidance: Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (europa.eu). Participants will remain in hospital for a minimum of 48 hours after dosing, although if the clinical picture requires it e.g. participant develops an adverse effect, then their hospital stay will be prolonged as necessary according to the clinical picture. All participants will also be routinely followed up for up to 6 weeks post infusion, with daily oxygen saturations being recorded until day 21. These timings have been identified based on the likely timelines of onset of adverse events were any to occur (as described below) and the likely persistence of the IMP (see below). The principal mechanism of clearance of this product is through the host immune response via recognition of mis-matched HLA markers on the donor VST. The initiation of the immunological recognition of the infused VST can take several days but may be quicker depending on the participant's immunological status, but once in effect the rejection of donor VST would be rapid. The monitoring period has been identified to ensure that the therapeutic window of the administered VST has been captured.

The initial dose of $1.5x10^6$ cells (equates to $2x10^4$ cells/kg based on standard 75kg weight) is considered appropriate based on experience observed with other adoptive T cell therapies (e.g. Feuchtinger et al, 2006) where no acute clinical side effects were documented after infusion of this dose of T cells. Blood components in the UK currently contain up to 10^6 leukocytes/unit (and up to $5x10^6$ leukocytes/unit between 1999 and 2004) with a very high safety level and no evidence of TA-GvHD. In addition, given the safety data accumulated for the EBV-CTL product in a patient group with very severe illness and immunosuppression, and treated with 4 doses of the product (each dose at $1\text{-}2x10^6$ cells/kg, equivalent to the highest target dose for the SARS-CoV-2 VSTs), it is anticipated that the risk of adverse effects following administration of the SARS-CoV-2 VSTs to the COVID-19 patient cohort is low.

Each batch of SARS-CoV-2 VSTs is tested for sterility using a validated test according to Ph. Eur 2.6.27. Each batch is only released after it has tested negative. Each batch of drug product must also pass testing for endotoxin (Ph. Eur. 2.6.14) and mycoplasma (Ph. Eur 2.6.7) prior to release. The risk of sterility failure is therefore controlled to a high degree.

Patients will be admitted to a suitable clinical area within the study site for administration and monitoring during the initial period of the trial. Patients will remain in hospital for at least 48 hours post administration of the product.

Risk associated with starting material and manufacturing process
Every effort to minimise any potential risk from adventitious agents has been taken for both starting material and reagents used during the manufacturing process. All materials of human or animal origin associated with the manufacturing process for the allogeneic SARS-CoV-2 VSTs are of acceptable safety with respect to adventitious agents.

Risk from excipient
To maintain the viability of the SARS-CoV-2 VSTs during cryopreservation, the CryoStor® CS10 component of the excipient includes DMSO. The concentration of DMSO in the product is low (approx. 6.7%) and as such is unlikely to cause severe side effects. DMSO is routinely used in the cryopreservation of haematopoietic stem / progenitor cells and other ATMPs such as CAR-T cells, and the SNBTS EBV CTL product used clinically by SNBTS, and is normally well tolerated. Any side effects that are likely to happen frequently (>10%) are normally mild and transient, including a feeling of warmth spreading up the arm, into the chest and then into the head, abdomen

and pelvis; and a garlic-like smell that lasts for several minutes to a few hours. Common (1-10%) side effects include nausea, low blood pressure, low heart rate, skipped beats, cough, headache, stinging on passing urine. Uncommon (0.1-1%) side effects include vomiting, diarrhoea, high blood pressure, pelvic discomfort and haemolysis. Rare (<0.1%) side effects include anaphylactic reactions.

Previous clinical experience
As detailed above SNBTS has manufactured allogeneic EBV-CTLs for many years which have been used clinically for treatment of EBV driven malignancies, in particular Post-Transplant Lymphoproliferative Disorder (PTLD). The clinical experience with this product has demonstrated a good safety record; it is therefore anticipated that the risk of side effects from administration of the SARS-CoV-2 VSTs to the COVID-19 patient cohort is low.

Infusion
An infusion requires a cannula to be inserted which may in turn cause brief pain, bleeding, bruising of the skin, soreness, swelling and possible infection at the infusion site.

Persistence and reactivity
COVID-19 patients with no underlying immunodeficiency generally retain an intact immune response throughout infection, though with evidence of perturbations to immune system function in severe infection (Qin et al 2020 ; Chen et al 2019, Zheng et al 2020). The VSTs are predominantly CD4+ central memory T cells which are able to replicate a number of times in response to virus antigens and differentiate into effector cells which would target the infection. Allogeneic cells are not likely to persist in recipients due to anti-HLA antibody and T cell responses, and therefore there is little likelihood of long-term persistence of the adoptively-transferred T cells.

Detection of the VSTs in the recipient after administration would be extremely difficult due to the small numbers administered in comparison with the complete T cell compartment, particularly in the low dose stages. In addition, after administration it is anticipated that the VSTs will sequester in infected tissues and therefore be essentially undetectable in peripheral blood. In light of the challenges involved, assessing persistence of the VSTs after administration is considered unlikely to be either feasible or clinically relevant, although exploratory testing will be considered for the two higher Dose Groups. Patients will undergo daily clinical safety assessments including full blood count and differential white cell count.

The specificty of the adoptive T cells is targeted primarily at SARS-CoV-2 virus-specific motifs, and therefore would have very low reactivity to recipient tissues. The effectiveness of the product is increased by matching HLA type to at least one allele, while the risk of GvHD is minimised by also ensuring at least one HLA mismatch.

It has been shown with other virus-specific T cell therapies that there is very low incidence of GvHD in recipients (Melenhorst et al 2010), and any significant side effects in patients receiving these VSTs is not expected. SNBTS's own experience of administering EBV-CTL products in over 100 immunocompromised patients corroborates this with only two cases of very mild transient dermal GvHD being reported.

Risk of tumorigenesis
There are no known published data on testing of differentiated somatic cell therapies such as VSTs for tumorigenic potential. All current data on tumorigenesis relates to

cell banks such as induced pluripotent stem cells or mesenchymal stromal cells, which have the potential for mutagenic events in manufacture (Ben-David et al 2010; Kim et al 2017). As the SARS-CoV-2 VSTs are differentiated allogeneic primary cells, with limited likelihood of persistence due to partial HLA matching, there is minimal risk for the potential of tumorigenesis from this product.

1.3 PRECAUTIONS

Trials with the drug product will be conducted to the principles of Good Clinical Practice (GCP, ICH E6) and in line with the Clinical Trial Regulations 2004 No. 1031. All revelant staff will be GCP trained.

In addition, to the normal review and approval process required, this trial also underwent review and approval by the local Phase 1 Committee and the Advanced Therapy medicinal Products (ATMP) Committee.

Prior to the use of the product in clinical trial, each product batch will have completed all necessary final product specification tests with acceptable results and undergone QA review and approval, followed by certification by the Qualified Person (QP) to verify that the product complies with its specification, that essential documentation is in place and that all information relating to the safety of the product has been reviewed and is acceptable. In addition, the HLA profile of the patient will be obtained and the batch of SARS-CoV-2 VSTs selected according to the HLA cross-matching strategy (please cross refer to section 5.1.6).

Safety oversight for the trial will be provided by an independent DMC, with the DMC membership including relevant experts in the field. The DMC will have oversight and input to the trial at all stages to maximise safety. The dose escalation study is designed such that there is initially 3 weeks between dosing (administration of infusion) the first patient and subsequent dosing of patients within the first dose group and from the next Dose Group. DMC review and approval will be required prior to progressing to the next Dose Group. Thereafter, the dosing schedule and decisions to proceed will be determined by the DMC, cross refer to section 5.2, 5.3 and Fig. 1.

The DMC will communicate its decision whether to authorise progression to the next dose and the dosing schedule for the next escalation dose. The decision will be communicated to the sponsor on an agreed template.

1.4 ADAPTIVE PROTOCOL DESIGN

This appendix has been written to be used in conjunction with the main DEFINE COVID-19 trial protocol which describes an overarching and adaptive trial design to test candidate therapies for COVID-19 positive patients.

The information detailed within this appendix applies only to SARS-CoV-2 VSTs.

2. INTERVENTION-SPECIFIC OBJECTIVES

2.1 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the safety of SARS-CoV-2 VSTs as add-on therapy to SoC in patients with COVID-19.	Safety will be assessed using: • Haematological and biochemical safety laboratory investigations • Directed cardio-respiratory physical examination • Vital signs (blood pressure / heart rate / respiratory rate, temperature) • Adverse events **No daily electrocardiogram (ECG) readings required for this appendix (only required at screening)*
Secondary	
To evaluate the improvement or deterioration of patients	Record changes to WHO ordinal scale and NEWS2 score
To evaluate the number of oxygen-free days.	Duration (days) of oxygen use and oxygen-free days.
Where oxygen is required, change in the ratio of the oxygen saturation to fraction of inspired oxygen concentration (SpO_2/FiO_2)	SpO_2/FiO_2, measured daily from baseline to 48hrs post infusion
To evaluate time to discharge	• Duration of total hospital stay, due to covid 19 • Duration to discharge following treatment *Evaluation of time to discharge will not be included as a secondary objective/endpoint for Appendix 3 as length of hospital stay of participants meeting cohort 2C or 2D criteria is not, in general, determined by their COVID infection.*
Secondary Non Essential – these data will be collected if available however, they are not essential to the core analysis.	
To evaluate SARS-CoV-2 viral status.	Qualitative and quantitative polymerase chain reaction (PCR) determination of SARS-CoV-2 in oropharyngeal/nasal swab and/or saliva samples and/or blood samples. *These samples will be collected as per the table of assessments in this appendix.*

3. DESIGN

This is an early dose escalation safety trial phase Ib/IIa interventional clinical trial with SARS-CoV-2 VSTs.

3.1 Choice of Treatment

COVID-19 caused by the SARS-CoV-2 virus is characterised by dysregulation of effector T cells and accumulation of exhausted T cells. Ineffective or absent T cell responses to viruses can be corrected by adoptive cellular therapy using donor-derived Virus-Specific T cells (VSTs). The SNBTS has several years' experience in the manufacture and clinical use of donor derived VSTs through the allogeneic EBV-CTL products which have been used clinically for treatment of EBV-driven malignancies, in particular post-transplant lymphoproliferative disorder (PTLD). This clinical experience includes a multicentred phase II clinical trial using the SNBTS first generation stock of partially HLA-matched allogeneic EBV-CTLs which demonstrated this treatment to be safe and effective. In addition, a second generation stock from 25 HLA-typed apheresis donors has been issued on a named patient basis under SNBTS Manufacturers' Specials (MS) licence 3473 which also demonstrated some efficacy with a good safety profile.

Blood donations from COVID-19 Convalescent Donors (CCD) contain CD4 and CD8 memory T cells specific for SARS-CoV-2 Spike, Nucleocapsid and Membrane antigens. SARS-CoV-2 peptides can be used to isolate VSTs using a GMP-compliant selection technology followed by rapid expansion *in vitro* using closed culture vessels and GMP-compliant reagents (Cooper et al. 2021). The allogeneic SARS-CoV-2 VSTs are manufactured in a single stage process directly from the starting material procured from suitable post COVID-19 recovered individuals. The starting material is procured by leukapheresis at the SNBTS Clinical Apheresis Unit at the Royal Infirmary Edinburgh under the terms of the SNBTS Tissue Establishment Licence (11010). A series of allogeneic HLA-matched donor T cell products is therefore being established to generate a stock of HLA-typed T cell products.

The early phase trial platform, DEFINE, aims to support promising novel and repurposed therapeutic assets but without prior information on use in COVID-19 in small cohorts of COVID-19 patients. Inclusion of allogeneic SARS-CoV-2 VSTs in the DEFINE trial by dose escalation will therefore assess the safety of this product.

3.2 Justification for design

A cautious dose escalation strategy from $2x10^4$ cells/kg to $2x10^6$ cells/kg (based on standard 75 kg weight) will be followed to ensure recipient safety. The initial dose of $2x10^4$/kg (target dose of $1.5x10^6$ cells) is considered appropriate based on experience observed with other adoptive T cell therapies where low numbers of transferred adenovirus-specific T cells were able to expand *in vivo*, with no acute clinical side effects documented after infusion (Feuchtinger et al, 2006).

4. STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

It is anticipated that 11 participants (maximum of 20, cross refer to section 5.2) with a recent (less than 2 weeks ago) confirmed SARS-CoV-2 infection (lateral flow test followed by confirmatory PCR test **or** PCR only test) whose O_2 saturation are 92% or higher, on air or with a maximum supplemental FiO_2 of 28% at the time of screening and for a minimum of 24 hours prior to commencement of infusion will be recruited. There will be 3 participants each in Dose Groups 1 and 2 who will receive initial escalating doses and 5 participants in Dose Group 3 who will receive the maximum dose. Any patients withdrawn prior to treatment can be replaced and will not count towards patient numbers.

4.2 TARGET POPULATION

Patients with confirmed SARS-CoV-2 infection will be recruited into this trial. Only patients who are confirmed COVID-19 positive with ≥92% O_2 saturations on air or with a maximum supplemental FiO_2 of 28% will be included.

Members of the clinical research team will liaise with members of the clinical care team to identify potential participants – only in-paitents will be approached for inclusion in this appendix. A member of the clinical care team, which may include embedded research nurses, will make the first approach regarding screening for eligibility. Following informal review of routinely available information by members of the routine healthcare team, the attending consultant will be approached to discuss enrolment in the trial.

All recruited participants who go on to receive the intervention (receive the VST infusion) will be provided with a study contact card. This card will contain details of the trial (including title, investigator and treatment received) and guidance for the participant along with emergency contact details. The card will also contain instructions and contact details should a female participant, or the female partner of a male participant become pregnant in the **28 days (4 weeks)** following administration of infusion. Any pregnancy notification will be reported and followed up in accordance with the relevant sponsor process applicable at the time of the notification.

Cohort 2C	Confirmed COVID-19 positive patients with ≥92% O_2 saturations on air.
Cohort 2D	Confirmed COVID-19 positive patients with ≥92% O_2 saturations on supplemental oxygen (maximum FiO_2 of 28% (2 – 6 L/min depending on delivery device used))

4.3 INCLUSION & EXCLUSION CRITERIA

Eligibility criteria for participants recruited to this treatment arm are outlined below.

Main Inclusion criteria (all appendicies):

- Provision of informed consent
- Aged at least 16 years
- COVID-19 positive test (lateral flow followed by confirmatory PCR or PCR only) result within last 14 days

- If the patient is of child bearing potential*, or is a male with a female partner with child bearing potential the patient, and their partner(s), agree to use medically-accepted contraception.

Appendix Specifc Inclusion Criteria and/or changes to above:
For this Appendix **all** above inclusion criteria must be met. In addition, the following criteria must also be met:

- Patient deemed capacitated to provide informed consent for themselves.
- Maintaining oxygen saturations of ≥92% (on air or with a maximum supplemental FiO_2 of 28%) at time of screening and for 24 hours prior to commencement of infusion.
- If the patient is of child bearing potential*, or is a male with a female partner with child bearing potential, the patient, and their partner(s), agree to use a **highly effective** method of contraception **for 4 weeks following the date of the infusion**. Methods considered highly effective are those that achieve a failure rate of less than 1% per year when used consistently. This includes:
    - Combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation
        - Oral
        - Intravaginal
        - Transdermal
    - Progesterone-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Intrauterine device (IUD)
    - Intrauterine hormone-releasing system (IUS)
    - Bilateral tubal occlusion
    - Vasectomised partner
    - Sexual abstinence

* A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Main Exclusion criteria (all appendicies):
- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1.73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Women who are pregnant or breastfeeding
- Participation in another clinical trial of an Investigational Medicinal Product (CTIMP)
- Known hypersensitivity to the therapy (ATIMP) or excipients

- Patients (or their partners) planning on donating sperm/eggs during the trial period
- Ongoing dialysis
- History of serious liver disease (Child Pugh score > 10 *(See section 6.3 for details)*)
- Severe uncontrolled diabetes mellitus
- Concomittant use of treatments for COVID-19 that are not recognised as locally approved standard care.
- In the Investigator's opinion, patient is unwilling or unable to comply with trial intervention (e.g. IMP/ATIMP administration plan), laboratory tests or other study procedures

Appendix Specifc Exclusion Criteria and/or changes to above:
For this Appendix **all** above exclusion criteria must be satisfied along with those detailed below:

- Patient unable to provide informed consent for themselves.
- O_2 saturations <92% on air or with a maximum supplemental FiO_2 of 28% at time of screening or during the 24 hours prior to commencement of infusion.
- Patients receiving corticosteroids.
- Individuals who are immunocompromised and/or immunosuppressed
- Individuals with haemoglobinopathies
- Patients who have received any vaccine (including COVID-19 vaccine) within the preceding 3 weeks, or are due any vaccine within the 6 week trial follow up period following the infusion (it may be problematic to discriminate a reaction to a vaccine from signs/symptoms of the ongoing infection or a reaction to the SARS-CoV-2 VSTs).

Confirmation of Eligibility

For this appendix, eligibility will be confirmed over a two stage process due to the time requirements of some criteria.

Initial eligibility confirmation will be done during the screening visit and confirm most of the inclusion exclusion criteria as detailed above.

The second eligibility check prior to commencement of infusion will be carried out during the baseline visit (day of infusion). As well as confirming that all inclusion and exclusion criteria have been reviewed and met, specific attention should be made to confirm the following:

- **O_2 saturations** have been maintained at ≥92% for the 24 hours (on air or with a maximum supplemental FiO_2 of 28%) prior to commencement of infusion.
- **COVID-19 positive test** was ≤14 days prior to the day of infusion (baseline visit).
- **Safety bloods** – results must be available for **samples taken NO MORE than 36 hours prior to the start of the infusion**. Where these are not available a pre-infusion sample should be taken. Results should be reported and checked prior to commencing infusion to ensure that participant meets eligibility criteria (specifically eGFR, ALT and Hb as detailed in above exclusion criteria). If bloods have been taken clinically within the stated timeframe, and ALL required results are available, these can be recorded on the CRF without the need to obtain further blood samples from the participant.

Notification of, and communication with ICU

Communication between the research team and the clinical Critical Care team will be via the Edinburgh Critical Care Research Group (ECCRG). The ECCRG have a team email (edcriticalcare@nhslothian.scot.nhs.uk) which is checked daily, and all members of the group's research nursing team can access. This will be the primary contact used for ICU notification. ECCRG will then notify clinical colleagues, as appropriate, of planned trial activities.

4.3.1.1 Contact prior to start of study (before recruitment begins)

It will be the responsibility of the research team, via the clinical project management team, to notify ICU when approval for the trial (specifically this appendix) has been granted and the team are ready to open / begin screening and recruitment activities. The following documents and information will be provided to the ICU team:

- The main protocol and the relevant protocol appendix
- Participant information sheet
- A copy of the Phase I Risk Assessment report and approval
- REC Approval
- MHRA Approval
- Sponsor Approval to Open (when available)
- Anticipated start date (if possible, at least 1 week prior to anticipated start date).

The ECCRG will be asked to confirm receipt of the above via email and notification of relevant member(s) of the ICU clinical team. The confirmation email will be filed in the appropriate section of the ISF.

4.3.1.2 Notification of amendments

The research team will notify ICU (via ECCRG) of any amendments to the protocol, and provide copies of the updated documentation. An email from ECCRG will be requested to confirm receipt of updated documentation and notification of relevant member(s) of ICU clinical team, this will be filed in the ISF.

4.3.1.3 Notification of Study visits (dosing)

The research team will notify ECCRG, by email, when a participant has been screened and deemed eligible. ECCRG will be provided with details of the planned date of dosing (to be confirmed when HLA matching complete and decision to proceed to dosing is made). ECCRG will notify ICU, who will advise of any potential resource issues or advise that there are no issues ahead of dosing. The advice from ICU (ECCRG) will be retained in the ISF. As no ICU bed is required to allow dosing to proceed, bed capacity will not be a determining factor, but the CI (or one of specific sub-investigators listed on page 19) should be made aware of any resource issues for ICU so that they may decide whether to proceed with dosing or not. The decision will be communicated to the project management team to be filed in the ISF. The decision to proceed will also be communicated to ICU.

4.3.1.4 End of trial notification

A minimum of 48 hours following dosing of the final participant the research team will notify ICU (via ECCRG) the trial recruitment has been completed, and no further dosing visits are planned. The project management team will request that ICU/ECCRG confidentially destroy any study documentation, including the trial protocol and appendix and the RA documentation, providing written (email) confirmation when this has been completed.

5. INVESTIGATIONAL MEDICINAL PRODUCT (ATIMP)

5.1 STUDY DRUG

Study Drug Identification

SARS-CoV-2 VSTs

Study Drug Owner

SNBTS

Marketing Authorisation Holder

N/A

Labelling and Packaging

Labelling and packaging of the IMP at the SNBTS's MIA(IMP) 3473 licensed site (15150390):

SNBTS, The Jack Copland Centre
52 Research Avenue North,
Heriot-Watt Research Park, Edinburgh EH14 4BE

Storage

The packaged SARS-CoV-2 VST product is stored at the SNBTS manufacturing site as described above at ≤-135°C.

Regulatory Release to Site

On receipt of an approved IMP order, the QP certified secondary packaged product is removed from LN_2 storage by SNBTS, dispatched and transported to the study site in a validated transport container as described in TCATS CTL 024. On receipt at the study site the product is thawed and reconstituted by trained SNBTS and trial staff, following the instructions provided by SNBTS (TCATS CTL 024), and with oversight by NHS Lothian pharmacy. For a brief description of HLA matching of product to patient and the reconstitution step at the study site please cross refer to sections a) and b) below.

a) IMP Selection

Products are selected from the SARS-CoV-2 VST stock according to the HLA matching approach described below; where a suitable product cannot be identified from the product stock the participant will not proceed to treatment and will be withdrawn from the trial. HLA typing of the participants is carried out at the SNBTS Histocompatability and Immunogenetics Laboratory based at the Royal Infirmary Edinburgh. The

laboratory is accredited by UKAS and European Federation for Immunogenetics and inspected under the terms of the SNBTS Blood Establishment Authorisation.

The matching approach optimises the number of HLA class I and II alleles shared between the patient and donor, prioritising matching for those alleles that restrict the peptides which are used in manufacture of the SARS-CoV-2 VSTs.
The hierarchy of matching is:

1. HLA class I and II alleles matched between VST and patient which correspond to the reported restriction characteristics of the peptides used in the manufacture of the SARS-CoV-2- VSTs, ensuring at least one mismatch to avoid the risk of TA-GVHD.
2. HLA Class I and II alleles matched between VST and patient which do not correspond to the reported restriction characteristics of the peptides used in the VST generation – but may still enable the generation of a response.
3. Where an option between HLA Class I or II matches in either of the above categories (1,2) is required – class II matches will be preferentially selected due to current knowledge of VST lineage composition.
4. If the patient has an HLA antibody directed towards HLA mismatches on the SARS-CoV-2 VST batch then the SARS-CoV-2 VST batch will be excluded from selection.
5. Where there is no match between SARS-CoV-2 VSTs and patient at HLA class I or II the participant will not proceed to treatment and will be withdrawn from the trial.

b) Reconsitution at Investigator Site

Reconstitution of the product will be carried out according to the instructions defined in SNBTS SOP TCATS CTL 024.

The product (maximum dose, equivalent to Dose Group 3) undergoes thawing at the investigator site prior to administration to patients by intravenous infusion through a blood administration set.

To allow dose escalation in Dose Groups 1 and 2, the unit of drug product undergoes a reconstitution process at the investigator site to allow the desired cell dose to be achieved. This involves a thawing and dispensing step prior to administration to the patient by intravenous infusion through a blood administration set i.e. thawing, removal of a volume of cells and transfer of this volume to cryoprotectant ([1:2] Plasma-Lyte® 148:CryoStor®CS10) to achieve the desired target cell dose in a target of 15mL total volume.

Post thawing (and dilution for the lower doses), the primary container of the product is labelled at the study site with a reconstitution label detailing the participant identification and expiry date post thaw/reconstitution step. Administration of the product will be initiated within 2 hours of thawing.

Destruction of Trial Drug

Note, in some circumstances, although unlikely, it may not be possible to administer the treatment, e.g. if the patient becomes unwell on the day of the planned infusion. If this situation arises the cells will be disposed of according to local policies if already thawed, otherwise returned to SNBTS. In this case, the patient will be withdrawn from the trial. Unused thawed drug product (lower two doses) and primary packaging post infusion will be disposed of according to local policies.

Investigators' Brochure (IB) and Reference Safety Information (RSI)

The Investigators' Brochure is a separate document held in the trial master file; a copy is also held in each investigator site file. These SARS-CoV-2 VSTs have not been administered in humans before. Therefore there are no known adverse reactions on which to base expectedness (please refer to section 6.3) of the IB.

5.2 DOSING REGIME

EMA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1) have been considered when drafting this dosing regime.

The quality of the product and benefit and risk assessment are detailed in the associated IMPD and section 1.2. The requirement for pre-clinical studies was discussed with the MHRA at a scientific advice meeting (23/09/2020), and further details are given in section 2.2 of the IMPD and section 1.2.1 above.

A dose escalation strategy from $2x10^4$ cells/kg to $2x10^6$ cells/kg (based on standard 75kg weight; see table below) will be administered to patients with COVID-19 infection, and patients will be followed up to ensure their safety (see Fig. 1).

Dose Group	Target cell dose/kg*	Total target cell dose
1	$2x10^4$ cells/kg	$1.5x10^6$cells
2	$2x10^5$ cells/kg	$15x10^6$cells
3	$2x10^6$ cells/kg	$150x10^6$cells

* based on standard 75kg weight

This dosing strategy is considered cautious based on the following:

- the initial dose of $1.5x10^6$ cells (equates to $2x10^4$ cells/kg based on standard 75kg weight) is considered appropriate based on experience observed with other adoptive T cell therapies (e.g. Feuchtinger *et al.*, 2006) where no clinical side effects were documented after infusion of this level of T cells.
- Blood components in the UK currently contain up to 10^6 leukocytes/unit (up to $5x10^6$ leukocytes/unit between 1999 and 2004) with very high safety level. Prior to introduction of routine leukodepletion in 1999 blood components contained between 10^8 and 10^9 leukocytes / unit)) with very high safety level as demonstrated by SHOT reporting (launched in 1996).
- EBV-CTL product manufactured by SNBTS, used in a patient group with very severe illness and immunosuppression, and treated with 4 doses of product (each dose at 1-$2x10^6$ cells/kg which is equivalent to the highest target dose for the SARS-CoV-2 VSTs) demonstrates a good safety record.
- First in human studies with genetically modified T cells typically use a dose escalation range between around $5x10^6$ and $5x10^9$ total cells.
- The total nucleated cell count reinfused during a Haematopoietic Stem Cell transplant procedure is in the order of 2.5-$5x10^{10}$.

The product will be administered in a hospital setting, with appropriate resuscitation equipment available should there be unexpected side effects. On the day of dosing the CI **or** an appropriately trained and delegated sub-investigator (and senior clinician) will be on site and available for the duration of the dosing visit and for 2 hours post dosing**. These individuals will be detailed on the delegation log and task appropriately delegated by the CI (RIE) / PI (WGH).**

Participants will remain in hospital for observation for a minimum of 48 hours after dosing, although if the clinical picture requires it e.g. participant develops an adverse effect, then their hospital stay will be prolonged as necessary according to the clinical picture and only discharged at the end of the 48 hour period if well. At discharge the participants will be provided with contact details for the trial team should they develop any new symptoms or side effects and will also be followed up at regular intervals up to 6 weeks post-infusion as described later in Table 1. Consideration has been given with reference to the Adverse Reactions considered below – if any adverse reactions were to occur the majority would occur within a matter of minutes to hours with the exception of ICANS and TA-GvHD which would occur within a few days or up to 6 weeks post-infusion respectively.

The SARS-CoV-2 VST product issued will be selected based on an HLA matching protocol (see section 5.1.6); where no suitable match can be found the participant will not proceed to treatment.

Initially, there will be 3 weeks between each patient within the first Dose Group and also before the start of the next patient Dose Group. DMC review and approval will be required prior to progressing to the next Dose Group. Thereafter, the dosing schedule and decisions to proceed will be determined by the DMC, but with a minimum of 3 days between successively treated participants in that Dose Group. Adverse reactions, vital signs (including SpO2, FiO2, RR, PR, temperature, blood pressure) and laboratory safety assessments (as defined in the Table 1 below) will submitted to the DMC for review at each stage. It is expected that any adverse reactions/ serious adverse reactions will be observed within 48 hours as is seen in the transfusion setting. 3 weeks dosing schedule for the first dose group was agreed with the MHRA (at an advisory Scientific Meeting on September 2020) and is considered a precautionary window should any unexpected reactions such as TAGvHD or anaphylaxis occur.

The DMC and Trial Steering Committee (TSC) will operate according to a defined DMC and TSC charter.

Dose escalation protocol: see Fig. 1 below.

The first three recipients will receive $2x10^4$ cells/kg (based on standard 75kg weight; target dose of $1.5x10^6$ cells). The product will be infused at a slow rate (approximately 3-5mls/min) followed by a normal saline (0.9%) flush. During and post-infusion patients will be monitored and managed according to hospital transfusion procedures. This involves pre-infusion checks of vital signs followed by post infusion checks of vital signs every 15 minutes for up to 2 hours. An appropriately delegated member of the research team will carry out the pre and post infusion checks and data collection. In general, this task will be undertaken by a research nurse, with an investigator (medic) present or contactable during this period. Patients will be monitored in hospital for 48 hours post administration of the product. It is expected that any Adverse Reactions (AR) that may occur are likely to be those that are encountered in the transfusion setting; any such reactions will be managed in the usual way, using the relevant

hospital protocols for transfusion related reactions and the appropriate expertise sought.

Further follow up, up to 6 weeks post-infusion, is detailed in the Table 1 below. During the first 21 days post infusion participants will also have daily recording of their oxygen saturation and oxygen therapy during this period. The final follow up time point at 6 weeks post-infusion has been identified as it is typically 20-40 days post-infusion that symptoms of TA-GvHD would become apparent if this adverse reaction were to occur.

If 1 participant out of the 3 of a Dose Group has an AR (as listed below) then 3 additional participants will be treated at the same dose. If 2 or more patients get an AR (as listed below), escalation will stop. This pattern is repeated for $2x10^5$ cells/kg ($15x10^6$ cells) (3 patients) and $2x10^6$ cells/kg ($150x10^6$ cells) doses (5 patients); based on 75kg standard weight. This will allow confirmation that these doses are tolerated and may also identify the maximum tolerated dose. In the unlikely event that 2 or more participants at the lowest dose ($2x10^4$ cells/kg) have a AR, this will be referred to the DMC and consideration given to ceasing the trial. The maximum tolerated dose (MTD), as defined by the dose escalation protocol and as agreed by the DMC will be determined. All data obtained will undergo Quality Control according to ECTU procedures applicable at the time the QC is undertaken.

The following potential ARs have been considered:

Acute Infusion Reactions are a known side effect of blood component transfusion and cell therapy administration though have not been seen in association with the SNBTS allogeneic EBV VST product. Common clinical symptoms are usually mild and may include fever, chills, sweating, flushing, abdominal pain, headache, nausea, back pain and rash and usually respond to slowing or stopping the infusion.

Anaphylactic Reactions may show overlapping symptoms with other allergic reactions in the early stages but progress to more severe symptoms including urticaria, throat tightness, dyspnoea, tachycardia and hypotension. No anaphylactic reactions have been seen in association with the SNBTS EBV VST product. The administration of SARS-CoV-2 VST will be performed in an inpatient unit able to diagnose and treat anaphylaxis. Intramuscular adrenaline injection will be used as first line treatment.

Cytokine Release Syndrome is seen primarily in association with administration of CD19 and CD22 CAR-T cells and is thought to be caused by rapid expansion of the autologous product in vivo and killing of target tumour cells. A number of studies have demonstrated that it is tissue damage marker release (DAMPs) which drives CRS in adoptive cell therapies (Morris et al 2021). This tissue damage then activates macrophages to release pro-inflammatory cytokines such as IL-6 which drive the CRS which can occur in COVID patients (Vanderbeke et al 2021). The high percentage of CD4 T helper cells in the manufactured VST therapy product for this clinical trial indicates that there is unlikely to be a pronounced cytotoxic response and therefore the risk of CRS due to administration of low doses of VST is considered to be very small. The proposed dose escalation is considered appropriate based on results from other antiviral T cell therapies where no clinical side effects were documented after infusion of similar T cells. A recent trial report of antiviral T cell therapy in COVID-19 patients assessed a cohort of 9 patients treated with between 10^7 and 10^8 VST (similar to the planned dosing in this trial) with no adverse events. with no adverse events (Perez-Martinez et al 2021). In addition, given the safety data accumulated for the SNBTS EBV-VST product in a patient group with very severe illness and immunosuppression treated with 4 doses of T cells (each dose at equivalent to the

highest target dose for the SARS-CoV-2 VSTs), it is anticipated that the risk of administration of the SARS-CoV-2 VSTs to the COVID-19 patient cohort is likely to be very low (individuals who are immunocompromised and/or immunosuppressed are excluded from the trial). CRS may include a prodromal syndrome characterised by flu-like syndrome followed by fever, nausea, headache, rash, hypoxia and hypotension. In severe cases, CRS may be characterised by haemodynamic instability and organ dysfunction. Routine monitoring will include frequent vital sign assessment and evaluation. Prodromal symptoms should be treated with symptomatic support and observation. More severe CRS may be treated with supplemental oxygen, fluids, vasopressin or dopamine, tocilizumab or corticosteroids. Severe symptoms may require high-flow oxygen by face mask or positive airway pressure ventilation for hypoxia or high dose vasopressors with norepinephrine, dopamine, or phenylephrine for haemodynamic support.

Immune Effector Cell-associated Neurotoxicity Syndrome (ICANS) is also seen in association with CD19 and CD22 CAR-T cell therapy but has not been seen in association with SNBTS EBV VST or other similar VSTs manufactured elsewhere. It presents with a variety of signs of neurological and cognitive impairment and can progress in more severe cases to depressed level of consciousness, coma, seizures, motor weakness, and cerebral oedema. Dexamethasone and methylprednisolone are recommended for moderate and severe ICANS.

Transfusion Associated Graft versus Host Disease (TA-GvHD) occurs due to recognition of patient HLA antigens by allogeneic T cells. This is a known problem in allogeneic Haematopoietic Stem Cell (HSC) Transplantation and prior to the implementation of universal leucodepletion was seen occasionally post blood component transfusion in immunocompromised patients or those where the donor was homozygous for an HLA haplotype shared with the recipient (such that the incoming T cells were not recognised as foreign by the patient's immune system). We have seen two cases of mild flare up of dermal GvHD with the EBV VST product, both in patients who had undergone prior allogeneic HSC transplantation and both of whom responded to topical steroids. We do not expect to see GvHD in patients in this clinical study because of the high degree of specificity of the T cells to SARS-CoV-2 peptides, because the patients are immune competent and because a degree of HLA mismatching will be ensured. If GvHD occurs, subjects will be treated with corticosteroids as first line followed by other immunosuppressive agents if required.

In the case of **Acute Infusion Reactions** (as detailed above), when the severity of the event is assessed as '**severe**', the trial will be paused to allow review by the DMC and Investigators.

In the case of **Anaphylactic Reactions (as detailed above), when the severity of the event is assessed as "moderate" or "severe",** the trial will be paused to allow review by the DMC and Investigators.

For **all other AR events detailed above**, should any of these occur, the trial will be paused to allow review by the DMC and Investigators.

The decision to proceed to the next dose will be agreed in compliance with the current version of ACCORD dose progression and stopping rules procedure (CR016). In brief the decision to proceed requires approval by the DMC, Co-Sponsor, CI and PI.

5.3 DOSE CHANGES

If possible, toxicities should be managed symptomatically. If toxicity occurs the appropriate treatment as per usual hospital procedure will be used to ameliorate signs and symptoms and specialist opinion sought as necessary.

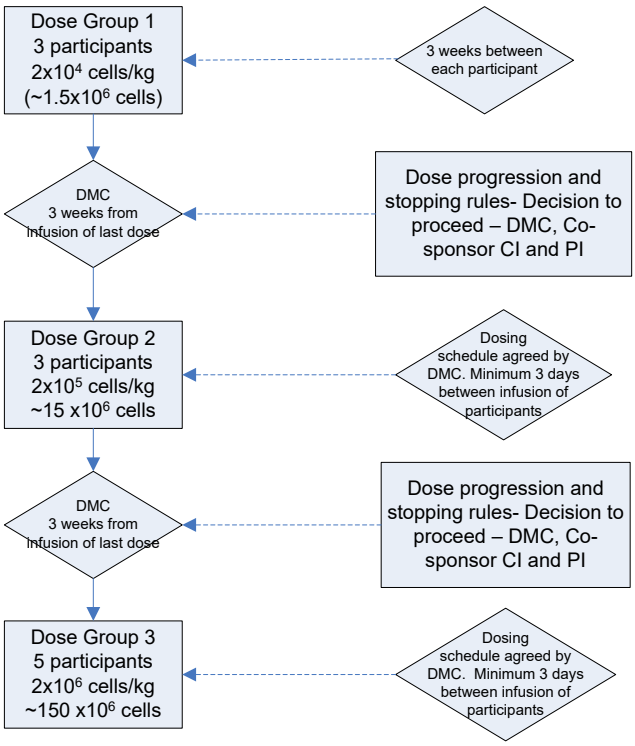

Figure 1 Dosing Schedule

5.4 PARTICIPANT COMPLIANCE

The SARS-CoV-2 VSTs infusions require administration by trial staff and therefore will be directly observed. Thawing and reconstitution of the product will be carried out by trained SNBTS staff and members of the trial team with NHS Lothian pharmacy oversight.

5.5 OVERDOSE

This will be unlikely as the cells will be prepared and then delivered in a sealed bag for infusion for the specific named patient, directly to trial staff. Reconstitution and dilution of product will be carried out by trained staff.

5.6 OTHER MEDICATIONS

All medication that the participant is taking at the time of enrolment will be recorded. Any changes or new medications added during the study will be recorded.

The generic drug name, daily dose, route of administration, treatment start/stop date and indication will be recorded.

Non-Investigational Medicinal Products

Permitted Medications

Any drug, if considered necessary for the participant, is permitted at the discretion of the Investigator.

Prohibited Medications

None

6. STUDY ASSESSMENTS

A table of assessments for this appendix is provided below.

Highlighted assessments are those specifc to this appendix.

Table 1 Study Assessments

Activities	Screening and Enrolment	Baseline /Administration of IMP Day 0 [1]	Day 1 post administration of IMP	Day 2 post admin. of IMP	Day 3-6	Day 7 (+/- 1 day)	Day 14 (+/- 1 day)	Day 21 (+/- 1 day)	6 weeks post administration (+/- 1 week)
	Eligibility								
Informed consent	✓								
Review and confirm eligibility	✓								
Reconfirm eligibility (in particular SpO_2 status for 24hours prior to infusion and safety bloods)		✓							
Urine pregnancy test	✓[2]								
Review of SARS-CoV-2 diagnostic test	✓								
Medical history	✓[3]								
12-lead Electrocardiogram	✓[4]								
HLA type	✓								
Anti-HLA Antibody test	✓							✓	
	Study intervention								
Allocation of Treatment		✓							
Administration of product		✓							
SoC treatment	✓	✓	✓	✓	✓[5,6]				

[1] Day 0 (day of infusion) must be 14 days or less from the date of positive SARS-CoV-2 test. Administration must be within 72 hours of screening.
[2] Pregnancy test at screening (to confirm eligibility) for women of childbearing potential.
[3] Includes estimated date of first symptoms and number of co-morbidities (e.g. respiratory, cardiovascular, metabolic, malignancy, endocrine, gastrointestinal, immunologic, renal).
[4] If the Electrocardiogram was taken as part of routine clinical care within the last 72 hours, results can be recorded and no additional assessments are required.
[5] If participant remains in hospital >48 hours post infusion and these assessments are done as part of routine clinical care at any point on the relevant calendar day then available results should be recorded. Research team not expected to do if not done by the clinical care team as part of routine care. This will not be recorded as a deviation.
[6] Whilst participant remains an in-patient

Activities	Screening and Enrolment	Baseline /Administration of IMP Day 0	Day 1 post administration of IMP	Day 2 post admin. of IMP	Day 3-6	Day 7 (+/- 1 day)	Day 14 (+/- 1 day)	Day 21 (+/- 1 day)	6 weeks post administration (+/- 1 week)
	Clinical study procedures and assessments								
NEWS2 Score and WHO ordinal scale		✓[7]	✓[7]	✓[7]	✓[7, 7]	✓[7]			
Vital signs including SpO2, FiO2, RR, PR, temperature,blood pressure	✓[7]	✓[8]	✓[7]	✓[7]	✓[7, 9, 10]	✓[7]			
Physical assessment (including general baseline medical examination, height, weight)	✓[7]								
Directed cardio-respiratory physical examination		✓[7]	✓[7]	✓[7]	✓[7, 9]	✓[9]			
Cough symptom score		✓[7]	✓[7]	✓[7]	✓[7, 9]	✓[7]		✓	
Child Pugh Score *(See section 6.3 for details)*	✓								
Oxygen saturation via Pulse oximeter					✓[11]	✓[11]	✓[11]	✓[11]	
Oxygen Therapy					✓[11]	✓[11]	✓[11]	✓[11]	

[7] If these assessments have been taken as part of routine clinical care on the same calendar day as the research assessment is to be conducted, results can be recorded and no additional assessments conducted.
[8] Vital sign monitoring pre and post infusion will be as per hospital tranfusion procedures. This involves pre-infusion checks of vital signs and then post-infusion checks of vital signs every 15 minutes (+/-5mins) for 2 hours.
[9] If participant remains in hospital >48 hours post infusion and these assessments are done as part of routine clinical care at any point on the relevant calendar day then available results should be recorded. Research team not expected to do if not done by the clinical care team as part of routine care. This will not be recorded as a deviation.
[10] Use SpO_2 result recorded in Participant Diary
[11] Daily from day 3 through to day 21 - in participant diary.

Activities	Screening and Enrolement	Baseline /Administration of IMP Day 0	Day 1 post administration of IMP	Day 2 post admin. of IMP	Day 3-6	Day 7 (+/- 1 day)	Day 14 (+/- 1 day)	Day 21 (+/- 1 day)	6 weeks post administration (+/- 1 week)
	Safety assessments								
Laboratory Safety Assessments[12]	✓[13]	✓[14, 15]	✓[7]	✓[7]	✓[7, 9]	✓[7]		✓[7]	
AE/SAE recording and assessment		✓	✓	✓	✓[7]	✓	✓	✓	✓
Survival status		✓	✓	✓	✓[7]	✓	✓	✓	✓
Graft Versus Host Disease check									✓[16]
	Research Samples[17]								
ICCK assay (15ml)[18]		✓		✓ (+/- 1 day)		✓		✓	
Plasma marker testing (9ml)[19]		✓		✓ (+/- 1 day)	✓[7] Day 4 only (+/- 1 day)	✓		✓	
SARS-CoV-2 oropharyngeal/nasal/ swab		✓		✓ (+/- 1 day)	✓[7] Day 4 only (+/- 1 day)	✓		✓	
SARS-CoV-2 Blood sample		✓		✓ (+/- 1 day)	✓[7] Day 4 only (+/- 1 day)	✓		✓	
Viral Load (saliva)		✓		✓ (+/- 1 day)	✓[7] Day 4 only (+/- 1 day)	✓		✓	

[12] **Biochemistry** – Urea, sodium, potassium, chloride, magnesium, bicarbonate, creatinine, C-reactive protein (CRP), ferritin, troponin; creatine kinase and eGFR (eGFR for screening and baseline only);
LFTs - Total bilirubin, ALT and AST (AST for screening and baseline only);
Haematology - FBC (haemoglobin, haematocrit, RCC, WCC and differential WCC (excluding eosinophils) and platelets);
Coagulation - activated partial thromboplastin time (aPTT), Prothrombin time (PT), Fibrinogen.

[13] If these assessments have been taken as part of routine clinical care within the last 72 hours, results can be recorded and no additional assessments are required.

[14] If screening safety assessment bloods were taken **no more than 36 hours prior** to the infusion being started these do not have to be repeated at baseline. If bloods were taken **>36hours** prior to infusion then new samples to be taken and results should be reported prior to commencement of infusion.

[15] Samples to be repeated 2-3 hours post infusion.

[16] Via phone call, participant asked whether they have any new symptoms such as skin rashes. It can take up to 20-40 days post infusion for symptoms of TA-GvHD to become apparent.

[17] These samples are desirable. If the participant declines, the sample will not be taken and will not be considered a deviation and non-collection will be recorded in the CRF.

[18] Only to be carried out on samples from participants receiving a target of $2x10^5$ cells/kg ($15x10^6$ cells dose) and $2x10^6$ cells/kg ($150x10^6$ cells dose) (if participant agrees)

[19] Plasma marker testing will include, for example, markers of inflammation and disease resolution.

6.1 SAMPLING

Haematology and biochemistry samples will be analysed in local NHS laboratories.

Research samples (VST blood sampling, ICCK and plasma marker testing) will be analysed at SNBTS and University of Edinburgh laboratories. Details of the sampling schedule for this study is outlined in a separate sample processing document held by the trial team. If testing of any research sample fails it will be discussed with the relevant participant to determine whether they would be willing to give a repeat sample, if it is operationally feasible to do so within the prerequisite timelines.

6.2 SAFETY ASSESSMENTS

Strict observation and stop/go policy as determined by the DMC will be adhered to in accordance with the above.

Standard safety assessments that will apply in all treatment arms within the DEFINE Trial are detailed in Section 6.1 of the main protocol.

Clinical Safety Laboratory Assessments

In addition to the safety assessments detailed in the main DEFINE Protocol, the treatment arm specific clinical safety laboratory assesssments are detailed below.

Fasting is not required before collection of laboratory samples.

Screening bloods (the following are the **minimum** blood results required for screening):

Biochemistry – Urea, Sodium, Potassium, Chloride, Magnesium, Bicarbonate, Creatinine, CRP, Ferritin, Troponin, CK and eGFR

LFTs - Total Bilirubin, ALT and AST

Haematology - FBC (haemoglobin, haematocrit, RCC, WCC and differential WCC (excluding eosinophils) and platelets).

Coagulation screen – Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Fibrinogen.

If these have been taken in the previous 72 hours and satisfy eligibility criteria, no repeat bloods are required.

Where a repeat sample is required at **baseline** (see table of assessments), the same clinical laboratory bloods required at screening will be collected.

For **post infusion and daily** (up to 48 hours post infusion), day 7 **day 21** clinical laboratory assessments, the same blood results are required as detailed for screening excluding eGFR and AST.

All tests detailed above have been confirmed as routinely performed within NHS Lothian labs.

Research samples for plasma markers and ICCK will be analysed by the University of Edinburgh and SNBTS and not recorded in the pCRF/database and therefore not available to the statistician. These tests will be analysed by a delegated member of the relevant lab team and included in the final analysis carried out by the research team. Full details of labs involved and work undertaken by each can be found in the Appendix specific **Work Instruction for DEFINE Clinical Trial Samples.**

6.3 STUDY ASSESSMENTS

Study assessment are outlined in the table of assessments above.

Below are details of the Childs Pugh Score, recorded as part of the eligibility criteria. However, please note, only the individual components are required to be collected and entered on to the database, which will then calculate the overall score.

Clinical and Lab Criteria	Points*		
	1	2	3
Encephalopathy	None	Mild to moderate (grade 1 or 2)	Severe (grade 3 or 4)
Ascites	None	Mild to moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	< 2	2-3	>3
Albumin (g/dL)	> 3.5	2.8-3.5	<2.8
Prothrombin time			
Seconds prolonged	<4	4-6	>6
International normalized ratio	<1.7	1.7-2.3	>2.3
Child-Turcotte-Pugh Class obtained by adding score for each parameter (total points) Class A = 5 to 6 points (least severe liver disease) Class B = 7 to 9 points (moderately severe liver disease) Class C = 10 to 15 points (most severe liver disease)			

Figure 2 - Child Pugh Score

Follow up visits at 7 and 21 days can be carried out in hospital (as an inpatient or outpatient) by an appropriately delegated member of the research team or by the Clinical Research Facility research nursing team. However, some patients may not be able to attend hospital for the follow up visit, so the option to have the follow up done at home by a member of the research team or by one of the Clinical Research Facility Community Research Nursing Team will be offered where appropriate. Where the participant is within the required isolation period following positive diagnosis of COVID-19 the team will follow the appropriate guidance relating to PPE and infection control measures applicable at the time of the visit.

Participants will be asked to record if they are receiving oxygen therapy (no FiO_2 details required) and daily oxygen saturation levels from day 3 to day 21 using the pulse oximeter provided by the trial. Recordings will be noted in a participant diary (provided on day 3). Training by the research team will be provided prior to discharge. In addition to the in person visits at days 7 and 21, a member of the research team will call the participant at day 14 to record oxygen therapy and pulse oximeter data and check for any adverse events.

The participant will be provided with 2 diaries, one at day 3 to cover from day 3 post infusion up to end of week 3 (day21) and the second will cover from week 4 (day 22) to week 6 post infusion (day 42). The first diary will collect oxygen therapy and pulse oximeter data as detailed above along with safety data (new symptoms) and concomitant medications. This will be collected by the research team at the in-person day 21 visit, and at the same visit the participant will be provided with the second diary. The second diary will collect safety data and concomitant medication data only. This will be returned in a stamped addressed envelope to the research team following the final entry at 6 weeks. The research team will remind the participant of process at 6 week follow-up call. If no diary is received by the end of week 7 post infusion, the research team will contact the participant by telephone to request return. Details can be collected over the phone (and documented in study documentation), but participant will still be asked to return diary. On receipt of the diary, it may be necessary to contact the participant to clarify incomplete or illegible data. This follow up will be performed as close to receipt of the diary as possible. Details of potential contact after 6 weeks is detailed in the participant information sheet.

6.4 STORAGE AND ANALYSIS OF SAMPLES

Safety blood samples will be analysed at relevant NHS laboratories and then disposed of locally as per standard NHS laboratory policy. SARS-CoV-2 and viral load samples will be tested at NHS Lothian Virology laboratory using standard processes. Research samples will be allocated to relevant laboratories and stored appropriately. Samples will only be stored as consented by trial participants. Refer also to section 6.1. Full details of labs involved and work undertaken by each can be found in the Appendix specific **Work Instruction for DEFINE Clinical Trial Samples.**

The following study samples listed in the master protocol will not be collected as part of this appendix:

- PK and biomarker samples.

7. STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE CALCULATION

Initial statistical input and oversight, at the time this appendix protocol was developed, was provided by Prof John Norrie (lead statistician for the DEFINE Platform until May2024) and the statistics team at ECTU. Lead statistician role transferred to Catriona Graham from May2024 following Prof John Norrie taking up a new post outwith the University of Edinburgh.

This is a dose escalation study with safety primary endpoints for 11 participants. No formal size calculation is required for this study. A formal sample size calculation that would be mandatory for a confirmatory phase III randomised trial is neither feasible nor appropriate. The small sample size at each dose point (n=3-5) means that it will be challenging to establish significance. The total number of patients treated in the study should be sufficient to compare against SOC data to detect differences, and establish significance using appropriate tests (e.g. Student T or Mann-Whitney U test depending upon data being parametric).

7.2 PROPOSED ANALYSES

The proposed analysis for Appendix 3 will be carried out by the Epidemiology and Statistics Core of the Edinburgh Clinical Research Facility, lead by Catriona Graham.

This Phase I safety study is not powered for subgroup analyses and so such analyses will be exploratory, and on a limited number of participants. Particularly at the lower doses of the product it is unlikely to see any efficacy from the treatment.

Due to the small sample sizes there will be no formal adjustment for missing data.

Safety data will be analysed on the as-treated data-set (anyone who was initiated on treatment) and will be presented descriptively.
The independent DMC will scrutinise accumulating data. Their first and foremost responsibility will be the safety of the participants, and the committee may terminate the study at any time on the grounds of safety. This study is unlikely to demonstrate efficacy particularly for participants treated with the initial lower doses and given the small number of participants at the maximum dose, however in the unlikely event of identifying overwhelming evidence of efficacy, the study would in that situation be adapted or stopped early.

8. PHARMACOVIGILANCE

Pharmacovigilance procedures are detailed within the main trial protocol.

8.1 ADDITIONAL REPORTING REQUIREMENTS

To comply with SNBTS' licencing requirements, all possibly related SAEs and SARs reported to ACCORD (using the ACCORD reporting form) will also be reported to SNBTS by e-mail to ***NSS.SAE-SUSAR-reports@nhs.scot*** within 24 hours of a report being receipted.

For this arm, all AEs and SAEs occurring in the Dose Group will be assessed for potential of the event to be a Serious Adverse Reaction (SAR) to the product: if the event is considered to be a potential SAR, it will be assessed for expectedness and reported using the applicable reporting requirements for serious unexpected serious adverse reactions (SUSAR) described in the master protocol.

The assessment of expectedness will be made against the reference safety information found in section 6.3 of the IB.

All SAE/SARs that occur in participants will be reported to the Sponsor as specified in the main protocol (i.e. within 24 hours of identification of the event).

Participants will be followed up as per the Table 1 above for 6 weeks post administration of the IMP. Particpants wil be requested to notify the team if any reactions relating to the infusion occur.

Due to the nature of the ATIMP, in the case of any relevant safety information regarding the procurement or the donor that became available and might have an impact on the participant, the sponsor must be informed immediately as those events might require actions such as urgent safety measures or substantial amendment.

All substantial changes to protocol/appendix 3 or amendments in response to a USM will, in addition to standard approval process, require phase I committee approval.

9. STUDY RECORD RETENTION

For this appendix of the DEFINE Trial documentation will be kept for a minimum of **30 years after expiration of the investigative product** (final batch prepared for administration in accordance with this appendix).

10. REFERENCES

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