PROTOCOL AB002

Multicenter, Randomized, Double-Blind, Placebo-Controlled, Proof of Concept Study of LSALT Peptide as Prevention of Acute Respiratory Distress Syndrome (ARDS) and Acute Kidney Injury (AKI) in Patients Infected with SARS-CoV-2 (COVID-19)

Investigational Product:	LSALT peptide
Indication:	LSALT peptide is indicated for prevention of acute respiratory distress syndrome and acute kidney injury in patients infected with confirmed COVID-19.
Phase:	2
Date:	June 9, 2020
Amendments:	1 (July 15, 2020) 2 (August 15, 2020) 3 (February 15, 2021) 4 (March 17, 2021)
Name and Affiliation of Principal Investigator (PI):	A list of the Principal Investigators is maintained in the trial master file
Name and Address of Sponsor:	Arch Biopartners Inc. 545 King Street West Toronto, Ontario M5V 1M1 Canada
GCP Statement:	This study will be performed in compliance with GCP, including the archiving of essential documents.

SYNOPSIS

TITLE: Multicenter, Randomized, Double-Blind, Placebo-Controlled, Proof of Concept Study of LSALT Peptide as Prevention of Acute Respiratory Distress Syndrome (ARDS) and Acute Kidney Injury (AKI) in Patients Infected with SARS-CoV-2 (COVID-19)

INVESTIGATIONAL PRODUCT: LSALT peptide

INDICATION: LSALT peptide is indicated for the prevention of ARDS and AKI in patients infected with confirmed COVID-19.

PHASE OF DEVELOPMENT: 2

INVESTIGATIONAL SITES/LOCATIONS: Multicenter

PRIMARY OBJECTIVE: To evaluate the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen [≥ 6 L/minute], or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR on Day 28 <10 mL/min/1.73 m^2.

SECONDARY OBJECTIVES: To evaluate the following:

- Incidence and time to mild, moderate, and severe ARDS per patient between treatment groups
- Ventilation-free days
- Time on nasal canula or oxygen mask
- 28-day mortality– both all-cause and attributable
- ICU length of stay (in days)
- Hospitalization length of stay (in days)
- Virologic clearance rate
- Incidence of other organ disorders
- Need and duration for extracorporeal membrane oxygenation (ECMO)
- Vasopressor-free days
- Clinical improvement in chest x-rays
- Change in baseline modified Medical Research Council (mMRC) dyspnea scale
- SOFA scores assessed periodically
- Change from baseline in liver function tests (ALT, AST, and total bilirubin levels)
- Change from baseline in SCr and eGFR
- Change from baseline in highly-sensitive troponin (hs-troponin)
- Change from baseline ACT, aPTT, and/or PT/INR levels
- Change from baseline for antiviral (SARS-CoV-2) immunoglobulins; IgG, IgM, and IgA at EOS.

HEALTH OUTCOMES OBJECTIVE:

- Total healthcare costs from admission to discharge.

EXPLORATORY OBJECTIVES: To evaluate:

- Change in serum cytokines including IL-1α, IL-1ß, and ferritin levels, as well as other exploratory biomarkers.
- Change in baseline antiviral immunoglobulins (IgG, IgM, IgA) at EOS.
- Pharmacokinetics of LSALT peptide over the study period.

STUDY DESIGN AND METHODOLOGY: This study is a parallel group, randomized, third-party blinded, multicenter study to assess safety and efficacy of LSALT peptide *versus* placebo in hospitalized patients with confirmed infection or recent confirmed infection with complications associated with COVID-19.

Patients will be followed for safety and efficacy up to Day 28, with Day 1 being the day of randomization to assess safety.

After assessing the risk of ARDS and satisfying all inclusion and exclusion criteria, the patient will be randomized to 5 mg LSALT peptide or blinded placebo to be given intravenously once daily for a maximum of 14 days. Physical and respiratory examinations, vital signs, and adverse events will be recorded throughout the study, including Day 28 (EOS). Blood chemistries, hematology, coagulation, urinalysis, ECG, SARS-CoV-2 tests, eGFR, and chest x-ray (CXR) will be assessed at Day 1 (Screening/Baseline) prior to initiation of study drug, and on Day 3, EOT, and at EOS, as well as when clinically indicated. The ECG at EOS will only be obtained if clinically indicated. An additional CXR will be obtained at time of clinical improvement. Cytokines/biomarkers and pharmacokinetics (PK) will be assessed at Day 1 (Screening/Baseline) prior to initiation of study drug, at 1 (mid-dose) and 2 hours (end of infusion) of drug therapy on Days 1, 3, EOT, and a single blood sample at EOS for cytokines/biomarkers only. Where applicable, a urinary pregnancy test will be obtained at Screening in women of childbearing potential. Questionnaires (APACHE II, SOFA) will be obtained at Baseline, Day 3, EOT, and EOS; venous blood gas (VBG) or HCO_3 (bicarbonate) levels may be substituted for arterial blood gas (ABG) if it is considered standard-of-care (SOC) or in the patient's best interest, and results in comparable APACHE II and SOFA scores. Other questionnaires (Berlin Definition and modified Medical Research Council Dyspnea Scale) will be assessed at Baseline, Day 3, EOT, and EOS. IgG, IgA, and IgM antiviral antibodies will be collected at Baseline and EOS. Patients will be maintained on the SOC per institutional guidelines, including prophylaxis or treatment of VTE, throughout the study.

A Data and Safety Monitoring Board (DSMB) will evaluate patients on a continuing basis for primarily safety assessments. Per the DSMB Charter, the DSMB will meet at least monthly if not more frequently based upon enrollment throughout the study period.

NUMBER OF PATIENTS (PLANNED): Thirty (30) patients will be randomized to active drug (LSALT peptide) and 30 patients will be randomized to matching placebo. This study will be third-party blind with only the Pharmacist at the site unblinded for the purpose of preparing drug/placebo for injection.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:
Key Inclusion Criteria:

Eligible patients must fulfill the following inclusion criteria:

1. Male and female hospitalized patients between 18 and 80 years of age at time of consent.
2. Clinical and laboratory diagnosis of COVID-19 infection. Patients must be positive for the SARS-CoV-2 by Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel or have an existing complication secondary to SARS-CoV-2 infection which was positive within 2 weeks of entry into the study. Further, patients must have at least <u>two of the following three</u> symptoms:
   - Fever (oral temperature ≥ 100.4 °F [> 38 °C]) with or without chills
   - Dyspnea or difficulty breathing (≥ 2 on mMRC dyspnea scale)
   - Nonproductive cough;
   - Or other signs and symptoms of established complications to SARS-CoV-2 infection (*e.g.*, coagulopathy, cardiomyopathy, acute kidney injury [AKI], and/or acute liver injury) within the limits of Exclusion Criteria #8.
3. Patients must present with moderate to severe illness as defined below:
   - <u>Moderate illness</u>: Patients who have evidence of lower respiratory disease by clinical assessment or imaging and an oxygen saturation (SpO_2) > 93% on room air at sea level
   - <u>Severe illness</u>: Patients who have a respiratory frequency > 30 breaths per minute (bpm), $SpO_2 \leq 93\%$ on room air at sea level, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300, or lung infiltrates > 50% (COVID-19 Treatment Guidelines Panel, 2020).
4. APACHE II score < 20 or establishment of survivability of the patient beyond 48 hours following randomization.
5. Therapies which have been shown to be beneficial and are included in standard COVID-19 treatment guidelines (*e.g.*, those of WHO or NIH, or institutional guidelines) are permitted.
6. Sexually active women of child-bearing potential (WCBP) must be using a medically acceptable method of birth control throughout the study and for at least 1 day following the end of study, and have a negative urine pregnancy test at the Screening visit. A WCBP is defined as a female who is biologically capable of becoming pregnant. A medically acceptable method of birth control includes intrauterine devices in place for at least 3 months, surgical sterilization, or the implant. In patients who are not sexually active, abstinence is an acceptable form of birth control and urine will be tested per protocol. Women who are of nonchild-bearing potential, *i.e.*, post-menopause, must have this condition captured in their medical history. Pregnant women and nursing mothers are excluded from this study.
7. Patient or Legally Authorized Representative (LAR) is available and willing to give written informed consent, after being properly informed of the nature and risks of the study and prior to engaging in any study-related procedures.

Key Exclusion Criteria:

The presence of any of the following excludes a patient from study enrollment:

1. Known sensitivity, allergy, or previous exposure to LSALT peptide.
2. Exposure to any investigational drug or device <90 days prior to entry into study.
3. Treatment with immunomodulators or immunosuppressant drugs, including but not limited to IL-6 inhibitors, TNF inhibitors, anti-IL-1 immunomodulators, and JAK inhibitors within five half-lives or 30 days (whichever is longer) prior to randomization and throughout the study period. However, should any of these treatments become SOC and incorporated into clinical treatment guidelines (*e.g.*, those of WHO or NIH, or institutional guidelines), the treatment is permitted. Further, low-dose oral prednisone (≤ 20 mg/day) and inhaled steroids (*e.g.*, for treatment of asthma) are allowed in the study.
4. Anticipated transfer to another hospital or medical center within 72 hours, which is not a study site.
5. Uncontrolled or poorly-treated active hepatitis B (HBV), hepatitis C (HepC), or HIV infection. Those subjects who are positive for HBV, HepC, or HIV but are well-controlled with low viral loads are allowed to participate in the study:
    - HBV low viral load defined as <20,000 IU/mL
    - HepC low viral load defined as <800,000 IU/mL
    - HIV low viral load defined as <5000 copies/mL.
6. Participation in another drug or device study at any time during this study, for example:
    - Ulinastatin 200,000 IU or greater
    - High dose intravenous Vitamin C
    - Budesonide and formoterol
    - Bevacizumab to prevent ARDS
    - Dornase alfa to reduce hypoxemia in ventilated trauma patients.
7. As indicated in the inclusion criteria, pregnant female patients are excluded from study. Further, female patients who are nursing are excluded from study.
8. Has any medical condition considered to be clinically significant and could potentially affect patient safety or study outcome, including but not limited to:
    - Acute or chronic kidney disease (stage-4 or -5 renal impairment; eGFR<30 mL/min/1.73 m^2 or hemodialysis)
    - End-stage malignancy undergoing treatment
    - Immunocompromised patients or those with medical/surgical conditions (*e.g.*, solid organ transplantation) which require chronic immunosuppression

- Chronic hematologic disease which, in the opinion of the PI, prohibits the patient from entering into study
- Acute liver injury with AST and/or ALT levels greater than 3x ULN, unless recent injury (within 2 weeks) likely due to COVID-19 infection
- History of coagulopathy of unknown etiology, as defined by abnormal ACT (only if part of SOC), aPTT, and/or PT/INR values at least 2-fold outside normal limits, and currently present at screening, and/or
- End-stage lung disease, acute lung injury, very severe chronic obstructive pulmonary disease (COPD) as assessed by the GOLD criteria (GOLD Stage IV), or mechanical ventilation.

TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION:

LSALT peptide or an equivalent volume of commercially-available 0.9% sodium chloride (saline) solution to a volume of 100 mL mini-IV bag by an unblinded Pharmacist or designate. The drug will be infused into the patient through a peripheral 18, 20, or 22 g intravenous catheter over 2 hours and under the supervision of the Principal Investigator. Study drug may also be administered through a central line. Study drug will be administered once daily at a consistent time throughout the study.

DURATION OF TREATMENT: Maximum of 14 days.

DISCONTINUATION FROM TREATMENT:
Reasons for permanent discontinuation of treatment include the following:

- Patient experiences two or more Grade 2 toxicities or one or more Grade 3 or 4 toxicity considered by the PI to be associated with LSALT peptide treatment (CTCAE v5.0, 2017)
- Patient requests to discontinue treatment or patient withdrawal of consent
- PI considers that it is not in the best interest of the patient to continue treatment due to an adverse event.
- Protocol deviation requiring discontinuation of study treatment for safety reasons, and/or
- Pregnancy.

Patients who discontinue treatment for any reason (except withdrawal of consent from study participation) will not be discontinued from the study and will continue to be followed for all assessments until Day 28, or if not possible then for all assessments necessary to determine the value of the primary endpoint at Day 28.

EFFICACY ENDPOINTS:

Primary efficacy endpoint:

- To evaluate the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen [≥ 6 L/minute], or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR on Day 28 <10 mL/min/1.73 m^2.

Secondary efficacy endpoints include:

- All-cause mortality
- The presence and severity of ARDS as an ordinal outcome of the proportion of patients who have none, mild, moderate, or severe ARDS
- Time to each of mild, moderate, and severe ARDS
- The number of ventilation-free days and ECMO-free days
- Time on nasal canula or oxygen mask
- Length of stay in ICU and hospital (admission to discharge)
- Virologic clearance rate
- Worst PaO_2/FiO_2 ratio following enrollment
- Change in PaO_2/FiO_2 ratio
- Vasopressor-free days
- Change from maximal radiographic damage to EOT
- Change in baseline mMRC score
- Change in APACHE II score
- Proportion of patients with extrapulmonary organ dysfunction using the daily SOFA score
- Change in liver function tests (ALT, AST, total bilirubin)
- Change in renal function tests (SCr, eGFR)
- Change in hs-troponin levels
- Change in ACT, aPTT, and/or PT/INR values
- Change in antiviral IgG, IgA, and IgM levels.

Health Outcomes endpoint:

- Total healthcare costs from admission to discharge between treatment groups.

Exploratory Endpoints:

- Change in serum cytokines including IL-1α, IL-1ß, and ferritin levels, as well as other exploratory biomarkers drawn at the same time as LSALT peptide concentrations.
- Change in baseline antiviral immunoglobulins (IgG, IgM, IgA) at EOS.

SAFETY ENDPOINTS:

- Treatment-emergent adverse events (TEAEs)
- Clinical laboratory tests
- Physical examinations
- Vital signs
- 12-lead electrocardiogram
- Radiologic findings

PHARMACOKINETICS (PK): Blood samples will be obtained at Baseline prior to the start of drug infusion on Day 1, then midpoint (1 hour after start of infusion), and immediately following the daily 2-hour infusions on Days 1, 3, and at EOT regardless of active or placebo therapy. These blood samples will be analyzed for parent LSALT peptide and associated fragments (metabolites). Cytokines will be measured at the same times to assess relationship between drug concentrations and cytokine levels.

STATISTICAL METHODS:

Sample Size: Sixty subjects are planned for enrollment (randomized and treated) in this study.

Efficacy Analyses: Continuous variables will be summarized with descriptive statistics (the number of non-missing values [n], mean, median, standard deviation [SD], minimum, and maximum). All categorical variables will be summarized with frequency counts and percentages, as applicable. Time to event variables will be summarized using Kaplan-Meier survival curves and compared between treatment groups using either Log-rank tests or Cox's Proportional Hazard model adjusting for baseline covariates, as necessary. For continuous variables assessed at multiple time points, a Mixed Model Repeated Measures (MMRM) approach will be used or Analysis of Covariance (ANCOVA) if assessed at a single time point post-baseline. For categorical endpoints Cochran-Mantel-Haenszel (CMH) methods will be used, or a logistic regression approach, in the case of continuous covariates. The primary efficacy analysis population will be the Full Analysis Set (FAS), which will include all subjects randomized who receive any part of at least one infusion of randomized treatment, assigned to treatment group as randomized.

Additional details regarding precise endpoint definitions (including transformations, as applicable), analysis model specifications and α-level control for primary and important secondary endpoints associated with multiplicity will be provided in a separate statistical analysis plan (SAP), to be finalized prior to unblinding the study.

Although significant missing data with respect to the primary endpoint is not anticipated for a study of this duration (due to the nature of the endpoint), methods for handling missing data and intercurrent events, including for secondary endpoints will be specified in the SAP. These methods will be consistent with missing data due to death as having a worst possible outcome.

Safety Analysis:

All patients randomized and who received any part of at least one infusion of randomized treatment will be evaluated for safety according to the treatment actually received. The safety analyses will include evaluation of the incidence of treatment-emergent AEs, SAEs, and AEs leading to discontinuation of study treatment tabulated by Overall, System Organ Class, and Preferred Term using the MedDRA coding system. Additional tables with AEs by severity and relationship to drug as assessed by the PI will be presented. Laboratory data, vital signs, radiologic evidence, drug and cytokine concentrations, and ECGs assessments will be evaluated over time on study using descriptive statistics, including changes from baseline. Shift analyses of relevant clinical laboratory parameters will be produced showing shifts across low, normal, and high categories.

DOCUMENT APPROVAL

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirements.

ARCH BIOPARTNERS REPRESENTATIVE

_____ _____

Signature

Daniel A. Muruve, MD Date 17-March-2021

PRINCIPAL INVESTIGATOR

_____ ___18-March-2021_____

Signature Date

Print Name: __Alain Tremblay_____

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1 LIST OF ABBREVIATIONS

ABG	Arterial blood gas
ACT	Activated clotting (coagulation) time
AE	Adverse event
AECC	American–European Consensus Conference
AKI	Acute kidney injury
ANCOVA	Analysis of covariance
APACHE II	Acute Physiology and Chronic Health Evaluation II
aPTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
$AUC_{0-\infty}$	Area under the concentration-time curve from $0 \rightarrow \infty$
BMI	Body mass index
CDC	Centers for Disease Control & Prevention
CHF	Congestive heart failure
C_{max}	Maximal concentration
CMH	Cochran-Mantel-Haenszel
COPD	Chronic obstruction pulmonary disease
COS-1	CV-1 in origin and carrying SV40 genetic material
CRF	Case report form
CRO	Contract research organization
DM	Diabetes mellitus
DPEP-1	Dipeptidase-1
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
eCRF	Electronic Case Report Form
EC	Ethics Committee
eGFR	Estimated glomerular filtration rate
EOS	End of study
EOT	End of treatment
FAS	Full analysis set
FDA	Food and Drug Administration
FiO_2	Fraction of inspired oxygen
GCP	Good Clinical Practice
HA	Hyaluronic acid
HBV	Hepatitis B virus
HED	Human equivalent dose
HepC	Hepatitis C virus
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
hs-troponin	Highly-sensitive troponin levels
ICF	Informed consent form
ICU	Intensive care unit

IL-X	Interleukin X (*e.g.*, IL-2 = interleukin 2)
IND	Investigational New Drug
IP-10	Interferon gamma-induced protein 10
IRB	Institutional Review Board
IRI	Ischemia/reperfusion injury
ITT	Intent-to-treat study population
IV	Intravenous
IWRS	Interactive Web Response System (for randomization)
LAR	Legal authorized representative
LIS	Lung injury score
LLN	Lower limit of normal
LPS	Lipopolysaccharide
MCP3	Monocyte-chemotactic protein 3
MedDRA	Medical dictionary for regulatory activities
MERS	Middle East Respiratory Syndrome
mITT	Modified intent-to-treat study population
mMRC	Modified Medical Research Council
MMRM	Mixed Model Repeated Measures
MOF	Multiorgan failure
MRT_{obs}	Mean residence time (observed)
MTD	Maximal tolerated dose
PaO_2	Partial pressure of oxygen
PECAM1	Platelet and endothelial cell adhesion molecule 1
PEEP	Positive end-expiratory pressure
PI	Principal Investigator
PK	Pharmacokinetics
PNGaseF	Peptide:N-glycosidase F
PO	Oral (*per os*)
PP	Per protocol study population
PT/INR	Prothrombin time/International normalized ratio
RRT	Renal replacement therapy
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS	Severe acute respiratory syndrome
SCr	Serum creatinine
SD	Standard deviation
SOC	Standard-of-Care
SOFA	Sequential Organ Failure Assessment score
SpO_2	Fraction of O_2-saturated Hgb relative to total Hgb in blood
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	Elimination half-life
TEAE	Treatment-emergent adverse event
TEC	Tubular epithelial cells

T_{max}	Time at C_{max} (maximal concentration)
TNF_{α}	Tumor necrosis factor – α
ULN	Upper limit of normal
USP	United States Pharmacopeia
VAS	Visual analog scale
VBG	Venous blood gas
VTE	Venous thromboembolism
WCBP	Women of child-bearing potential
WIS	Working internal standard solution
ZO-1	*Zonula occludens* – 1

2 ETHICAL CONDUCT OF THE STUDY AND REGULATORY REQUIREMENTS

2.1 Institutional Review Board (IRB) or Ethics Committee (EC)

An Institutional Review Board (IRB) or Ethics Committee (EC) will review the study protocol and any amendments. The IRB will also review the informed consent forms, their updates (if any), and any written materials given to the patients. A list of all IRBs and contact information will be included in the study report.

2.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, GCP, and applicable regulatory requirements.

2.3 Patient Information and Consent

The PI, or designate, will obtain a freely given written consent from each patient or Legal Authorized Representative (LAR) after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the study that are relevant to the patient's or LAR's decision to participate. The consent forms must be signed and dated by the patient or LAR before he/she is exposed to any protocol-specific procedure.

The PI, or designee, will explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify.

The patient or the patient's LAR will receive a copy of the patient information and the signed informed consent form(s).

The patient or the patient's LAR will be informed if new information becomes available that may be relevant to his/her willingness to continue participation in the study.

Each patient or patient's LAR will be informed that a monitor, in accordance with applicable regulatory requirements, may review the portions of their source records and source data related to the study. Data protection and confidentiality will be handled in compliance with local laws.

3 INTRODUCTION

3.1 COVID-19 Background

Recently, a global outbreak of a novel coronavirus named Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2; also listed as 2019-nCoV in the literature) and the disease it produces named Coronavirus Disease 2019 (COVID-19), has infected 2,355,676 total cases with 162,032 deaths globally (19-April-2020; Johns Hopkins, 2020). Similar to influenza, COVID-19 presents with fever > 100 °F, dry cough, difficulty breathing, and fatigue, and, in severe cases, bilateral pneumonia (Wu and McGoogan, 2020; Jin *et al*, 2020). Diagnosis of COVID-19, or any coronavirus, is based on the symptoms and confirmed through laboratory testing. Human-to-human transmission of COVID-19 has rapidly occurred, urging individuals to exercise social and physical distancing (*e.g.*, 6 feet apart). Those who may be infected yet asymptomatic highlight the need to implement social distancing for all people (Ahmed *et al*, 2018; Lau *et al*, 2020). Recently, there are two FDA-approved treatments, chloroquine and hydroxychloroquine ± azithromycin, to treat SARS-CoV-2 under emergency use authorization (CDC, 2020a). However, there is neither FDA-approved antiviral therapy nor a vaccine authorized to treat or protect against COVID-19, yet more than 300 studies have been initiated looking at dozens of promising therapeutic interventions (Mullard, 2020). Patients with severe symptoms should go directly to an emergency department or contact their primary care provider and likely will be hospitalized, in particular if community-acquired viral pneumonia is diagnosed (Grasselli *et al*, 2020). Criteria for progressive COVID-19 disease *vs.* disease improvement/stabilization include (Liu *et al*, 2020):

- Age (66 *vs.* 37 years, U=4.932, P=0.001)
- History of smoking (27.3% *vs.* 3.0%, χ^2=9.291, P=0.018)
- Maximum body temperature at admission (38.2 °F *vs.* 37.5 °F, U=2.057, P=0.027)
- Proportion with respiratory failure (54.5% *vs.* 20.9%, χ^2=5.611, P=0.028) and respiratory rate (34 *vs.* 24 breaths/minute, U=4.030, P=0.004)
- Receive high-level respiratory support (χ^2=16.01, P=0.018)
- C-reactive protein (38.9 *vs.* 10.6 mg/L, U=1.315, P=0.024)
- Albumin (36.62 *vs.* 41.27 g/L, U=2.843, P=0.006).

Others, including one report of 99 cases of COVID-19 in Wuhan China and another of 201 patients in the same area, found similar signs and symptoms to diagnose community-acquired viral pneumonia (Chen *et al*, 2020; Huang *et al*, 2020; Wu *et al*, 2020).

Due to rapid escalation of this pandemic, clinicians and scientists have initiated experimental treatments for prevention and treatment of COVID-19. Raoult and colleagues recently described their findings on the effects of 600 mg hydroxychloroquine daily in attenuating the symptoms of COVID-19 and reducing viral load (Gautret *et al*, 2020). Depending upon the clinical picture of each patient, at the clinician's discretion, azithromycin (Z-Pak 1500 mg over 5 days) was added to the regimen in this single arm, open-label, four site study at the Méditerranée Infection University Hospital Institute. A total of 20 hospitalized patients with confirmed COVID-19 infection were treated with hydroxychloroquine for up to 10 days and compared with 16 control patients. At Day 6, 70% of hydroxychloroquine-treated patients were eradicated compared with 12.5% in the

control group (*P*=0.001). Remdesivir, an investigational antiviral with activity against SARS-CoV-2 (Wang *et al*, 2020), manufactured by Gilead and undergoing compassionate trials, is being studied in a large ongoing clinical trial; cohorts studying the effects of individual and combination drugs, such as hydroxychloroquine, lopinavir/ritonavir, and interferon-ß-1A, are also being studied in this prospective, multinational trial involving 3100 patients (NCT04315948). Remdesivir was very recently authorized by the FDA on May 1, 2020 for the emergency use in the treatment of COVID-19 (FDA, 2020).

There is also an ongoing post-exposure prophylaxis study with hydroxychloroquine and azithromycin (NCT04308668). In many cases (*e.g.*, vitamin C infusions for the treatment of severe 2019-nCoV pneumonia; NCT04264533), success of the treatments will not be known for several months to years.

Despite these preliminary and uncontrolled reports of potential efficacy, none of the potential antiviral therapies under consideration are proven until proper placebo-controlled studies are completed. Furthermore, it is becoming apparent that once cytokine release syndrome and respiratory failure occur in COVID-19 patients, these events are believed to be largely virus-independent and caused primarily by excessive and uncontrolled host immune/inflammatory responses (Moore and June, 2020; Henderson *et al*, 2020). A large proportion of patients with critical illness related to viral pneumonia still die despite appropriate antiviral therapy (Ayres, 2020). Furthermore, uncontrolled host immune responses, also known as cytokine storms, drive the need for critical care, ventilatory support, and ensuing mortality from COVID-19.

There is mounting evidence that organs other than, or in addition to lungs are damaged by COVID-19 (Zhai *et al*, 2020; Klok *et al*, 2020; Shi *et al*, 2020). A recent report from 13 hospitals on Long Island NY involving 5,449 patients over a 5-week period highlights this issue (Hirsch *et al*, 2020). The vast majority of patients were diagnosed with acute kidney injury (AKI) within 1 month of mechanical ventilation in SARS-CoV-2-infected patients. The odds ratio for need for ventilation and AKI was 10.7. At the time of the publication, 780 (39%) were still admitted in the hospitals, 519 (26%) had been discharged, and 694 (35%) had died. Other organs such as the liver, gastrointestinal tract, and the heart have been similarly compromised in COVID-19 patients. Thus, there is an urgent need to devise approaches to complement antiviral therapies to control the host immune/inflammatory response.

3.2 Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is a life-threatening inflammatory response to triggers such as infections and sepsis, resulting in acute lung injury (ALI) and respiratory failure (Jenne and Kubes, 2015; Thompson *et al*, 2017). The prevalence of ARDS in 459 ICUs from 50 countries was 10.4% (3022 ARDS patients of 29,144 ICU patients; Bellani *et al*, 2016), with more than 75% being diagnosed with ARDS in the first 48 hours of admission to the ICU. This prevalence was divided into mild (30.9%), moderate ARDS (46.6%), and severe ARDS (23.4%). Hospital mortality was 34.9%, 40.3%, and 46.1% for those with mild, moderate, and severe ARDS, respectively. Others have reported a similar mortality rate (Cochi *et* al, 2016). The incidence of ARDS was highest in the United States (80.1 cases per 100,000 person-years) and lowest in Iceland

(3.65 cases per 100,000 person-years), likely due to patient characteristics including gender, race, and age, and early recognition of the signs and symptoms of ARDS (Pham and Rubenfield, 2017; Eworuke *et al*, 2018).

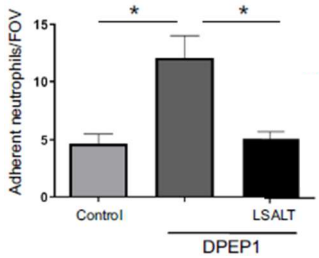
Acute respiratory distress syndrome (ARDS) developed in 17–29% of hospitalized patients with COVID-19, and secondary infections developed in 10% (Huang *et al,* 2020; Chen *et al*, 2020). In one report, the median time from symptom onset to ARDS was 8 days (Wang *et al*, 2020). Among 201 patients with confirmed COVID-19, 41.8% developed ARDS, and among these patients, 52.4% died (Wu *et al*, 2020). Comorbidities, such as hypertension and diabetes, as well as those presenting with dyspnea, were found in patients who developed ARDS compared with those who did not. Risk factors for ARDS in this special population who died from ARDS-related disease were older age, neutrophilia, and underlying or evolving organ and coagulation dysfunction.

The cascade of pattern recognition receptor activation, upregulation of pro-inflammatory cytokines, and ultimately recruitment of leukocytes to the affected tissue, in this case alveoli, results in the release of cytotoxins, ensuing cytotoxicity and cellular death. A number of interventions have been implemented over the last decade including (1) positive end-expiratory pressure (PEEP) to keep alveoli open for oxygenation of the deoxygenated blood, (2) induction of paralysis in the patient maintained on a lung-protective mechanical ventilator, and (3) prone positioning (patient lying face-down) for 16–18 hours per day to prevent primary aspiration (Festic *et al*, 2015; Albert, 2012). All interventions have reduced the mortality from 41% to 16%, however, these measures are supportive, at best, but at least reduce mortality in patients with ARDS. There remains no therapeutic modality to prevent the development of ARDS nor target its specific pathophysiology once diagnosed (Rawal *et al*, 2018). Trials with aspirin and inhaled budesonide/formoterol, amongst others, have been studied but despite some advances, mortality and morbidity remain high (Panka *et al*, 2017; Pais *et al*, 2018). Since 2010, ARDS mortality rates of in-hospital, ICU, 28/30-day, and 60-day mortality were 45, 38, 30, and 32%, respectively (Máca *et al*, 2017); thus, at least a third of patients with ARDS do not survive hospitalization. Others (Bellani *et al*, 2016) have shown mortality rates as high as 46% for those with severe ARDS, defined by a PaO_2/FIO_2 <100 mmHg based on the Berlin Definition (Ranieri *et al*, 2012).

A keystone to preventing and managing ARDS is understanding the interaction between leukocytes, primarily neutrophils, and endothelial cells which line the vascular lumen of the lung allowing the exchange of oxygen between alveoli and blood (Mammoto and Mammoto, 2019). As part of the endothelial glycocalyx, which on its own may be a target in the cascade of events related to ARDS (Porras *et al*, 2018; Ozolina *et al*, 2016), these small capillary vessels provide a critical pathway for delivery of oxygen, nutrients, and cellular components to local tissues while also eliminating waste products carbon dioxide and blood. The recruitment of leukocytes to the site of injury in the lung actually results in "friendly fire" since cytokines expressed and released by neutrophils in response to the damaged cells by the disease state (*e.g.*, infection) also result in "bystander" cytotoxicity of previously normal tissue and end-organ damage (Narasaraju *et al*, 2011; Collins *et al*, 2013). Further, interactions between neutrophils and inflammatory mediators at the site of damage result in increased stiffness of the neutrophil and inability to deform due to rearrangement of the cytoskeleton within the capillary bed, leading to vascular congestion and ischemia (Doerschuk, 2000; Williams and Chambers, 2014).

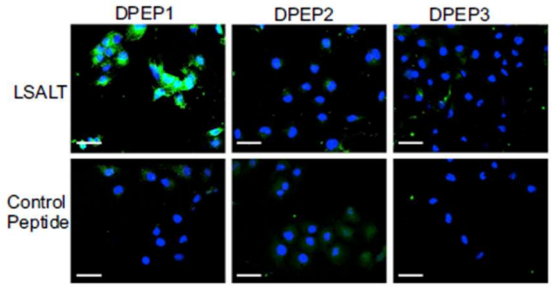
Discovered by Arch Biopartners scientists, dipeptidase-1 (DPEP-1) is a vascular adhesion receptor for recruitment of neutrophils and other immune responders to organs of pathogenic inflammation, including the lung, liver, and kidneys (Choudhury *et al*, 2019). Thus, DPEP-1 is a viable target for therapeutic intervention to halt attraction of neutrophils as well abrogate binding of the neutrophil to endothelial cells (Figure 1). The therapeutic intervention of the LSALT peptide is discussed later, but it specifically binds to DPEP-1 and not DPEP-2 nor DPEP-3 (Figure 2). More information is found in Section 3.3.

Figure 1. Human neutrophils bound DPEP-1 COS1 monolayers with/without LSALT



Used with permission (Choudhury *et al*, 2019).

Figure 2. LSALT peptide (green) binding to cells expressing DPEP1, DPEP2, DPEP3



Used with permission (Choudhury et al, 2019).

There is no cure for ARDS, only patient supportive strategies (American Lung Association, 2020). Current therapies include lung-protective ventilation with lower tidal volumes, thereby reducing neutrophil accumulation in the alveoli and lower concentrations of IL-6, IL-8, and TNF_{α} (Parsons *et al*, 2005; Ranieri *et al*, 1999). Extracorporeal membrane oxygenation (ECMO) is often used for severe ARDS to allow lung healing/repair and reverse respiratory failure (Peek *et al*, 2009). High-frequency oscillatory ventilation, with its rapid delivery of low tidal volumes and a respiratory rate in the range of 60 to 900 breaths/minute, has also been utilized in ARDS patients to avoid complications found with traditional ventilators (Meyers *et* al, 2019; Chan *et al*, 2007). Other measures are outlined in Rawal *et al*, 2018. All interventions do not prevent ARDS, rather stabilize any ongoing lung injury, and in some cases, reduce overall mortality (Saguil and Fargo, 2012).

It is important to understand that multi-organ dysfunction associated with ARDS, such as acute kidney injury (AKI; Cheng *et al*, 2020; Martinez-Rojas *et al*, 2020), cardiomyopathies (Shi *et al*, 2020; Akhmerov and Marban, 2020), VTE (Llitjos *et al*, 2020; Zhai *et al*, 2020), acute liver failure (Xu *et al*, 2020; Lee *et al*, 2020), amongst others also occur alone in COVID-19. Some reports accurately suggest that ARDS also arises from non-pulmonary conditions including acute

pancreatitis, sepsis, trauma, pneumonia, burns, elective surgery, and amniotic fluid embolisms (Fan *et al*, 2018; Cutts *et al*, 2016). A recent review of 357 patients with ARDS who did not have acute or chronic renal impairment prior to ARDS (an exclusion criteria for their primary analysis) revealed the following facts:

- 68.3% developed AKI of which half (48.4%) were stage III severe AKI
- Median time of onset was 4 days
- Higher BMI (> 30 kg/m^2), history of DM, high SOFA score (3 – 4), and acidosis were risk factors for Stage III AKI (Panitchote *et al,* 2019).

Others have shown similar results associating ARDS with the development of nephropathies (Liu *et al*, 2012; Tignanelli *et al*, 2018; Darmon *et al*, 2014). ARDS is also associated with acute liver failure which increases in severity as the severity of ARDS increases (Kallet *et al*, 2019). In this single-center retrospective study, 1747 patients with ARDS were evaluated for prevalence, severity, and mortality by organ system. Up to four nonpulmonary organ dysfunctions were common at the onset of ARDS: trauma-associated ARDS had the lowest mortality rate and decreased in prevalence with increasing ARDS severity. In contrast, aspiration-associated ARDS had the highest mortality rate, second only to sepsis, which increased in prevalence with increasing severity. COVID-19 has been linked to pulmonary damage through the angiotensin-converting enzyme 2 (ACE2), which is the primary receptor of SARS-CoV-2 (Yang *et al*, 2020). Since ACE2 is extensively expressed in the vascular endothelial cells of the heart, kidney, liver, intestine, and testis, amongst others, it is hypothesized that coronavirus will bind to ACE2 in these organs as well, resulting in multiple organ failure secondary to activated neutrophils expressing cytokines (Varga *et al*, 2020; Fu *et al*, 2020; Henderson *et al*, 2020).

Since late 2019, intensivists treating COVID-19 patients have identified acute lung injury which approximates ARDS, largely a result of activated neutrophils at the infected alveolar endothelial level. The Berlin definition of mild ARDS is 200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm H_2O (Table 1; Donahoe, 2011). However, when supplemental oxygen is administered to a patient, there are ways to determine whether a VQ mismatch is the source of poor oxygenation or if there is a right to left shunt that is causative. The rule of thumb for oxygenation improvement is that for every 10% change in FiO_2, there should be an accompanying increase in the arterial PaO_2 of 7 mmHg. For example, a patient is receiving 50% FiO_2 by facemask and due to a drop in pulse oximetry, the patient is then placed on 100% by non-rebreather (mask). The expected increase in PaO_2 related to the 50% increase in FIO_2 (50→100) should be 35 mmHg (7x5=35). If the increase in PaO_2 is not at least or greater than 35 mmHg, then there is a right to left shunt (Figure 3), suggesting an increased risk of cellular anaerobic metabolism at end organ, which could lead to the need for intubation.

The NIAID Ordinal Scale could capture this information: 1) Death; 2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 3) Hospitalized, on non-invasive ventilation or high flow oxygen devices; 4) Hospitalized, requiring supplemental oxygen; 5) Hospitalized, not requiring supplemental oxygen – requiring ongoing medical care (COVID-19 related or otherwise); 6) Hospitalized, not requiring supplemental oxygen – no longer requires ongoing medical care; 7) Not hospitalized, limitation on activities and/or requiring home oxygen; 8) Not hospitalized, no limitations on activities. Any change from scale #4 to scale #3 would suggest worsening respiratory status and likely progression to ARDS. This would be the

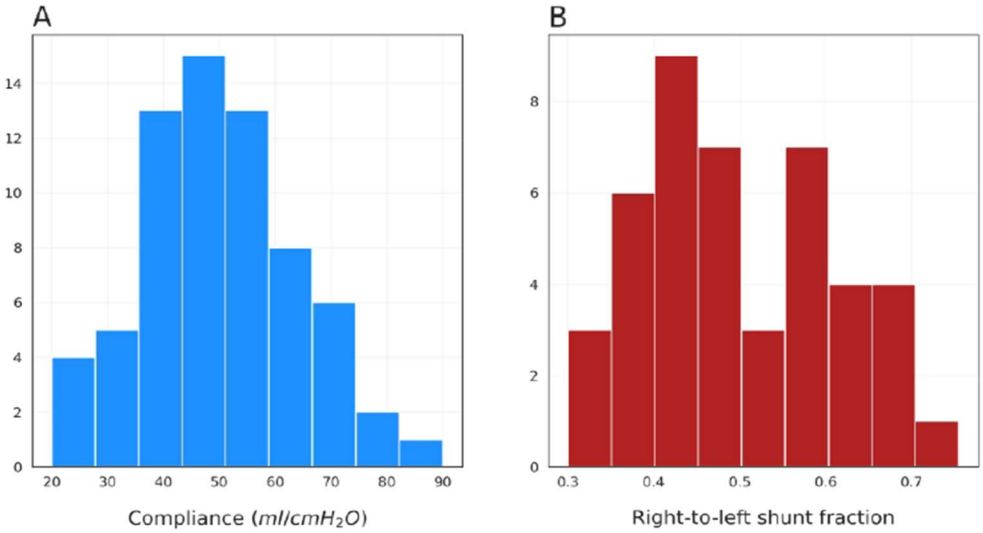
ideal time for intervention to prevent ARDS, although this scale does not obviate clinical judgement and the decision may occur prior to scale #4.

In COVID-19 patients, review of arterial blood gas data at the time of admission to the ICU and pre-intubation, suggest there is a signal of uncoupling of the lungs ability to augment oxygenation. This inability is multifactorial related to poor diffusion, VQ mismatch caused by viral pneumonia, and microvascular thrombosis resulting in right to left shunt physiology (Gattinoni *et al*, 2020; Figure 3). Augmented PEEP in this setting can be deleterious, due to inappropriate redistribution of cardiac output to areas of thrombosis or poor diffusion caused by damage to the capillaries infected by COVID-19 (Greenland *et al*, 2020; Gattinoni *et al*, 2020), although others continue with moderate to high levels of PEEP in these patients (Matthay *et al*, 2020; Poston *et al*, 2020; Alhazzani *et al*, 2020).

Scientists are also exploring the role of neutrophil extracellular traps (NETs) in pulmonary disease, including ventilator-associated nosocomial pneumonia, community-acquired pneumonia, virus-related acute lung injury, and pneumonia-related ARDS (Mikacenic *et al*, 2018; Ebrahimi *et al*, 2018; Lefrançais *et al*, 2018; Bendib *et al*, 2019; Twaddell *et al*, 2019). Indeed, Bendib and colleagues speculate on the potential of NETs as therapeutic targets in ARDS (Bendib *et al*, 2019). The formation of NETs has been linked to disease severity and mortality, including recruitment of macrophages to the active site which secrete IL-1ß which in turn induces IL-6. All-together, the ensuing cytokine storm leads to respiratory decompensation and other untoward events (Zhu *et al*, 2020; Barnes *et al*, 2020; Kaplan and Radic, 2012; Henderson *et al*, 2020).

By halting the upstream neutrophil-associated cascade of events, LSALT peptide may be an option to more fully understand the therapeutic benefits of NETs clinically as well as harm to normal tissue and organs. Further research with patient-centric outcomes will be a focus of subsequent research with LSALT peptide.

Figure 3. Respiratory system compliance is associated with right to left shunt fraction



A. Respiratory system compliance of 50.2 ± 14.3 mL/cmH_2O (N=16 patients)
B. Shunt fraction of 0.50 ± 0.11 (Used with permission, Gattinoni *et al*, 2020)

As discussed above, reports are emerging during this viral crisis that damage to the lungs are in part due to an evolving cytokine storm (Mehta *et al*, 2020; Ruan *et al*, 2020; Yang Y *et al*, 2020). Biomarkers such as the interleukins (specifically interleukin-1ß, IL-1ra, IL-6, IFN$_{\gamma}$, and TNF$_{\alpha}$, amongst others), interferon gamma-induced protein 10 (CXCL10, IP-10), and serum ferritin are elevated in patients with COVID-19 with evolving acute lung injury (Xu *et al*, 2020; Ye *et al*, 2020). We will study the prognostic value of these biomarkers and their relevance to the severity of ARDS (Jiang *et al*, 2005; Liu *et al*, 2020) and COVID-19 (Zhang *et al*, 2020; Cao X, 2020).

Our study is focused on COVID-19 pneumonia which, not surprisingly, had higher prevalence and greater mortality with increasing severity of ARDS of all nonpulmonary etiologies, likely due to increasing hypoxemia, fluid overload, and ongoing ischemia. We will also assess multiple organ dysfunction to more broadly evaluate viral damage. Clearly there is an unmet medical need to find a therapeutic intervention to prevent the onset of ARDS and other acute organ dysfunction in patients with COVID-19.

3.2.1 Scoring systems

3.2.1.1 Berlin ARDS definition

ARDS was first described by Ashbaugh and colleagues in 1967, describing a syndrome characterized by the acute onset of dyspnea, severe hypoxemia, diffuse lung infiltrates, and decreased lung parenchymal system compliance in the absence of evidence for congestive heart failure (Ashbaugh *et al*, 1967; Cutts *et al*, 2016). In 1988, Murray *et al*. introduced the Lung Injury Score (LIS), an assessment tool for ARDS, which incorporates four parameters on a scale of 0 → 4: (1) ratio of PaO_2 to FiO_2, (2) total respiratory compliance, (3) level of PEEP, and (4) extent of radiologic infiltrates (Murray *et al*, 1988). Six years later, the American-European Consensus Conference (AECC) on ARDS defined ARDS as respiratory failure of acute onset with a PaO_2/FiO_2 ratio < 200 mmHg independent of PEEP, bilateral infiltrates on front chest x-ray, and a pulmonary capillary wedge pressure ≤ 18 mmHg or no evidence of left atrial hypertension (Bernard *et al*, 1994; Raghavendran and Napolitano, 2011).

The AECC definition was readily adopted by clinical researchers and clinicians; however, issues with the original definition required some adjustments, including PaO_2/FiO_2 ratios adjusted for ventilator settings, increase reliability of the chest x-ray criteria, and distinguishing hydrostatic edema (Phua *et al*, 2008). The European Society of Intensive Care Medicine endorsed by the Society of Clinical Care Medicine and the American Thoracic Society gained consensus on new guidelines known as the Berlin Definition (Ranieri *et al*, 2012; Ferguson *et al*, 2012). The degree of hypoxemia divided the Berlin Definition into three categories (Table 1).

Table 1. The Berlin Definition of Acute Respiratory Distress Syndrome

	Acute Respiratory Distress Syndrome (ARDS)
Timing	Within 1 week of a known clinical insult or new/worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities – not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (*e.g.*, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm H_2O[c]

Moderate	100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg with PEEP ≥ 5 cm H_2O
Severe	PaO_2/FiO_2 ≤ 100 mmHg with PEEP ≥ 5 cm H_2O

[a]Chest radiograph or computed tomography scan
[b]If altitude is >1000 m, the correction factor should be calculated as follows: [PaO_2/FiO_2 x (barometric pressure/760)]
[c]This may be delivered noninvasively in the mild acute respiratory distress syndrome group

3.2.1.2 Sequential Organ Failure Assessment score (SOFA)

There are many quantitative scoring instruments to assess the number and severity of multiple organ dysfunctions, including the Denver Postinjury Multiple Organ Failure score (Sauaia *et al*, 2009), Marshall Multiple Organ Dysfunction Score (MODS; Marshall *et al*, 1995), and the Predisposition, Infection, Response, and Organ Failure (PIRO) Sepsis Classification System (Granja *et al*, 2013), amongst others. The sequential organ failure assessment score (SOFA score; Table 2), previously known as the sepsis-related organ failure assessment score (Vincent *et al*, 1996), is used to track a patient's status during the stay in an ICU to determine the extent of organ function or rate of failure. This score has been compared with the other scoring systems, and with few exceptions, results in better sensitivity, specificity, and a balance between the two for mortality and organ dysfunction (Hutchings *et al*, 2017; Granja *et al*, 2013; Fröhlich *et al*, 2016; Soo *et al*, 209; Ulvik *et al*, 2007). The SOFA score is based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal, and neurologic systems.

Table 2. The SOFA Score*

Organ System, Measurement	SOFA score				
	0	1	2	3	4
Respiration PaO_2/FiO_2	NL	< 400	< 300	< 200[a]	< 100[a]
Coagulation Platelets x10^3/mm^3	NL	< 150	< 100	< 50	< 20
Liver Bilirubin, mg/dL (µmol/L)	NL	1.2 – 1.9 (20 – 32)	2.0 – 5.9 (33 – 101)	6.0 – 119	> 12.0 > 204
Cardiovascular Hypotension	NL	MAP < 70 mmHg	Dopamine ≤5 or dobutamine (any dose)[b]	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central Nervous System Glasgow Coma Scale	NL	13 – 14	10 – 12	6 – 9	< 6
Renal Creatinine mg/dL (µmol/L) or Urine output	NL	1.2 – 1.9 (110 – 170)	2.0 – 3.4 (171 – 299)	3.5 – 4.9 (300 – 440) or <500 mL/day	>5.0 (>440) or <200 mL/day

*Source: Vincent *et al*, 1996
NL = Normal
[a]With respiratory support (*e.g.,* ventilator)
[b]Adrenergic agents administered for at least 1 hour (doses given are µg/kg/min)

3.3 LSALT peptide

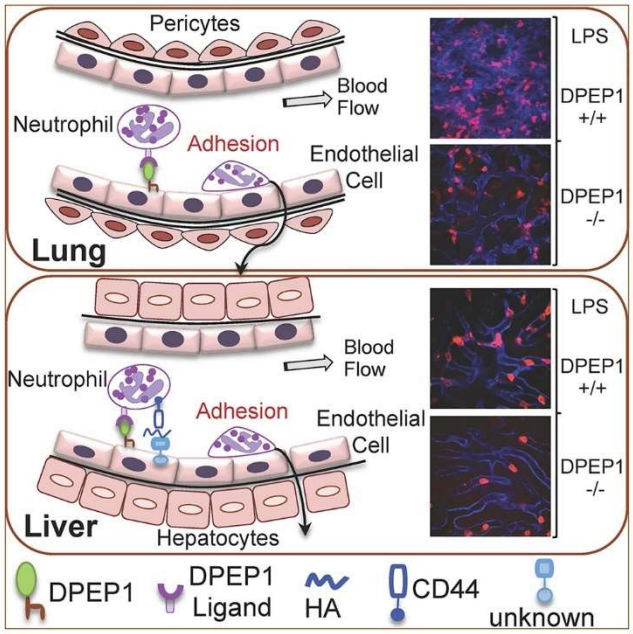
3.3.1 Inflammation and Organ Injury

Inflammation is a common pathway that contributes to the pathogenesis of many diseases caused by infection, allograft rejection, hypoxia, and autoimmunity. Although inflammation is regulated

by many complex biological processes and can be activated by numerous microbial and non-microbial stimuli, a common framework of events exists regardless of disease context which includes activation of pattern recognition receptors, upregulation of pro-inflammatory cytokines, and recruitment of leukocytes to affected tissues (Kopf *et al*, 2010; Nourshargh and Alon, 2014; Takeuchi and Akira, 2010). The interactions between recruited leukocytes and endothelial cells has been studied in detail to understand the exact processes that allows immune cells to bind to and infiltrate into injured or infected tissues. Endothelial receptors for leukocyte adhesion, including various selectins (*e.g.*, E-selectin), integrins and members of the immunoglobulin family (*e.g.*, PECAM1), have been identified yet other unknown receptors remain. Although inflammation is beneficial in eliminating microorganisms, abnormal and damaged cells during disease, it can also cause organ dysfunction and failure due to an excessive or unrelenting response that involves the overproduction of cytokines, cytotoxicity, and cell death (Kuchroo *et al*, 2012).

LSALT, a peptide drug with the sequence NH3-LSALTPSPSWLKYKAL-COOH, binds to dipeptidase-1 (DPEP-1) but does not inhibit its biologic enzymatic activity, potentially minimizing off-target or other adverse effects. LSALT peptide inhibits leukocyte recruitment in multiple experimental disease models through the direct inhibition of leukocyte adhesion to DPEP-1 present in lungs, kidney, and liver (Choudhury *et al*, 2019; Figure **4**). DPEP-1 represents a new molecular pathway for leukocyte adhesion discovered by Arch Biopartners scientists.

Figure 4. Graphic Illustration of Neutrophils Binding to DPEP-1.



Used with permission (Choudhury *et al*, 2019).

LSALT peptide was discovered by using an unbiased *in vivo* approach where a combinatorial phage display library17 was used to isolate specific peptide-displaying-phage that homed to the liver and lungs of mice during an inflammatory condition. In brief, neutrophils were isolated from C57/BL6 mice. RNA isolated from these neutrophils were then converted into cDNA. These

neutrophil-derived cDNA's were then fused into the coat protein gene of T7 phage thereby generating a library called T7N. This resulted in a random pool of T7 phages which presented various neutrophil peptides on the surface. These phage display libraries were subtracted by depleting the phage that bound to background cells or primary murine unstimulated lung endothelium. Unbound phage were then isolated from the culture and injected into neutrophil-depleted mice after lipopolysaccharide (LPS) stimulation. Phage that bound to the lung and liver endothelium were recovered, isolated and re-injected into another group of mice to enrich for the specific homing phage. This process was repeated five times *in vivo* to identify enriched phage clones that specifically homed to the lungs and liver. After isolation, phage were screened on the basis of their ability to inhibit neutrophil recruitment in the liver sinusoids in the presence of LPS by intravital microscopy. A specific peptide-displaying phage was isolated that inhibited the adhesion of neutrophils in the liver sinusoids in response to LPS *in vivo*. This phage subclone expressed the peptide NH3-LSALTPSPSWLKYKAL-COOH and designated as LSALT peptide.

3.3.2 Pre-Clinical Data for Dipeptidase-I and LSALT Peptide in AKI

3.3.2.1 DPEP-1 expression and function in kidney, liver, and lung

DPEP-1 expression and localization were characterized in renal tubular epithelial cells (TEC) cultured from human nephrectomy tissue samples were labeled with either zonula occludens-1 (ZO-1), a tight junction protein found on TEC, and DPEP-1. Confocal microscopy confirmed the expression of DPEP-1 on the surface of TEC. Additional characterization of DPEP-1 using immunohistochemical analysis revealed high expression levels in mouse kidney epithelium and endothelium as well as expression in lung and liver endothelium. Similar expression patterns have been confirmed in human lung, liver, and kidney. Using immunoblotting, DPEP-1 expression was found in both total kidney lysates and isolated TEC which confirmed the microscopy findings. Although DPEP-1 is predicted to have a size of 42kDa, a 55kDa DPEP-1 protein was also detected due to the presence of a GPI anchor as well as post-translational glycosylation as demonstrated by the effects of PNGaseF. Functional activity of DPEP-1 was determined in various samples using fluorescent enzyme activity assay that demonstrated high levels of dipeptidase expression or activity in both human and mouse lung, liver, and kidney tissue.

3.3.2.2 Specificity of LSALT Peptide binding to DPEP-1

DPEP-1 as a binding target for LSALT peptide was confirmed by overexpressing rat, canine, or human DPEP-1 in Cos-1 cells and treated with fluorescently labeled LSALT peptide. Analysis with fluorescent confocal microscopy of these cells demonstrated that LSALT peptide was bound only to cells expressing DPEP-1. This specific interaction was confirmed using a biotin-labeled LSALT peptide immunoprecipitation assay of DPEP-1. Total protein lysates from DPEP-1 overexpressing cells were treated with biotin-labeled LSALT peptide and the protein complex was pulled down with neutravidin conjugated beads before analysis by immunoblotting using anti-DPEP-1 antibody. Immunoblot analysis revealed that DPEP-1 interacted with LSALT peptide but not non-specific control peptide indicating specificity of LSALT peptide binding to DPEP-1.

To ensure LSALT peptide would not target similar proteins, other members of the dipeptidase family were also assessed. Human DPEP-1, DPEP-2, and DPEP-3 were transiently expressed in

Cos-1 cells before being treated with fluorescent-labeled LSALT peptide. Analysis by fluorescent microscopy revealed that LSALT peptide bound to DPEP-1 expressing cells but not DPEP-2 or DPEP-3 expressing cells.

DPEP-1 enzyme activity was tested using a fluorometric assay originally described by Heywood and Hooper in 1995 (Heywood and Hooper, 1995). Enzymatic activity of DPEP-1 was confirmed in lysates from human, rat, and mouse derived DPEP-1 expressing cells. Cilastatin or penicillamine, known functional inhibitors of DPEP-1, abrogated the enzymatic activity of both human and mouse DPEP-1. When cells expressing human DPEP-1 were treated with LSALT peptide, no significant decrease in DPEP-1 enzymatic activity was detected indicating that LSALT peptide binds to DPEP-1 but does not inhibit its enzymatic activity.

To determine whether the catalytic region of DPEP-1 was required for LSALT peptide interaction, a catalytically inert human DPEP-1 (E141D) was transiently expressed in Cos-1 cells and treated with fluorescent LSALT peptide. Analysis by fluorescent microscopy demonstrated removal of catalytic activity had no effect on LSALT peptide binding on cells as compared to normal DPEP-1 or negative mutant controls (H215). Loss of enzymatic activity in the catalytically inert DPEP-1 (E>D) was confirmed using the fluorometric assay (Gaber *et al*, 2011). Protein expression level of catalytically inert DPEP-1 (E141D) in Cos-1 cells was also confirmed by immunoblotting.

In a human *in vitro* static neutrophil adhesion assay, LSALT peptide inhibited neutrophil adhesion to activated endothelium at concentrations as low as 1 μg/mL.

3.3.2.3 DPEP-1 plays a role in renal ischemia reperfusion injury

Renal ischemia/reperfusion injury (IRI) is a major cause of acute kidney injury (AKI). A reduction in renal blood flow followed by reperfusion during patient recovery occurs in numerous clinical contexts including cardiac surgery and kidney transplantation. Renal IRI results in inflammation that includes leukocyte infiltration, renal hemorrhage, and tubular cell necrosis. Inflammation plays a significant role in the pathophysiology of renal IRI, however there are currently no therapeutic strategies in human AKI that directly target this pathway. To determine the potential impact of LSALT peptide in renal IRI, a survival model of murine renal IRI was employed where a vascular clamp is placed on the renal pedicle of a unilateral nephrectomized mouse to induce warm ischemia and subsequently released to induce reperfusion injury. A significant inflammatory response can be observed in the kidney as early as 2 hours of renal IRI with large numbers of monocytes and neutrophils infiltrating into the tubular/interstitial space. Furthermore, because of immune cell infiltration and extravasation, loss of endothelial integrity can also be observed as blood vessels become leaky and no longer retain their web-like organization.

Phenotypically, mice undergoing renal IRI developed oliguria/anuria over the course of 2 days due to loss of kidney function measured as an increase in serum creatinine (SCr). Treatment of mice with LSALT peptide (10 μg/kg) prior to renal IRI ameliorated AKI allowing mice to maintain normal urine output and preserving renal function. A similar effect on AKI was also seen with cilastatin (35 μg/kg), a chemical inhibitor of DPEP-1, albeit less effective than LSALT peptide.

Furthermore, dose response experiments demonstrated that LSALT peptide effectively inhibited the inflammatory response to renal IRI at a dose as low as 100 ng/kg.

Use of another DPEP-1 targeting peptide, GFE-1 (10 μg/kg), also demonstrated protection against renal IRI induced inflammation in a similar manner as LSALT peptide (10 μg/kg; Choudhury thesis, 2018).

3.3.2.4 DPEP-1 plays a role in lung & renal inflammation during endotoxemia and sepsis

Sepsis is the result of overwhelming bacterial infection and systemic activation of inflammatory pathways. Accumulation of leukocytes in tissues/organs is responsible for many sepsis manifestations including lung injury (ARDS) and AKI.

To evaluate the effect of the LSALT peptide in models of sepsis, mice were administered lethal doses of lipopolysaccharide (LPS) with or without pre-treatment with the LSALT peptide (given as a single dose before the LPS). All mice receiving LPS intravenously alone succumbed within 48 hours. Mice pretreated with a single dose of LSALT peptide survived. Pathology of the lungs in mice LPS plus LSALT peptide demonstrated significantly less inflammation and injury compared to mice receiving LPS alone. Similarly, LSALT peptide prevented the accumulation of neutrophils and monocytes in the kidney. In a more physiologic model of sepsis, LSALT peptide was also protective in terms of survival and AKI. Mice underwent cecal ligation and puncture and followed for 48 hours. Mice were untreated (no antibiotics) or pre-treated with a single dose of the LSALT peptide. Mice receiving the LSALT peptide demonstrated less kidney inflammation at 6 hours and improved kidney function and survival at 48 hours. Thus, LSALT peptide protects mice from the lethal effects of LPS.

3.3.3 Drug Metabolism and Pharmacokinetics

Plasma levels of LSALT peptide were measured post-intravenous dosing of 100, 250, and 450 mg/kg in rats. Terminal elimination half-lives of 0.66 – 1.17 hr and MRT_{obs} values of 0.58 – 0.85 hr were observed for LSALT peptide. The $AUC_{0-\infty}$ was linear and close to proportional with dose.

LSALT peptide is rapidly degraded in human plasma into smaller proteolytic fragments, likely from endopeptidase activity as measured by mass spectrometry. In mouse studies, at least one LSALT proteolytic fragment remains biologically active. Some of the proteolytic fragments have been detected in human plasma for as long as 120 minutes. LSALT peptide is undetectable in whole blood in the absence of phosphoric acid due to proteolytic digestion. LSALT peptide however is compatible with blood and does not induce red blood cell hemolysis. Work is ongoing to identify the LSALT peptide volume of distribution, tissue biodistribution, and excretion.

3.3.4 Toxicology and Safety

The maximum tolerated dose (MTD) of LSALT peptide in mice and rats is 100 and 450 mg/kg, respectively, which is several logs above the minimum effective dose of 100 ng/kg to prevent organ inflammation and injury in mice. In dogs, the MTD of LSALT is 20 mg/kg. In all mouse studies, no LSALT peptide-related toxicity has been observed at therapeutic doses.

3.3.4.1 14-Day GLP Toxicology Studies

3.3.4.1.1 Rat GLP Toxicology Study

A GLP study assessed the potential toxicity and toxicokinetics of LSALT peptide when administered by intravenous injection to Sprague-Dawley rats once daily for 14 days. Three dose groups of 10, 30, and 50 mg/kg/day were evaluated. The control group was administered the vehicle used to prepare the LSALT peptide dosing formulations (0.9% sodium chloride, USP). The LSALT peptide dose formulations and the control vehicle were administered to rats by intravenous bolus injection at a dose volume of 1 mL/kg based on the most recent body weight of each animal. The progression or regression of any effects was evaluated during an additional 14-day treatment-free, recovery period in the control and high-dose groups.

Male and female rats [strain: Crl: CD® (SD) BR-Sprague-Dawley; Charles River] were acclimated for this study for 16 days. During the acclimation period, observations for clinical signs and ophthalmology were conducted and body weights as well as food consumption were measured. Following the pre-study evaluations, rats were randomized by body weight to control and test groups.

There were no statistically significant differences in body weight, body weight changes, or food consumption between the treated and control groups during the study. Similarly, there were no ophthalmological or neurological findings considered to be related to treatment with the test item. Changes in hematology and clinical chemistry were mild, generally within normal ranges, and unlikely of clinical significance. Further, there were no gross findings that could be related to the test item treatment. There were no statistically significant findings on organ weights that could be attributed to the test item treatment.

LSALT peptide administered to Sprague-Dawley rats by intravenous bolus administration daily, for 14 days in the dose range of 10 to 50 mg/kg/day, was well-tolerated with no test-item-related effects observed upon evaluation of clinical observations, body weights, food consumption, clinical pathology (chemistry/hematology), gross pathology, or histopathology.

The NOAEL in rats is 50 mg/kg.

3.3.4.1.2 Dog GLP Toxicology Study

A GLP study assessed the potential toxicology and toxicokinetics of LSALT peptide when administered by intravenous infusion to Beagle dogs once daily for 14 days. The control group was administered the vehicle used to prepare the LSALT peptide dosing formulations (0.9% sodium chloride, USP). Three dose groups of 2.5, 7.5, and 15 mg/kg were evaluated. The LSALT peptide dose formulation and the control vehicle were administered to animals by intravenous infusion at a dose volume of 2 mL/kg based on the most recent body weight of each animal. The progression or regression of any effects was evaluated during an additional 14-day treatment-free, recovery period in the control and high dose groups.

LSALT peptide administered to Beagle dogs by daily intravenous infusions for 14 days in the dose range of 2.5 to 7.5 mg/kg was well tolerated with no test-item related clinical observations noted.

The dose level of 15 mg/kg resulted in manifestation of pseudo-allergic (anaphylactoid) infusion reactions. The clinical signs appeared shortly after dosing and consisted of reddening of the skin around the eyes and mouth, head flicking, facial edema, hives/reddening on the pinnae, and/or reddened eyes and muzzle. Pseudo-allergic reactions were typically observed throughout the entire treatment phase duration. Affected animals were treated with diphenhydramine by IV injection. These reactions occurred during infusion and disappeared shortly after the infusion was completed. Other clinical signs were sporadic and not considered to be related to treatment with the test item. These reactions may likely diminish by lengthening the time of infusion for delivering daily doses ≥ 15 mg/kg.

Pseudo-allergic reactions following intravenous administration of peptides/proteins in dogs have been reported and occur in response to the infusion of biotherapeutics. Non-clinical infusion reactions do not necessarily predict responses in humans (Mease *et al*, 2017; Cohn *et al*, 2007; Roselló *et al*, 2017).

Repeated dosing in the dose range of 2.5 to 15 mg/kg had no statistically significant effects. There were no differences in vital signs, body weight, or food consumption between the treated and control groups during the study. Changes in hematology and clinical chemistry were mild, generally within normal ranges, and unlikely of clinical significance. No changes in gross pathology and histopathology were attributed to the test item. Gross findings noted upon necropsy of Main Study animals included a white-tan area of capsular thickening and contraction in the spleen of one mid-dose female and marked enlargement, redness, and protrusion of the right third eyelid in the eye of one high-dose female. These gross findings were considered incidental and unlikely to be test item-related. There were no statistically significant findings on organ weights that could be attributed to the test item treatment.

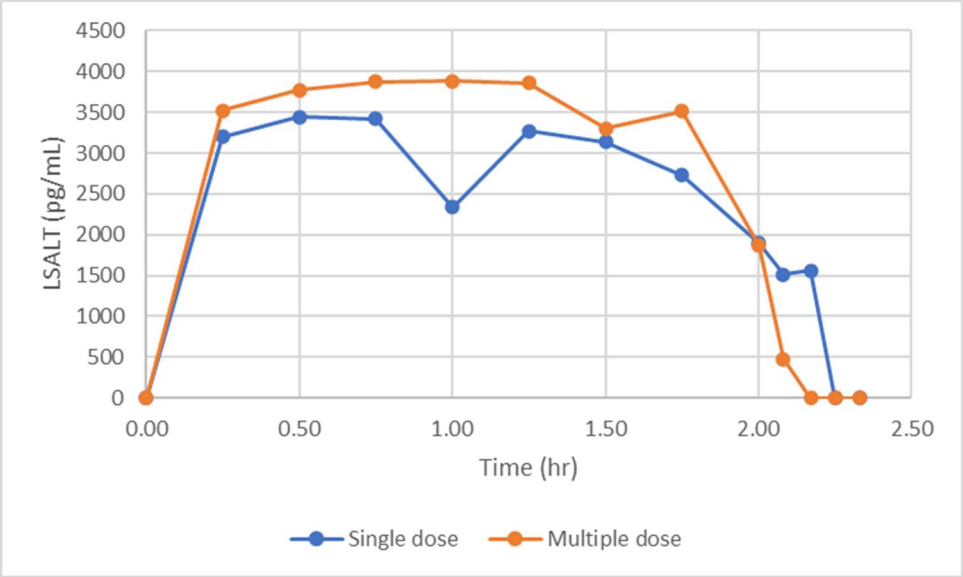
The NOAEL in dogs is 2.5 mg/kg.

3.3.5 Preliminary Safety in Humans

The first 6 cohorts of this study have been completed including subjects receiving a 5 mg dose daily for 3 consecutive days. No significant adverse effects were reported in any subjects receiving a single or multiple doses of the LSALT peptide (range 0.1 mg to 5 mg IV daily or IV daily for 3 days). There were no drug-related clinical adverse events. No significant changes in clinical chemistry, hematology and other laboratory parameters were observed from baseline and compared to subjects receiving placebo.

3.3.6 Pharmacokinetics

Figure 5. Intact LSALT peptide 5 mg single and multiple dose plasma concentrations



As seen in Figure 5, there was rapid elimination of intact LSALT peptide after the end of the infusion (2 hours) with an estimated mean ± SD $t_{½}$ of 0.41 ± 0.147 and 0.21 ± 0.0753 hours for the single dose and multiple dose, respectively. Apparent clearance was 580 ± 155 and 990 ± 146 L/hr for the two regimens.

3.4 Study Rationale

According to a report by Asher Mullard, more than 180 clinical trials have emerged since the first description of SARS-CoV-2 in late 2019 in China (Mullard, 2020). A further 150 trials are in the wings to be started in the next few months. Thus, at least 300 trials will either be ongoing or initiated studying various cures for SARS-CoV-2, ranging from antivirals such as remdesivir, lopinavir, and ritonavir, to repurposed drugs such as hydroxychloroquine, azithromycin, and other immunomodulators, to cellular and vitamin interventions. The question raised in this article is: "Do we need 300 trials? Is that a good use of resources?"

Coupled with this very recent article (published April 18, 2020) is an article by Janelle Ayres (published on April 17, 2020) questioning the priority of successful treatments of infectious diseases, which is obviously a primary endpoint of every ID study, without addressing concern for patients who progress to life-threatening sequelae of COVID-19 infections, including ARDS, septic shock, and multi-organ failure. These are serious conditions that result from immune responses to the virus (as discussed in the Introduction of this protocol). About one-quarter of patients presumably eradicated from a viral infection still die due to the underlying conditions resulting from host immune responses to the viral infection (Versluys and Boelens, 2018; Louis *et al*, 2012). Dr. Ayres asks a very important question in her article: It is equally as important to ask

the question: "How do we *survive* infections?" as it is to ask the familiar question: "How do we *fight* infections?"

Acute respiratory distress syndrome (ARDS) is a devastating complication for patients infected with microorganisms, including COVID-19. Currently, there are no effective therapies to prevent or minimize ARDS. Inflammation is an important factor in the pathogenesis of lung injury related to viral pneumonia and COVID-19. Recent pathologic and molecular studies confirm a prominent role for neutrophils and other inflammatory cells in the pathogenesis of COVID-19 associated lung injury (Zhou et al, 2020; Varga et al 2020).

Similarly, acute kidney injury (AKI) associated with COVID19 has important clinical implications with more than two thirds of patients experiencing prolonged hospitalization and/or death (Hirsch et al, 2020). While the pathophysiology of COVID19-associated AKI is not fully understood, inflammation related to direct viral infection of kidney cells, renal ischemia or systemic illness (sepsis or cytokine storm) are believed to play a role (Batlle *et al*, 2020; Diao *et al*, 2020).

Given its favorable toxicology and clinical pharmacology profiles, the administration of LSALT peptide to target lung and kidney inflammation and prevent or minimize ARDS or AKI during a COVID-19 infection has the potential to provide a significant long-term clinical impact. During the course of their disease, approximately 17 – 42% of COVID-19 hospitalized patients develop ARDS within 8 days of admission and almost half of those patients died while hospitalized (Chen *et al*, 2020; Huang *et al*, 2020; Wang *et al*, 2020; Wu *et al*, 2020). 36.6% of patients developed AKI in the series by Hirsch with more than two thirds of patients dying or requiring prolonged hospitalization. Of the patients who were discharged, a large proportion were left with significant kidney dysfunction (Hirsch et al, 2020). How many had cleared the virus is unknown from the literature.

Clearly, a therapeutic intervention that prevents life-threatening ARDS is needed. Even a 20 – 30% reduction in ARDS incidence or severity during COVID-19 would have a significant impact on clinical outcomes and health systems, including mortality, critical care, and ventilatory capacity. LSALT peptide has shown activity in animal models of inflammation including lung, kidney, and liver injury in the context of bacterial sepsis and its safety profile has been well-described in healthy adult subjects. Thus, the critical next step is the proof-of-concept Phase 2 study in hospitalized patients with COVID-19. We are attempting to answer the question: "How do we *survive* infections?"

3.5 Dose Justification

In order to identify a potential starting dose in the Phase 2 clinical trial in adult patients with COVID-19 using the existing data, toxicology data from the 14-day dog toxicology study were relied on; these are considered to be the most appropriate as the NOAEL in rats (50 mg/kg) is much less sensitive than the dog. In the 14-day dog toxicology study, the NOAEL dose was established as 2.5 mg/kg/day. The FDA's Guidance for Industry entitled "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" (FDA, 2005) was used to determine the human equivalent dose (HED) for an infected

adult weighing 70 kg. This patient represents the mid-range of the weight of COVID-19 patients intended to be included in the Phase 2 trial. The dog NOAEL dose of 2.5 mg/kg/day converts to a HED of 1.4 mg/kg/day. Applying a safety factor of 10 results in an initial starting dose of approximately 0.14 mg/kg/day or 10 mg/day. The study dose of 5 mg over 2 hours is substantially less than the HED and safe according to the Phase 1 results.

4 STUDY OBJECTIVES AND ENDPOINTS

4.1 Study Objectives

4.1.1 Primary Objective

To evaluate the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen [≥ 6 L/minute], or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR[1] on Day 28 <10 mL/min/1.73 m^2.

4.1.2 Secondary Objectives

To evaluate the following:

- Incidence and time to mild, moderate, and severe ARDS between treatment groups
- Ventilation-free days
- Time on nasal canula or oxygen mask
- 28-day mortality– both all-cause and attributable
- ICU length of stay (in days)
- Hospitalization length of stay (in days)
- Virologic clearance rate
- Incidence of other organ disorders
- Need and duration for extracorporeal membrane oxygenation (ECMO)
- Vasopressor-free days
- Clinical improvement in chest x-rays
- Change in baseline modified Medical Research Council (mMRC) dyspnea scale
- SOFA scores assessed periodically
- Change from baseline in liver function tests (ALT, AST, and/or total bilirubin)
- Change from baseline in SCr and eGFR

[1] eGFR is calculated from the serum creatinine according to the $eGFR_{CKD\text{-}EPI}$ equation (Levey *et al*, 2009):
eGFR (mL/min/1.73 m^2) = 141 × min(S_{cr}/κ,1)α × max(S_{cr}/κ, 1) - 1.209 × 0.993Age × 1.018 [if female] * 1.159 [if black], where S_{cr} = serum creatinine; κ = 0.7 for females and 0.9 for males; α = -0.329 for females and -0.411 for males; min indicates the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1.

- Change from baseline in highly-sensitive troponin (hs-troponin)
- Change from baseline ACT, aPTT, and/or PT/INR levels
- Change from baseline for antiviral immunoglobulins (IgG, IgM, and IgA) at EOS.

4.1.3 Health Outcomes Objective

- Total healthcare costs from admission to discharge (Omer *et al,* 2020).

4.1.4 Exploratory Objectives

- Change in serum cytokines including IL-1α, hs-troponin, and ferritin levels, as well as other exploratory biomarkers drawn at the same time as LSALT peptide levels.
- Change in baseline antiviral immunoglobulins (IgG, IgM, IgA) at EOS.
- Pharmacokinetics of LSALT peptide and fragments (metabolites) over the study period.

4.2 Study Endpoints

4.2.1 Primary Efficacy Endpoint

To evaluate the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen [≥ 6 L/min], or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR on Day 28 <10 mL/min/1.73 m^2.

4.2.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include:

- All-cause mortality
- The presence and severity of ADRS as an ordinal outcome of the proportion of patients who have none, mild, moderate, or severe ARDS
- Time to each of mild, moderate, and severe ARDS
- The number of ventilation-free days and ECMO-free days
- Time on nasal canula or oxygen mask
- Length of stay in ICU and hospital (admission to discharge)
- Virologic clearance rate
- Worst PaO_2/FiO_2 ratio following enrollment
- Change in PaO_2/FiO_2 ratio
- Vasopressor-free days
- Change from maximal radiographic damage to EOT
- Change in baseline mMRC score

- Change in APACHE II score
- Proportion of patients with extrapulmonary organ dysfunction using the daily SOFA score
- Change in liver function tests (ALT, AST, total bilirubin)
- Change in renal function tests (SCr, eGFR)
- Change in hs-troponin levels
- Change in ACT, aPTT, and/or PT/INR values
- Change in antiviral IgG, IgA, and IgM levels.

4.2.3 Health Outcomes endpoint:

- Total healthcare costs from admission to discharge between treatment groups.

4.2.4 Exploratory Endpoints:

- Change in serum cytokines including IL-1α, IL-1ß, and ferritin levels, as well as other exploratory biomarkers drawn at the same time as LSALT peptide concentrations.
- Change in baseline antiviral immunoglobulins (IgG, IgM, IgA) at EOS.

4.2.5 Safety Endpoints

Safety endpoints include:

- Treatment-emergent adverse events (TEAEs)
- Clinical laboratory tests
- Vital signs
- Physical examinations
- 12-lead ECG
- Radiologic findings.

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design and Plan – Description

This is a global, multicenter, randomized, double-blind, placebo-controlled, proof of concept study of LSALT peptide as prevention of acute respiratory distress syndrome (ARDS) and acute kidney injury in patients infected with SARS-CoV-2 (COVID-19).

After assessing the risk of ARDS and satisfying all inclusion and exclusion criteria, the patient will be randomized to 5 mg LSALT peptide or blinded placebo to be given intravenously once daily for a maximum of 14 days. Physical and respiratory examinations, vital signs, and adverse events will be recorded throughout the study, including Day 28 (EOS). Blood chemistries, hematology, coagulation, urinalysis, ECG, SARS-CoV-2 tests, eGFR, and chest x-ray (CXR) will be assessed at Day 1 (Screening/Baseline) prior to initiation of study drug, and on Day 3, EOT, and at EOS, as well as when clinically indicated. The ECG at EOS will only be obtained if

clinically indicated. An additional CXR will be obtained at time of clinical improvement. Cytokines/biomarkers and pharmacokinetics (PK) will be assessed at Day 1 (Screening/Baseline) prior to initiation of study drug, and on Days 1, 3, and EOT at 1 (mid-dose) and 2 hours (end of infusion) of drug therapy, and a single blood sample at EOS for cytokines/biomarkers only. Where applicable, a urinary pregnancy test will be obtained at Screening in women of childbearing potential. When clinically indicated, questionnaires (APACHE II, SOFA) will be obtained at Baseline, EOT, and EOS; venous blood gas (VBG) or HCO_3 (bicarbonate) levels may be substituted for arterial blood gas (ABG) if it is standard-of-care (SOC) or in the patient's best interest, and results in comparable APACHE II and SOFA scores (Brown et al, 2016). Other questionnaires (Berlin Definition and modified Medical Research Council Dyspnea Scale) will be assessed at Baseline, Day 3, EOT, and EOS. IgG, IgA, and IgM antiviral antibodies will be collected at Baseline and EOS. Patients will be maintained on the SOC per institutional guidelines, including prophylaxis or treatment of VTE, throughout the study.

Patients will be randomized to one of two blinded treatment regimens:

1. 100 mL of 5 mg IV LSALT peptide infusion over 2 hours daily
2. 100 mL of drug-free IV saline infusion over 2 hours daily.

Duration of therapy will be a maximum of 14 days. Patients will be maintained on the standard of care (SOC) for the treatment of COVID-19 as defined by institutional guidelines and/or physician practice. Co-morbidities and concomitant medications will be reviewed daily and documented in the patient's eCRF. The risk of venous thromboembolism (VTE), a potential consequence of SARS-CoV-2 infection, will be assessed and prophylaxis will be included as SOC for every patient (Zhai *et al,* 2020; Klok *et al*, 2020). Please refer to antithrombotic therapy in patients with COVID-19 (COVID-19 Treatment Guidelines Panel, Antithrombotic Therapy in Patients with COVID-19, p197; 17-December-2020).

5.2 Number of Patients (Planned)

Thirty (30) patients will be randomized to active drug (LSALT peptide) and 30 patients will be randomized to matching placebo. Patients and, if necessary, the Legal Authorized Representative (LAR), the Investigational Staff, and the Sponsor and its representatives will not know the randomization schemata. The Pharmacist at the site will be unblinded and prepare drug/placebo for injection.

A Data and Safety Monitoring Board (DSMB) will evaluate patients on a continuing basis for primarily safety assessments but also the adequacy of treatment based upon clinical data of the patient, target drug concentrations, and data obtained from the AB001 PK study in healthy subjects to offer recommendations to the Sponsor. In the absence of adverse events or any safety issues during the course of study, the DSMB may recommend continuance or suspension of the study due to futility or safety issues. The DSMB will meet at least monthly if not more frequently based upon enrollment throughout the study period. Per protocol, at no time will patients be dosed for more than 14 days of therapy. The DSMB Charter will outline all processes for changes to the protocol and protocol design prior to the first patient being enrolled into study.

5.3 Discussion of Study Design

This double-blind, placebo-controlled, proof-of-concept Phase 2 study is designed to evaluate LSALT peptide compared with placebo in a defined number of adult hospitalized patients with the diagnosis of COVID-19. This is a 1:1 randomization comparing 5 mg LSALT peptide daily with matching placebo. LSALT peptide or drug-free saline will begin immediately once the patient meets all inclusion/exclusion criteria at the start of hospitalization and followed with physical and respiratory exams, blood chemistries, coagulation, hematology, ECGs, SARS-CoV-2 tests, CXRs, biomarkers, and PK will be recorded periodically throughout the 28 day study. When clinically indicated, questionnaires (APACHE II, SOFA) will be obtained at Baseline, EOT, and EOS; venous blood gas (VBG) or HCO_3 (bicarbonate) levels may be substituted for arterial blood gas (ABG) if it is SOC or in the patient's best interest, and results in comparable APACHE II and SOFA scores (Brown et al, 2016). Other questionnaires (Berlin Definition and modified Medical Research Council Dyspnea Scale) will be assessed at Baseline, Day 3, EOT, and EOS. Patients will be maintained on SOC, including prophylaxis or treatment for VTE, per institutional guidelines throughout the study. Dosing will end on Day 14, or at cure or increasing severity of ARDS, or at death. Patients will be followed clinically, and safety and efficacy measures will be recorded at end of study (EOS; Day 28) or death, whichever occurs first.

5.4 Selection of Study Population

5.4.1 Inclusion Criteria

Eligible patients must fulfill the following inclusion criteria:

1. Male or female hospitalized patients between 18 and 80 years of age at time of consent.
2. Clinical and laboratory diagnosis of COVID-19 infection. Patients must be positive for the SARS-CoV-2 by Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel or have an existing complication secondary to SARS-CoV-2 infection which was positive within 2 weeks of entry into the study. Further, patients must have at least <u>two of the following three</u> symptoms:
   - Fever (oral temperature ≥ 100.4 °F [> 38 °C]) with or without chills
   - Dyspnea or difficulty breathing (≥ 2 on mMRC dyspnea scale)
   - Nonproductive cough (Stokes *et al*, 2020),
   - Or other signs and symptoms of established complications to SARS-CoV-2 infection (*e.g.*, coagulopathy, cardiomyopathy, acute kidney injury [AKI], and/or acute liver injury) within the limits of Exclusion Criteria #8.
3. Patients must present with moderate to severe illness as defined below:
   - <u>Moderate illness</u>: Patients who have evidence of lower respiratory disease by clinical assessment or imaging and an oxygen saturation (SpO_2) > 93% on room air at sea level
   - <u>Severe illness</u>: Patients who have a respiratory frequency > 30 breaths per minute (bpm), $SpO_2 \leq 93\%$ on room air at sea level, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300, or lung infiltrates > 50% (COVID-19 Treatment Guidelines Panel, 2020).
4. APACHE II score < 20 or establishment of survivability of the patient beyond 48 hours

following randomization.

5. Therapies which have been shown to be beneficial and are included in standard-of-care COVID-19 treatment guidelines (*e.g.*, those of WHO or NIH, or institutional guidelines) are permitted.

6. Sexually active women of child-bearing potential (WCBP) must be using a medically acceptable method of birth control throughout the study and for at least 1 day following the end of study, and have a negative urine pregnancy test at the Screening visit. A WCBP is defined as a female who is biologically capable of becoming pregnant. A medically acceptable method of birth control includes intrauterine devices in place for at least 3 months, surgical sterilization, or the implant (ACOG, 2020). In patients who are not sexually active, abstinence is an acceptable form of birth control and urine will be tested per protocol. Women who are of nonchild-bearing potential, i.e., post-menopause, must have this condition captured in their medical history. Pregnant women and nursing mothers are excluded from this study.

7. Patient or Legally Authorized Representative (LAR) is available and willing to give written informed consent, after being properly informed of the nature and risks of the study and prior to engaging in any study-related procedures.

5.4.2 Exclusion Criteria

The presence of any of the following excludes a patient from study enrollment:

1. Known sensitivity, allergy, or previous exposure to LSALT peptide.
2. Exposure to any investigational drug or device <90 days prior to entry into study.
3. Treatment with immunomodulators or immunosuppressant drugs, including but not limited to IL-6 inhibitors, TNF inhibitors, anti-IL-1 immunomodulators, and JAK inhibitors within five half-lives or 30 days (whichever is longer) prior to randomization and throughout the study period. However, should any of these treatments become SOC and incorporated into clinical treatment guidelines (*e.g.*, those of WHO or NIH, or institutional guidelines), the treatment is permitted. Further, low-dose oral prednisone (≤ 20 mg/day) and inhaled steroids (*e.g.*, for treatment of asthma) are allowed in the study.
4. Anticipated transfer to another hospital or medical center within 72 hours, which is not a study site.
5. Uncontrolled or poorly-treated active hepatitis B (HBV), hepatitis C (HepC), or HIV infection. Those subjects who are positive for HBV, HepC, or HIV but are well-controlled with low viral loads are allowed to participate in the study:
    - HBV low viral load defined as <20,000 IU/mL
    - HepC low viral load defined as <800,000 IU/mL
    - HIV low viral load defined as <5000 copies/mL.
6. Participation in another drug or device study at any time during this study, for example:
    - Ulinastatin 200,000 IU or greater (ClinicalTrials.org NCT03089957)
    - High dose intravenous Vitamin C (ClinicalTrials.org NCT042645333)

- Budesonide and formoterol (Festic et al, 2017)
- Bevacizumab to prevent ARDS (NCT01314066)
- Dornase alfa to reduce hypoxemia in ventilated trauma patients (Pottecher *et al*, 2020).

7. As indicated in the inclusion criteria, pregnant female patients are excluded from study. Further, female patients who are nursing are excluded from study.
8. Has any medical condition considered to be clinically significant and could potentially affect patient safety or study outcome, including but not limited to:

    - Acute kidney injury (Thomas *et al*, 2015) or chronic kidney disease (stage-4 and stage-5 renal impairment [Wongrakpanich *et al*, 2017; Agarwal, 2016]; hemodialysis or eGFR < 30 mL/min/1.73 m^2; [CKD-EPI equation; Levey *et al*, 2009)
    - End-stage malignancy undergoing treatment
    - Immunocompromised patients or those with medical/surgical conditions (*e.g.*, solid organ transplantation) which require chronic immunosuppression
    - Chronic hematologic disease which, in the opinion of the PI, prohibits the patient from entering into study
    - Acute liver injury with AST and/or ALT levels greater than 3x ULN, unless recent injury (within 2 weeks) likely due to COVID-19 infection
    - History of coagulopathy of unknown etiology, as defined by abnormal ACT (only if part of SOC), aPTT, and/or PT/INR values at least 2-fold outside normal limits, and currently present at screening, and/or
    - End-stage lung disease, acute lung injury, very severe chronic obstructive pulmonary disease (COPD) as assessed by the GOLD criteria (GOLD Stage IV; Vogelmeier *et al*, 2017), or mechanical ventilation.

5.4.3 Removal of Patient from Therapy

Reasons for permanent discontinuation of treatment include the following:

- Patient experiences two or more Grade 2 toxicities or one or more Grade 3 or 4 toxicity considered by the PI to be associated with LSALT peptide treatment (CTCAE v5.0, 2017)
- Patient requests to discontinue treatment or patient withdrawal of consent
- PI considers that it is not in the best interest of the patient to continue treatment due to an adverse event.
- Protocol deviation requiring discontinuation of study treatment for safety reasons, and/or
- Pregnancy.

Patients who discontinue treatment for any reason (except withdrawal of consent from study participation) will not be discontinued from the study and will continue to be followed for all

assessments until Day 28, or if not possible then for all assessments necessary to determine the value of the primary endpoint at Day 28.

5.4.3.1 Procedures for Early Termination of Treatment

For any discontinuation, the PI will obtain all the required details and document the date and the main reason for the premature termination. If the reason for discontinuation is an AE, the specific event or the main laboratory abnormality will be recorded in the electronic case report form (eCRF). The PI will make thorough efforts to document the outcome. The PI will attempt to continue to follow the patient for the full duration of the study and continue planned assessments. If circumstances prevent the patient from completing all scheduled evaluations, every attempt will be made to complete all procedures listed in Section 8.2.5.

6 STUDY PRODUCT

6.1 Study Medication Supply

LSALT peptide and matching placebo will be sent to the sites per the randomization schemata. Drug receipt/administration logs and temperature logs will be maintained by the study site using the Drug Accountability Form. The unblinded Pharmacist/designate will ensure that the total number of vials in the drug dispatch log is present in the shipment from the Sponsor. The Pharmacist/designate will also ensure that there is no evidence of breakage of the drug vials. The Sponsor/designate must be notified immediately if any discrepancy is identified in the drug or temperature log or if any breakage occurs.

6.1.1 Description and Formulation of Study Product

LSALT peptide will be supplied to the study site in aqueous solution at -20°C as a single concentration (1 mg/mL) in a glass vial with restricted access to the site Pharmacist or designate. The LSALT peptide vials will be labeled in English or the local language in the country of study and according to local regulations (Appendix 1).

6.1.2 Study Drug Dosage and Preparation

LSALT peptide or an equivalent volume of 0.9% sodium chloride (NaCl) will be diluted in 0.9% sodium chloride solution to a volume of 100 mL mini-IV bag by an unblinded Pharmacist or designate. The drug will be infused into the patient through a peripheral 18, 20, or 22 g intravenous catheter or through a central line over 2 hours and under the supervision of the study investigator.

6.1.3 Pharmacy records

The unblinded Pharmacist or designate will be responsible for ensuring that the study site maintains an accurate record of the LSALT peptide inventory using the Drug Accountability Form. A coded label will be affixed to the patient eCRF to identify placebo and LSALT peptide-treated subjects.

6.1.4 Conditions for Storage and Use

All study medication will be stored in the research pharmacy prior to dispensing, or in a locked freezer accessible only to the pharmacy team. Study drug (LSALT peptide and commercially-available drug-free saline) should be stored frozen at -20°C (-4°F) in a temperature-monitored

freezer; a record of daily temperatures during the study will be reviewed by the Sponsor or delegate. Refer to the Pharmacy Manual on specifics of storage and other related information.

6.2 Study Drug Administration

The dose will be one of the following for each patient:

A. 100 mL of 5 mg IV LSALT peptide infusion over 2 hours once daily

B. 100 mL drug-free IV saline infusion over 2 hours once daily.

The frozen study drugs (LSALT peptide or saline) will be warmed to room temperature by the unblinded Pharmacist before dispensing to the investigators for adding to the infusion bags. As warming time may vary, every effort should be made to allow ample time to the unblinded Pharmacist for the completion of the warming procedure. Post-preparation, the drug product and placebo will be stored for no longer than 4 hours at room temperature (68°F/20°C) or no longer than 24 hours when refrigerated (35°F/2°C) prior to administration to the patient to minimize the risk of excessive growth of adventitious microbial contamination. Times of post-preparation to infusion and storage temperatures will be documented in the pharmacy records and eCRF.

Study drug will be administered once daily at a consistent time throughout the study. A missed dose of study medication can be administered up to 8 hours before the next scheduled dose; otherwise, the missed dose should not be administered to the patient and the deviation should be noted on the eCRF.

6.2.1 Patient Numbering

Each patient will be assigned a unique 6-digit patient number by the study staff. The patient number will consist of a 3-digit clinical investigational site number assigned by the Sponsor, followed by a 3-digit patient number (*e.g.*, 001) assigned by the study staff.

The clinical site and/or pharmacy, if applicable, is responsible for maintaining a current log of patient number assignments and bottle numbers of the investigational product administered to each patient. The patient's unique patient number is required to be entered on all clinical investigation documentation (*i.e.*, eCRFs, labeling of clinical materials and samples containers, drug accountability logs, *etc*.).

6.2.2 Method of Assigning Patients to Treatment Groups

This is a double-blind, placebo-controlled study. The actual treatment given to individual patients is determined by a randomization schedule. A fixed block randomization schedule will be prepared to assign patients to 1 of 2 treatments: LSALT peptide 5 mg once daily or matching placebo in a 1:1 allocation ratio.

6.2.3 Dispensing, Compliance and Accountability

The study drug must be used only as directed in the protocol. The Pharmacist/delegate will keep accurate records of the quantities of the study drug dispensed/distributed, used, and returned by each patient in the eCRF system.

The Sponsor or delegate will provide drug product accountability forms to assist the Pharmacist/delegate, when applicable, in maintaining current and accurate inventory records

covering receipt, dispensing, and the return of study drug supplies. When a shipment is received, the Pharmacist will verify the quantities received and return the acknowledgment to the Arch Biopartners Clinical Trial Material (CTM) Coordinator. The drug product will not be used without Arch Biopartners or designate approval in writing. The unblinded Pharmacist will record study drug accountability which includes the coded identification of the patient to whom the study drug is dispensed, the quantity and the date of dispensing, and any returned or unused study drug, as well as a full record of CTM storage temperatures. This record is in addition to any study drug accountability information recorded on the eCRF. These records will be readily available for inspection by an unblinded monitor and/or Arch Biopartners audits and are open to regulatory authority inspection at any time.

Noncompliance is defined as receiving less than 80% or more than 120% of study drug during the study period. Noncompliance should be discussed with the Project Manager, or delegate, and is to be noted both in the source documentation and in the eCRF.

At the end of the study, all unused study drug will be destroyed on site according to site procedures. If the site does not have procedures in place for on-site destruction, the study drug will be returned to the Arch Biopartners CTM Coordinator or designate for destruction.

Based on entries in the site electronic study drug accountability forms, it must be possible to reconcile study drug delivered with those used and returned. All study drug must be accounted for and all discrepancies investigated and documented appropriately.

The unblinded Pharmacist is responsible for maintaining accountability for the receipt, dispensing, and return of all study medication to the clinic.

6.3 Prior and Concomitant Therapy

Medications having the potential to interfere with the evaluation of efficacy or safety are excluded throughout the trial (review Sections 5.4.1 Inclusion criteria and 5.4.2 Exclusion criteria). Current therapies which have been shown to be efficacious and are included in standard COVID-19 treatment guidelines (*e.g.*, WHO or US NIH, institutional guidelines) are permitted if not part of another investigational study. All concomitant medication usage needs to be documented in the eCRF.

7 EFFICACY AND SAFETY ASSESSMENTS

7.1 Efficacy Assessments

7.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen [≥ 6 L/minute], or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR on Day 28 <10 mL/min/1.73 m^2.

7.1.2 Secondary Efficacy Endpoints

- All-cause mortality

- The presence and severity of ADRS as an ordinal outcome of the proportion of patients who have none, mild, moderate, or severe ARDS
- Time to each of mild, moderate, and severe ARDS
- The number of ventilation-free days and ECMO-free days
- Time on nasal canula or oxygen mask
- Length of stay in ICU and hospital (admission to discharge)
- Virologic clearance rate
- Worst PaO_2/FiO_2 ratio following enrollment
- Change in PaO_2/FiO_2 ratio
- Vasopressor-free days
- Change from maximal radiographic damage to EOT
- Change in baseline mMRC score
- Change in APACHE II score
- Proportion of patients with extrapulmonary organ dysfunction using the daily SOFA score
- Change in liver function tests (ALT, AST, total bilirubin)
- Change in renal function tests (SCr, eGFR)
- Change in hs-troponin levels
- Change in ACT, aPTT, and/or PT/INR values
- Change in antiviral IgG, IgA, and IgM levels.

7.1.3 Health Outcomes endpoint

- Total healthcare costs from admission to discharge between treatment groups.

7.1.4 Exploratory Endpoints

- Change in serum cytokines including IL-1α, IL-1ß, and ferritin levels, as well as other exploratory biomarkers drawn at the same time as LSALT peptide concentrations.
- Change in baseline antiviral immunoglobulins (IgG, IgM, and IgA) at EOS.

Additional measures may be added. Data will be analyzed for the modified intent-to-treat (mITT) and per protocol (PP) populations.

7.2 Pharmacokinetic Variables

Blood samples for determination of LSALT peptide concentrations and related metabolites (degradation products) and cytokines will be obtained at Baseline prior to initiation of drug, and at the mid-point of the 2-hour drug infusion and immediately following the end of the 2-hour infusion on Days 1, 3, and EOT A single blood sample will be obtained at EOS for measurement of cytokines/biomarkers only.

7.3 Safety Assessment

Safety will be assessed by changes from Baseline in:

- TEAEs
- Clinical laboratory tests (Appendix 2)
- Vital signs including pulse rate, blood pressure, body temperature, respiration rate, body weight
- Physical examinations
- 12-lead ECG
- Radiologic findings.

Additional assessments may be conducted as considered appropriate. Clinical labs, vital signs, ECG measurements, and radiologic findings may be repeated if needed to corroborate or refute abnormal findings. Both the original and replicate assessments should be recorded in the eCRF.

7.3.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient participating in a clinical trial. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the study medication, whether or not considered related to the study medication. AEs will be collected from the initiation of treatment until 30 days following the final dose. Pre-existing known clinically significant conditions observed at screening should be recorded as medical history.

This definition also includes accidental injuries, reasons for any change in medication (drug and/or dose), reasons for re-admission to a hospital, or reasons for surgical procedures (unless for minor elective surgery for a pre-existing condition). It also includes adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the study medication. Any laboratory abnormality assessed as clinically significant by the PI must be recorded as an adverse event.

A treatment-emergent adverse event (TEAE) is also defined as any adverse event occurring after start of study medication and within the time of residual drug effect (30 days after the last administration of the study medication), or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of study medication and within the time of residual drug effect.

Adverse events should be recorded as diagnoses, if available. If not, separate sign(s) and symptom(s) are recorded. One diagnosis/symptom should be entered per record.

Note that death is not an adverse event but the cause of death is. An exception is the adverse event of sudden death of unknown cause. Note that hospitalization is not an adverse event; however, the reason for hospitalization is. Procedures are not adverse events; the reasons for conducting the procedures are. In general, only the reason for conducting the procedure will be captured as an adverse event. However, if deemed necessary by the PI, a procedure can be captured along with the reason for conducting the procedure.

An overdose or medication error is not an adverse event unless it is temporally associated with an unfavorable or unintended sign or symptom.

While pregnancy in and of itself is not a SAE, for the purposes of this trial, should a pregnancy occur in a subject or subject's partner, it should be reported on a Pregnancy Reporting form within 24 hrs of being aware and be followed until an outcome is received.

7.3.1.1 Serious Adverse Events

Each AE is to be classified by the PI as serious or non-serious. A serious adverse event (SAE) is any untoward medical occurrence or effect that occurs at any dose:

- Results in death
- Is life-threatening (*i.e.,* an immediate risk of death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is associated with a congenital anomaly/birth defect
- Is an important medical event.

An adverse event caused by an overdose or medication error is considered serious if a criterion listed in the definition above is fulfilled.

Important adverse events that may not result in death, may not be life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient's safety or may require medical or surgical intervention to prevent one of the outcomes listed above.

Serious adverse events also include any other event that the PI or Sponsor judges to be serious or which is defined as serious by the regulatory agency.

The PI is to report all directly observed adverse events and all adverse events spontaneously reported by the trial patient using concise medical terminology.

7.3.1.2 Procedures for Assessing, Recording, and Reporting Adverse Events and Serious Adverse Events

Throughout the duration of the study, the PI will closely monitor each patient for evidence of drug intolerance and for the development of clinical or laboratory evidence of adverse events. All AEs (expected or unexpected) which occur during the course of the study, whether observed by the PI or by the patient, and whether or not thought to be drug-related, will be reported and followed until resolution or until they become stable.

The description of the AE will include description of the event, start date, stop date, intensity, if it was serious, relationship to test drug, change in test drug dosage, if the patient died, and if treatment was required. Events will be recorded as one of the following severity/causality categories below (CTCAE v5.0):

Severity	Definition
Mild (Grade 1)	Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
Moderate (Grade 2)	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (*e.g.*, meal preparation, shopping, using telephone, managing money, *etc.*)
Severe (Grade 3)	Medically significant but not immediately life-threatening; prolongation of hospitalization indicated; disabling; limited self-care (*e.g.*, bathing, dressing, feeding self, using toilet, *etc.*)
Very severe (Grade 4)	Life-threatening consequences; urgent intervention indicated
Death (Grade 5)	Death related to AE

Category	Definition
Unrelated	Clearly and incontrovertibly due only to extraneous causes and does not meet criteria listed under possible or probable.
Unlikely	Does not follow a reasonable temporal sequence from administration. May have been produced by the patient's clinical state or by environmental factors or other therapies administered.
Possible	Follows a reasonable temporal sequence from administration but may have been also produced by the patient's clinical state, environmental factors or other therapies administered.
Probable	Clear-cut temporal association with administration with improvement on cessation of investigational medicinal product or reduction in dose. Reappears upon rechallenge. Follows a known pattern of response to the investigational medicinal product.
Definitely	There is evidence of exposure to the test product, for example, reliable history or acceptable compliance assessment; the temporal sequence of the AE onset relative to the drug is reasonable; the AE is most likely to be explained by the drug treatment than by another cause; the challenge is positive; re-challenge (if feasible) is positive; the AE shows a pattern consistent with previous knowledge of the drug treatment.

Adverse events with the causality assessed as possible, probable, and definitely are categorized as related to study medication and are called adverse drug reactions.

7.3.1.2.1 Serious Adverse Events (SAE) Reporting

Any SAE will be reported to the Sponsor or designate *via* telephone, fax, e-mail, or in-person, within 24 hours of knowledge by the Investigator, and then in writing as soon as possible, but no later than 7 calendar days after first knowledge of the SAE.

The notification must be directed to the following for North American sites:

Peter Polos, MD

Peter.Polos@SyneosHealth.com
(908) 963-7783

and

SafetyReporting@SyneosHealth.com or
FAX: (877) 464-7787

For Turkish sites, the following should be contacted:

Yaprak Çalğar, MD
YaprakC@MonitorCRO.com
90 (545) 261 19 75

and

ab002pharmacovigilance@monitorcro.com.

7.3.1.2.2 Fatal or Life-threatening Serious, Unexpected Adverse Drug Reactions

The Sponsor or designate is responsible for notifying regulatory agencies of fatal or life-threatening serious, unexpected adverse drug reactions (by telephone, facsimile transmission or in writing) as soon as possible, but no later than 7 calendar days after becoming aware of the information. Additionally, within 8 days after having informed the agency(ies), a complete report must be submitted, including an assessment of the importance and implication of any findings. Syneos Health Canada will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

It is the responsibility of the clinical site to report as soon as possible, but no later than 7 calendar days after first knowledge by the Investigator, fatal or life-threatening serious, unexpected adverse drug reactions to the Independent Ethics Committee (IEC) responsible for the study.

7.3.1.2.3 Other Suspected, Unexpected, Serious Adverse Drug Reactions (SUSAR)

The Sponsor is responsible for notifying regulatory agencies of all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening as soon as possible, but no later than 15 calendar days after becoming aware of the information. Syneos Health Canada will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

7.3.2 Other Assessments

7.3.2.1 Clinical Laboratory Tests (Appendix 2)

- Hematology Complete blood count: total white blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), total red blood cells (RBC, hemoglobin [Hgb], hematocrit, mean corpuscular volume, mean corpuscular Hgb, and mean corpuscular Hgb concentration), and platelets.

- Biochemistry Sodium, potassium, glucose, blood urea nitrogen, chloride, creatinine and eGFR, calcium, bilirubin (total), alkaline phosphatase, aspartate transaminase, alanine

transaminase, gamma-glutamyl transferase, total protein, albumin, and carbon dioxide (CO_2).

- Coagulation Activated Partial Thromboplastin Time (aPTT), Prothrombin time (PT), International Normalized Ratio (INR), and activated clotting (or coagulation) time (ACT), the latter only if part of SOC at the investigational site.

- Urinalysis Dipstick for presence of blood, glucose, ketones, and protein; pH, color (if applicable), transparency, specific gravity, and urobilinogen.

Blood and urine samples will be obtained at Screening, and prior to the drug infusion on Days 3, EOT, and at EOS, and as clinically indicated for evaluation of blood chemistries, hematology, coagulation, and urinalysis.

7.3.2.2 LSALT Peptide and Metabolite Pharmacokinetics

Blood samples will be drawn at Baseline prior to initiation of drug administration, at the mid-point (1 hour) of the 2-hour infusion and immediately following the end of the 2-hour infusion on Days 1, 3, and at EOT for measurement of LSALT peptide and metabolite(s) analysis (See Section 8.1 Study Flow Chart). The time of the blood sample collection and the time of the most recent dose prior to the blood sample collection will be recorded accurately on the eCRF. Samples of blood will be centrifuged to obtain plasma; refer to the Laboratory Manual for Pharmacokinetic Sample Collection.

7.3.2.3 Measurement of Cytokines and Other Biomarkers

Blood samples will be drawn at Baseline prior to initiation of drug administration, at the mid-point (1 hour) of the 2-hour infusion and immediately following the end of the 2-hour infusion on Days 1, 3, EOT, and a single blood draw at EOS for measurement of cytokines and other biomarkers (See Section 8.1 Study Flow Chart). Samples will be analyzed for the following biomarkers: IL-1α, IL-1β, hs-troponin, and ferritin levels, as well as other exploratory biomarkers. Please refer to the Laboratory Manual for more specific instructions.

7.3.2.4 Cardiovascular Assessments

Vital signs including supine pulse rate and blood pressure will be measured at Screening, and on Days 3, EOT, and EOS. ECGs (12-lead) will be recorded at Baseline pre-dose, Day 3, EOT, and at EOS (if clinically indicated). Highly-sensitive troponin levels will be compared from baseline and between treatment groups throughout the study (refer above to Section 7.3.2.3).

7.3.2.5 Pulmonary Assessments

Chest x-rays will be obtained at Baseline, Day 3, at time of clinical improvement, at EOT, at EOS, and where clinically indicated. All x-rays will be recorded in the eCRF. Chest CT will be obtained as per clinical practice at the study site.

8 STUDY PROCEDURES AND FLOW CHART

8.1 Study Flow Chart

Calendar	Admission[a]	Admission[b]	Treatment Period	Treatment Period	EOT	EOS
Day	1	1	1 – 14 (max)	3	Variable	28±3
Sign informed consent	X					
Assign screening number	X					
Inclusion/exclusion criteria	X					
Review demographics	X					
Review medical history	X					
Concomitant meds[c]	X		X	X	X	X
Physical exam[c]	X		X	X	X	X
Respiratory exam[c]	X		X	X	X	X
Urine pregnancy in WCBP	X					
Clinical labs/Urine tests[c,d]	X			X	X	X
12-lead ECG[e]	X			X	X	X
Vital signs[c,f]	X		X	X	X	X
eGFR[b,d]	X			X	X	X
SARS-CoV-2 testing swabs[c,g]	X			X	X	X
Chest x-ray[h]		X		X	X	X
PK and biomarker samples[i]		X		X	X	X
Randomization		X				
Study drug administration		X	X	X	X	
AE assessments		X	X	X	X	X
SARS-CoV-2 IgG, IgA, IgM levels		X				X
Berlin Definition[j,k]		X		X	X	X
SOFA score[j,l]		X		X	X	X
mMRC score[j,m]		X		X	X	X
APACHE II score[j,n]		X		X	X	X
Health Outcomes						X

[a] Screening to determine eligibility (prior to randomization)
[b] Baseline after Screening and prior to the start of study drug unless otherwise indicated.
[c] Performed at Screening, Day 3, and EOT prior to drug administration, and at EOS.
[d] Hematology, biochemistry, coagulation, and urinalysis will be obtained and eGFR will be calculated (Appendix 2), and when clinically indicated.
[e] 12-Lead ECG (supine) will be recorded at time 0 (pre-dose) at Screening, Day 3, EOT, and at EOS (only if clinically indicated). Also, an ECG will be obtained during the study when clinically indicated.
[f] Blood pressure (supine), pulse rate (supine), body temperature, respiration rate (supine), actual body weight (kg), and height (cm). Note: Height will only be measured at Screening.
[g] Swabs (nasal, throat, sputum, or lower respiratory tract) at Screening, Day 3, EOT, and EOS.
[h] Chest x-rays at Baseline (Day 1), Day 3, at time of clinical improvement, at EOT, and EOS. A chest x-ray will also be obtained if clinically warranted and recorded in the eCRF.
[i] Blood draws at Baseline pre-dose, at the mid-point (1 hr) and at the end of 2-hr infusion of study drug on Days 1, 3, and EOT, and a single blood draw at EOS.
[j] Obtained at Baseline, Day 3, when clinically indicated, at EOT, and at EOS.
[k] Appendix 3
[l] Appendix 4
[m] Appendix 5
[n] Appendix 6

8.2 Study Visits

8.2.1 Screening (Day 1a)

- Obtain written informed consent from the patient
- Assign screening number
- Review Inclusion/Exclusion criteria with the patient
- Collect demographics and medical history
- Document concomitant drugs, including drug name, dose, and frequency
- Urine pregnancy test for female patients of child-bearing potential
- Perform physical examination including respiratory exam
- Laboratory tests including hematology, biochemistry, coagulation, and urinalysis
- 12-lead ECG
- Measure vital signs, including blood pressure (sitting), pulse rate, body temperature, respiration rate, actual body weight (kg), and height (cm)
- Swab nasal, throat, sputum, or lower respiratory tract source for SARS-CoV-2.
- Calculate eGFR

8.2.2 Baseline Visit (Day 1b)

- Obtain chest x-ray
- Collect blood samples (5 mL each) at Baseline prior to start of drug infusion, at one hour post start of infusion, and end of the daily 2-hour drug infusion for PK, cytokines, and antibody analysis. Prepare the samples per Section 7.3.2.2.
- Review and record any TEAEs
- Complete the (1) Berlin Definition (Appendix 3), (2) SOFA score (Appendix 4), (3) modified Medical Research Council Dyspnea Scale (Appendix 5), and (4) APACHE II score (Appendix 6) pre-dose.
- Randomize the patient
- Administer IV drug over 2 hours (after completion of all other assessments and procedures on Day 1)

8.2.3 During Treatment Period (Days 1, 2, 4 – 14 (maximum))

- Document concomitant drugs, including drug name, dose, and frequency

- Perform physical examination including respiratory exam
- Measure vital signs, including blood pressure (sitting), pulse rate, body temperature, respiration rate, and actual body weight (kg) daily or as clinically indicated – note: no need to document height.
- Administer IV drug over 2 hours
- Review and record any TEAEs.

8.2.4 During Treatment Period (Day 3 and EOT, and when clinically indicated)

- Document concomitant drugs, including drug name, dose, and frequency
- Perform physical examination including respiratory exam
- Obtain chest x-ray on Day 3, in the event of clinical improvement, and at EOT, and when clinically indicated
- 12-lead ECG
- Laboratory tests including hematology, biochemistry, coagulation, and urinalysis on Day 3, EOT, and if clinically indicated
- Measure vital signs, including blood pressure (sitting), pulse rate, body temperature, respiration rate, and actual body weight (kg) daily or as clinically indicated – note: no need to document height.
- Calculate eGFR
- Administer IV drug over 2 hours
- Collect blood samples (5 mL each) at mid-point and end of the 2-hour drug infusion for PK and biomarker analysis. Prepare the samples per Section 7.3.2.2.
- Review and record any TEAEs
- Swab nasal, throat, sputum, or lower respiratory tract source for SARS-CoV-2 on Days 4, 7, 10, and 14 (if necessary).
- Complete the (1) Berlin Definition (Appendix 3), (2) SOFA score (Appendix 4), and (3) modified Medical Research Council Dyspnea Scale (Appendix 5), and (4) APACHE II score (Appendix 6; only if clinically indicated).

8.2.5 End of Treatment (EOT)

The EOT visit refers to the completion of the last dose of study drug. All study assessments outlined during the Treatment Period are to be performed. For a complete list of assessments, refer to the Study Flow Chart.

8.2.6 End of Study (Day 28 EOS ± 3 days)

- Document concomitant drugs, including drug name, dose, and frequency
- Perform physical examination including respiratory exam
- Laboratory tests including hematology, biochemistry, coagulation, and urinalysis, and antiviral antibodies (IgG, IgM, IgA)
- Collect blood sample in the morning for biomarker analyses. Prepare the samples per Section 7.3.2.2.
- 12-lead ECG (if clinically indicated)
- Obtain chest x-ray
- Measure vital signs, including blood pressure (sitting), pulse rate, body temperature, respiration rate, and actual body weight (kg)
- Calculate eGFR
- Review and record any TEAEs
- Swab nasal, throat, sputum, or lower respiratory tract source for SARS-CoV-2 if not eradicated on Day 14.
- Complete the (1) Berlin Definition (Appendix 3), (2) SOFA score (Appendix 4), and (3) modified Medical Research Council Dyspnea Scale (Appendix 5), and (4) APACHE II score (Appendix 6) on Day 28 (EOS).
- Record healthcare resource utilization.

8.2.7 Premature Withdrawal / Early Termination Visit

All attempts should be made to perform all the tests for Day 28 at the Withdrawal/Early Termination visit for patients who may withdraw consent for further participation in the study before completion.

8.2.8 Unscheduled Visits

If a patient returns to the site prior to their next scheduled study visit for assessment of an adverse event (*e.g.*, before Day 28), or at the request of the Investigator, the Unscheduled Visit forms of the eCRF should be completed. Procedures and examinations conducted at the Unscheduled Visit

are at the discretion of the Investigator. If the patient withdraws consent for further participation at the Unscheduled Visit, the eCRF forms for the Premature Withdrawal / Early Termination Visit, and the End of Study Form should be completed rather than the Unscheduled Visit form.

8.2.9 Appropriateness of Measurements

Prevention of ARDS will reduce morbidity and mortality in infected patients, amongst others.

8.2.10 Data Quality Assurance

Steps to be taken to assure the accuracy and reliability of data include the selection of qualified PIs and appropriate study centers, review of protocol procedures with the PI and associated personnel prior to the study, and periodic monitoring and site audits by the Sponsor or their designee. The data will be entered into the clinical trial database and verified for accuracy.

9 STATISTICAL METHODS PLANNED AND SAMPLE SIZE

9.1 Determination of Sample Size

The sample size (n=60) was determined based on medical judgement and a desire to assess safety in a limited number of patients, prior to further study and patient availability: 30 patients (active) and 30 patients (placebo).

9.2 Patient Populations

9.2.1 Full Analysis Set (FAS)

The Full Analysis Set is defined as patients who received any amount of at least one dose of study medication, with assignment to treatment as randomized.

9.2.2 Per Protocol (PP) Population

The Per Protocol population is defined as patients who meet all inclusion/exclusion criteria, were within the acceptable compliance range (≥80% and ≤120%), and completed the study with no major protocol violations. The Per Protocol population will be finalized prior to breaking the blind for final reporting.

9.2.3 Safety Population

The safety population is defined as all patients who received at least one dose (partial or complete) of the study medication, assigned to treatment according to the treatment actually received.

9.3 Statistical Methods

In general, continuous variables will be summarized with descriptive statistics (the number of non-missing values [n], mean, median, standard deviation [SD], minimum, and maximum). All categorical variables will be summarized with frequency counts and percentages, as applicable.

A detailed statistical analysis plan (SAP) will be developed which will describe all analyses to be performed. The SAP will be finalized prior to database lock and unblinding.

9.3.1 Demographic, Medical, and Disease History

All baseline characteristics, demographics, and medical history (by body system) will be summarized by treatment within each age cohort as well as overall. For continuous variables, n, mean, standard deviation, median, minimum, and maximum will be presented. For categorical variables, frequency counts and percentages will be presented. No formal statistical analysis methods will be employed.

Summary and/or data listings of the prior, concomitant medications, in particular AEDs, and class of medication will be provided.

9.3.2 Efficacy Analyses

The primary efficacy endpoint is the proportion of subjects alive and free of respiratory failure (*e.g.*, need for non-invasive or invasive mechanical ventilation, high flow oxygen (≥ 6 L/minute), or ECMO) and free of the need for continued renal replacement therapy (RRT) on Day 28. The need for continued RRT at Day 28 will be defined as either dialysis in the past 3 days (Day 26, 27, or 28) or an eGFR on Day 28 <10 mL/min/1.73 m^2.

Analyses for the primary and the key secondary endpoints will be performed on the FAS and PP populations, with the analysis on the FAS as the primary analysis; all other efficacy endpoints will be analyzed on the FAS population only.

Time to event variables will be summarized using Kaplan-Meier survival curves and compared between treatment groups using either Log-rank tests or Cox's Proportional Hazard model adjusting for baseline covariates, as necessary. For continuous variables assessed at multiple time points a Mixed Model Repeated Measures (MMRM) approach will be used or Analysis of Covariance (ANCOVA) if assessed at a single time point post-baseline. For categorical endpoints Cochran-Mantel-Haenszel (CMH) methods will be used, or a logistic regression approach, in the case of continuous covariates.

Additional details regarding precise endpoint definitions (including transformations, as applicable), analysis model specifications and alpha-level control for primary and important secondary endpoints associated with multiplicity will be provided in the SAP, to be finalized prior to unblinding the study.

9.3.3 Pharmacokinetic Analysis

All plasma concentration data will be summarized using N, mean, SD, % coefficient of variation, median, minimum, and maximum by treatment and cohort. Attempts will be made to correlate LSALT peptide concentrations with cytokine levels.

9.3.4 Safety Analysis

9.3.4.1 Adverse Events

All safety analyses will be conducted on all patients who are in the Safety Population. AEs will be coded to system organ class and preferred term using MedDRA version 20.0 or higher. All AEs occurring after the initiation of the study treatment (TEAEs) will be collected, including events present at baseline that worsened during the study.

AEs will be summarized by treatment group to provide comparison among the treatment groups with respect to incidence of AEs (the number of patients reporting at least one episode of a specific AE), incidence of AEs by severity within body system, incidence of AEs by attribution within body system, and incidence of AEs causing withdrawal and incidence of SAEs. Regarding severity and attribution summaries, the most extreme outcome (highest severity and closest to study drug related) will be used for those patients who experience the same AE on more than one occasion.

Written narratives will be provided for all serious, unexpected, or other significant AEs that are judged to be of special interest because of their clinical importance.

9.3.4.2 Clinical Laboratory

Clinical Laboratory examination will be performed prior to treatment and during treatment.

Descriptive statistics will be presented for the observed values and change from baseline of each of the clinical laboratory results by treatment and visit. Shift tables describing abnormality shifts from baseline to after treatment and follow-up (Day 28) will be created.

9.3.4.3 Vital Signs, ECG, and Chest X-rays

Vital signs, 12-lead ECG parameters, and chest x-rays will be summarized using descriptive statistics by treatment including changes from baseline.

9.3.5 Interim Analysis

No interim analysis apart from that provided to the DSMB for the purpose of ensuring safety of patients will be conducted.

9.3.6 Study Stopping Rules

The progress of the study will be monitored by the DSMB on an ongoing basis according to the DSMB Charter with meetings at monthly intervals or more frequently dependent upon the rate of enrollment. These meetings will include an assessment of differences in the number of subjects with an unfavorable outcome for the primary endpoint, as well as observed imbalances in severity or risk factors and the totality of data, including adverse event profiles not associated with an unfavorable primary endpoint value. Consideration will also be given to the limited information available in the early stages of the study and a potential for unfavorable trends with very few subjects due to chance alone.

9.3.6.1 Safety Indices

- The observed adverse event profile of the patients on the treatment arm exceeding the incidence and/or severity of adverse events observed in the placebo arm to a significant degree at any time during the study.

- Primary endpoint: there will be no more than a 1-subject difference in the number of subjects with an unfavorable outcome for the primary endpoint after the first 6 subjects completed active treatment compared with placebo (in favor of placebo). Subsequently, for the total number of 18 subjects, there will be no more than a 2-subject difference in the primary endpoint (in favor of placebo) and no more than a 3-subject difference for the remaining subjects. Otherwise, the DSMB will consider stopping the study for safety reasons.

For the primary endpoint, each of these scenarios is considered unlikely in the presence of at least a modest relative reduction in the incidence rate of only 20 percent (*i.e.*, a rate of 22.4% for LSALT from an assumed incidence rate of 28% for placebo), with probabilities in the range of 6% or less for these unfavorable outcomes at the end of each cohort, under this assumption.

9.3.7 Missing Data

Although significant missing data with respect to the primary endpoint is not anticipated for a study of this duration (due to the nature of the endpoint), methods for handling missing data and intercurrent events, including for secondary endpoints will be specified in the SAP. These methods will be consistent with missing data due to death as having a worst possible outcome.

10 QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Source Data and Records

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, data sheets provided by the Sponsor or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The PI will permit study-related monitoring, audit(s), IRB review(s), and regulatory inspection(s), with direct access to all the required source records.

All study records will be retained for a period of time as defined by the regulatory authority for the country in which the investigation is conducted. Generally, this means at least 2 years following the date on which the drug is approved by the regulatory authority for marketing for the purposes that were the subject of the investigation. In other situations, (*e.g.*, where the investigation is not in support of or as part of an application for a research or marketing permit), a period of 2 years following the date on which the entire clinical program is completed, terminated, or discontinued or the investigational application under which the investigation is being conducted is terminated or withdrawn by the regulatory authorities.

In the event the PI retires, relocates, or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to any other person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor. The PI must contact the Sponsor prior to disposal of any records related to this study.

10.2 Reporting of Results

The electronic Case Report Form (eCRF) is an integral part of the study and subsequent reports. The eCRF must be used to capture all study data recorded in the patient's medical record. The eCRF must be kept current to reflect patient status during the course of the study.

The monitor is responsible for performing on-site monitoring at regular intervals throughout the study to verify adherence to the protocol; verify adherence to local regulations on the conduct of clinical research; and ensure completeness, accuracy, and consistency of the data entered in the eCRF.

Arch Biopartners or its designate will monitor completed eCRFs. An eCRF will also be completed for each screened patient.

All protocol-required information collected during the study must be entered by the PI, or designated representative, in the eCRF, an internet-based electronic data collection system. All details of the eCRF completion and correction will be explained to the PI and his staff. The management module of the eCRF includes edit check and query systems that seamlessly integrate with the data entry system. All modifications to the data in the eCRF are tracked by an electronic audit trail (date and identity of the person making the change are instantaneously recorded). The eCRF will be 21CFR Part 11 compliant.

If the PI authorizes other persons to make entries in the eCRF, the names, positions, and signatures of these persons must be supplied to the Sponsor.

The PI, or designated representative, should complete the eCRF as soon as possible after data are collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. By design, an explanation must be provided for all missing data, altered data, and/or out of range data.

The completed eCRF must be reviewed and signed by the PI named in the study protocol or by a designated sub-Investigator.

Final monitored and audited eCRFs will be provided by the Sponsor to the sites at the end of the study in the format of a PDF file.

10.3 Confidentiality of Patient Data

The PI will ensure that the confidentiality of the patients' data will be preserved. In the eCRF or any other documents submitted to the Sponsor, the patients will not be identified by their names, but by an identification system, which consists of a uniquely assigned number in the study. The PI will maintain documents not meant for submission to the Sponsor (*e.g.*, the confidential patient identification code and the signed informed consent forms) in strict confidence.

11 REPORTING AND PUBLICATION

11.1 Confidentiality of Study Data

Any information relating to the study drug or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The PI and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to Arch Biopartners.

12 REFERENCES

ACOG, 2020. Birth Control. American College of Obstetricians and Gynecologists. https://www.-acog.org/patient-resources/faqs/especially-for-teens/birth-control. Accessed 16-May-2020.

Agarwal R. Defining end-stage renal disease in clinical trials: a framework for adjudication. *Nephrol Dial Transplant*. 2016;31:864-7.

Ahmed F, Zviedrite N, Uzicanin A. Effectiveness of workplace social distancing measures in reducing influenza transmission: a systematic review. *BMC Public Health*. 2018;18:518. https://doi.org/10.1186/s12889-018-5446-1.

Albert RK. The role of ventilation-induced surfactant dysfunction and atelectasis in causing acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2012;185(7):702-8.

Alhazzani W, Møller MH, Arabi YM, *et al.* Surviving sepsis campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). *Intensive Care Med*. 2020;28:1-34.

American Lung Association, April 15, 2020. https://www.lung.org/lung-health-diseases/lung-disease-lookup/ards/ards-treatment-and-recovery. Accessed 24-Jan-2021.

Ashbaugh DG, Bigelow DB, Petty TI, Levine BE. *Lancet* 1967;2(7511):319-23. https://doi.org/10.2026/S0140-6736(67)90168-7.

Ayres JS. Surviving COVID-19: A disease tolerance perspective. *Sci Adv*. 2020. https://doi.org/10.1126/sciadv.abc1518.

Barnes BJ, Adrover JM, Baxter-Stoltzfus A, *et al*. Targeting potential drivers of COVID-19 : Neutrophil extracellular traps. *J Exp Med*. 2020;217(6). https://doi.org/10.1084/jem.20200652.

Batlle D, Soler MJ, Sparks MA, *et al*. Acute kidney injury in COVID-19: Emerging evidence of a distinct pathophysiology. *J Am Soc Nephrol*. 2020. https://doi.org/10.1681/ASN.2020040419.

Bellani G, Laffey JG, Pham T, *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. *JAMA*. 2016;315(8) :788-800.

Bendib I, de Chaisemartin L, Dessap AM, Chollet-Martin S, de Prost N. Understanding the role of neutrophil extracellular traps in patients with severe pneumonia and ARDS. *Chest*. 2019. https://doi.org/10.1016/j.chest.2019.08.2179.

Bernard GR, Artigas A, Brigham KL, *et al.* The American-European Consensus Conference on ARDS: Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;149:818-24.

Bhatraju PK, Ghassemieh BJ, Nichols M, *et al*. COVID-19 in critically ill in the Seattle region – Case series. *N Engl J Med*. 2020. https://www.doi.org/10.1056/NEJMoa2004500.

Brown SM, Grissom CK, Moss M, *et al.* Nonlinear imputation of PaO_2/FiO_2 from SoO_2/FiO_2 among patients with acute respiratory distress syndrome. *Chest.* 2016;150(2):307-13.

Cao X. COVID-19: immunopathology and its implications for therapy. *Nature Rev Immunol*. 2020. https://doi.org/10.1038/s41577-020-0308-3.

Cascella M, Rajnik M, Cuomo A, *et al*. Features, evaluation and treatment of coronavirus (COVID-19) [Updated 2020 Apr 6]. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2020 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/.

CDC, 2020a. Coronavirus Disease 2019. Information for clinicians on investigational therapeutics for patients with COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/hcp/therapeutic-options.html. Accessed April 19, 2020.

CDC, 2020b. Testing for COVID-19. http://cdc.gov/coronavirus/2019-ncov/symptoms-testing/-testing.html. Accessed 18-Apr-2020.

CDC, 2017. Definitions of symptoms for reportable illnesses. https://www.cdc.gov/quarantine/air-/reporting-deaths-illness/definitions-symptoms-reportable-illnesses.html. Accessed 18-Apr-2020.

Chan KP, Stewart TE, Mehta S. High-frequency oscillatory ventilation for adult patients with ARDS. *Chest.* 2007;131:1907-16.

Chen N, Zhou M, Dong X, Qu J, Gong F. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395:507-13.

Cheng Y, Luo R, Wang K, *et al.* Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* 2020;97:829-38.

Choudhury SR. Identification of dipeptidase-1 as an organ-selective adhesion receptor utilized by neutrophils and metastatic cancer cells in the liver and lungs (Unpublished doctoral thesis). University of Calgary, Calgary, AB. 2018. doi:10.11575/PRISM/31901 http://hdl.handle.net/-1880/106619.

Choudhury SR, Babes L, Rahn JJ, *et al*. Dipeptidase-1 is an adhesion receptor for neutrophil recruitment in lungs and liver. *Cell*. 2019;178(5):1205-21. doi: 10.1016/j.cell.2019.07.017.

Cochi SE, Kempker JA, Annangi S, Kramer MR, Martin GS. Mortality trends of acute respiratory distress syndrome in the United States from 1999 to 2013. *Ann Am Thorac Soc*. 2016;13(10):-1742-51. https://doi.org/10.1513/AnnalsATS.201512-8410C.

Cohn LA, Kerl ME, Lenox CE, Livingston RS, Dodam JR. Response of healthy dogs to infusions of human serum albumin. *Am J Vet Res*. 2007;68:657-63.

Collins SR, Blank RS, Deatherage LS, Dull RO. The endothelial glycocalyx: Emerging concepts in pulmonary edema and acute lung injury. *Anesth Analg*. 2013;117(3):664-74.

COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://files.covid19treatmentguidelines.-nih.gov/guidelines/covid19treatmentguidelines.pdf. Accessed 23-January-2021.

CTCAE v5.0. Common Terminology Criteria for Adverse Events (CTCAE) v5.0. https://ctep.-cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. Accessed 16-May-2020.

Cutts S, Talboys R, Paspula C, Ail D, Premphe EM, Fanous R. History of acute respiratory syndrome. *Lancet.* 2016;4:547-8. http://dx.doi.org/10.1016/S2213-2600(16)30087-X.

Darmon M, Clec'h C, Adrie C, *et al.* Acute respiratory distress syndrome and risk of AKI among critically ill patients. *Clin J Am Soc Nephrol*. 2014;9(8);1347-53.

Diao B, Wang C, Wang R, *et al*. Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. Preprint at medRxiv 2020.03.04.20031120; doi: https://doi.org/10.1101/2020.03.04.20031120

Doerschuk CM. Leukocyte trafficking in alveoli and airway passages. *Respir Res*. 2000;1:136-40.

Donahoe M. Acute respiratory distress syndrome: A clinical review. *Pulm Circ*. 2011;1:192-211.

Ebrahimi F, Giaglis S, Hahn S, *et al.* Markers of neutrophil extracellular traps predict adverse outcome in community-acquired pneumonia: secondary analysis of a randomised controlled trial. *Eur Respir J*. 2018;5(4). Pii: 1701389.

Eworuke E, Major JM, Gilbert McClain LI. National incidence rates for acute respiratory distress syndrome (ARDS) and ARDS cause-specific factors in the United States (2006 – 2014). *J Crit Care*. 2018;47:192-7. https://doi.org/10.1016/j.jcrc.2018.07.002.

Fan E, Brodie D, Slutsky AS. Acute respiratory distress syndrome: Advances in diagnosis and treatment. *JAMA*. 2018;319(7):698-710.

FDA 2020. Communication May 1 2020. Authorization for emergency use of remdesivir for the treatment of hospitalized 2019 coronavirus disease (COVID-19). https://www.fda.gov/media/-137564/download Accessed 16-May-2020.

FDA Guidance for Industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. US Department of Health and Human Services, Food and Drug Administration, July 2005.

Ferguson ND, Fan E, Camporota L, *et al.* The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. *Intensive Care Med*. 2012;38(10):1573-82.

Ferluga M, Lucangelo U, Blanch L. Dead space in acute respiratory distress syndrome. *Ann Transl Med*. 2018;6. doi: 10.21037/atm.2018.09.46.

Festic E, Kor DJ, Gajic O. Prevention of acute respiratory distress syndrome. *Curr Opin Crit Care*. 2015;21:82-90.

Festic E, Carr GE, Cartin-Ceba R, *et al.* Randomized clinical trial of a combination of an inhaled corticosteroid and beta agonist in patients at risk of developing the acute respiratory distress syndrome. *Crit Care Med*. 2017;45:798-805.

Fröhlich M, Wafaisade A, Mansuri A, *et al.* Which score should be used for organ failure? Comparison of the MODS, Denver- and SOFA- scores. *Scand J Trauma, Resuscit Emerg Med*. 2016;24:130. doi: 10.1186/s13049-016-0321-5.

Fu J, Zhou B, Zhang L, *et al.* Expressions and significances of the *angiotensin-converting enzyme 2* gene, the receptor of SARS-CoV-2 for COVID-19. *Mol Biol Rep.* 2020; May 14. https://www.doi.org/10.1007/s11033-020-05478-4.

Fujita J, Yamadori I, Kamei T, Yasufumi Y, Takahara J. Neutrophilic endotheliitis in patients with idiopathic pulmonary fibrosis. *Chest.* 1995;108(2):589.

Gaber AO, Mulgaonkar S, Kahan BD, *et al*. YSPSL (rPSGL-1g) for improvement of early renal allograft function: a double-blind, placebo-controlled, multi-center Phase IIa study. *Clin Transplant*. 2011;25:523-33.

Gattinoni L, Coppola S, Cressoni M, Busana M, Chiumello D. COVID-19 does not lead to a "typical" acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2020. doi: 10.1164/-rccm.202003-0817LE.

Gautret P, Lagier J-C, Parola P, *et al.* Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020; In press.

Government of Canada, 2020. Coronavirus disease (COVID-19): Symptoms and treatment. https://www.canada.ca/-en/public-health/services/diseases/2019-novel-coronavirus-infection/symptoms.html. Accessed 24-Mar-2020.

Granja C, Póvoa P, Lobo C, Teixeira-Pinto A, Carneiro A, Costa-Pereira A. The predisposition, infection, response and organ failure (PIRO) sepsis classification system: results of hospital mortality using a novel concept and methodological approach. *PloS ONE*. 2013;8(1): e53885. doi: 10.1371/journal.pone.0053885.

Grasselli G, Zangrillo A, Zanella A, *et al.* Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA.* 2020. https://doi.org/10.1001/jama.2020.5394.

Greenland JR, Michelow MD, Wang L, London MJ. COVID-19 Infection: Implications for perioperative and critical care physicians. *Anesthesiology*. 2020. Doi: 10.1097/ALN.0000000-000003302.

Henderson LA, Canna SW, Schulert GS, *et al.* On the alert for cytokine storm: Immunopathology in COVID-19. *Arthritis Rheumatol.* 2020; Apr 15. https://www.doi.org/10.1002/art.41285.

Heywood SP, Hooper NM. Development and application of fluorometric assay for mammalian membrane dipeptidase. *Anal Biochem*. 1995;226:10-4.

Hirsch JS, Ng JH, Ross DW, *et al.* Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int.* 2020. https://www.doi.org/10.1016/j.kint.2020.05.006.

Huang C, Wang Y, Li X, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020. In press.

Hutchings L, Watkinson P, Young JD, Willett K. Defining multiple organ failure after major trauma: A comparison of the Denver, Sequential Organ Failure Assessment and Marshall scoring systems. *J Trauma Acute Care Surg*. 2017;82(3):534-41.

Jenne CN, Kubes P. Virus-induced NETs – Critical component of host defense or pathogenic mediator? *PLOS Pathogens*. 2015;11(1). E1004546. doi: 10.1371/journal.ppat.1004546.

Jin Y-H, Cai L, Cheng Z-S, *et al*. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Military Med Res*. 2020;7:4. https://doi.org/10.1186/s40779-020-0233-6.

Johns Hopkins 2020. https://coronavirus.jhu.edu/map.html.

Kallet RH, Lipnick MS, Zhuo H, Pangilinan LP, Gomez A. Characteristics of nonpulmonary organ dysfunction at onset of ARDS based on the Berlin definition. *Respir Care*. 2019;64(5):493-501.

Kaplan MJ, Radic M. Neutrophil extracellular traps (NETs): Double-edged swords of innate immunity. *J Immunol*. 2012; 189(6):2689-95.

Klok FA, Kruip MJHA, van der Meer NJM, *et al.* Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* 2020. https://www.doi.org/10.1016/-j.thromres.2020.04.013.

Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13(10):818-29.

Kopf M, Bachmann MF, Marsland BJ. Averting inflammation by targeting the cytokine environment. *Nat Rev Drug Discovery*. 2010;9:703-18.

Kuchroo VK, Ohashi PS, Sartor RB, Vinuesa CG. Dysregulation of immune homeostasis in autoimmune diseases. *Nat Med*. 2012;18:42-7.

Lau H, Khosrawipour V, Kocbach P, *et al*. The positive impact of lockdown in Wuhan on containing the COVID-19 outbreak in China. *J Travel Med*. 2020. https://doi.org/10.1093/jtm/-taaa037.

Lee I-C, Huo T-I, Huang Y-H. Gastrointestinal and liver manifestations in patients with COVID-19. *J Chin Med Assoc.* 2020. https://www.doi.org/10.1097/JCMA.0000000000000319.

Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. *JCI Insight*. 2018;3(3). pii:98178.

Levey AS, Stevens LA, Schmid CH, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604-12.

Liu KD, Thompson BT, Ancukiewicz M, *et al.* Acute kidney injury in patients with acute lung injury: Impact of fluid accumulation on classification of acute kidney injury and associated outcomes. *Crit Care Med*. 2011;39(12):2665-71.

Liu Q, Zhou Y-h, Yang Z-q. The cytokine storm of severe influenza and development of immunomodulatory therapy. *Cell Mol Immunol*. 2016;13:3-10.

Liu W, Tao Z-W, Wang L, *et al*. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chin Med J*. 2020. https://www.doi.org/10.1097/CM9.0000000000000775.

Llitjos J-F, Leclerc M, Chochois C, *et al.* High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J Thromb Haemost.* 2020 Apr 22. https://www.doi.org/10.1111/jth.14869.

Louis JK, Yang S, Acosta C, *et al.* Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1)pdm09. *Clin Infect Dis*. 2012;55:1198-1204.

Máca J, Ondřej J, Holub M, *et al*. Past and present ARDS mortality rates: A systematic review. *Respir Care*. 2017;62(1):113-22.

Mammoto A, Mammoto T. Vascular niche in lung alveolar development, homeostasis, and regeneration. *Front Bioeng Biotechnol*. 2019;7:318. doi: 10.3389/fbioe.2019.00318.

Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit Care Med*. 1995;23:1638-52.

Martinez-Rojas MA, Vega-Vega O, Bobadilla NA. Is the kidney a target of SARS-CoV-2? *Am J Physiol Renal Physiol.* 2020 May 15. https://www.doi.org/10.1152/ajprenal.00160.2020.

Matthay MA, Aldrich JM, Gotts JE. Treatment for severe acute respiratory distress syndrome from COVID-19. *Lancet Respir Med*. 2020. https://doi.org/10.1016/S2213-2600(20)30127-2.

Mease KM, Kimzey AL, Lansita JA. Biomarkers for nonclinical infusion reactions in marketed biotherapeutics and considerations for study design. *Curr Opin Toxicol*. 2017;4:1-15. doi: 10.1016/j.cotox.2017.03.005.

Mehta P, McAuley DF, Brown M, *et al*. COVID-19: consider cytokine storms and immunosuppression. *Lancet.* 2020. https://doi.org/10.1016/S0140-6736(20)30628-0.

Meyers M, Rodrigues N, Ari A. High-frequency oscillatory ventilation: A narrative review. *Can J Respir Ther*. 2019;55:40-6. doi: https://doi.org/10.29390/cjrt-2019-004.

Mikacenic C, Moore R, Dmyterko V, *et al*. Neutrophil extracellular traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. *Crit Care*. 2018;22(1). https://doi.org/10.1186/s13054-018-2290-8.

Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science*. 2020. https://doi.org/10.1126/science.abb8925.

Mullard A. Flooded by the torrent: the COVID-19 drug pipeline. *Lancet*. 2020;395:1245-6.

Munari AB, Gulart AA, dos Santos K, Venâcio RS, Karloh M, Mayer AF. Modified Medical Research Council dyspnea scale in GOLD classification better reflects physical activities of daily living. *Respir Care*. 2018;62(1):77-85. Questionnaire is found at http://www.cscc.unc.edu/-spir/public/UNLICOMMMRCModifiedMedicalResearchCouncilDyspneaScale08252011.pdf

Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis*. 1988;138:720-3.NCT03089957 ClinicalTrials.gov. Prevention of Ulinastatin on Acute Respiratory Distress Syndrome. https://clinicaltrials.-gov/ct2/show/NCT03089957. Accessed 26-Mar-2020.

Muscedere JG, Day A, Heyland DK. Mortality, attributable mortality, and clinical events as end points for clinical trials of ventilator-associated pneumonia and hospital-acquired pneumonia. *Clin Infect Dis*. 2010;51(S1):S120-5.

Narasaraju T, Yang E, Samy RP. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol*. 2011:179(1). doi: 10.1016/j.ajpath.2011.03.013.

NCT04264533 ClinicalTrials.gov. Vitamin C infusion for the treatment of severe 2019-nCoV infected pneumonia. https://clinicaltrials.gov/ct2/show/NCT04264533. Accessed 26-Mar-2020.

NCT04315948 ClinicalTrials.gov. Trial of treatments for COVID-19 in hospitalized adults. https://clinicaltrials.gov/ct2/results?cond=&term=NCT04315948&cntry=&state=&city=&dist= Accessed 24-Jan-2021.

Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity*. 2014;41:694-707.

Omer SB, Malani P, delRio C. The COVID-19 pandemic in the US. A clinical update. *JAMA*. 2020. https://www.doi.org/10.1001/jama.2020.5788.

Ozolina A, Sarkele M, Sabelnikovs O, *et al*. Activation of coagulation and fibrinolysis in acute respiratory distress syndrome: a prospective pilot study. *Front Med (Lausanne)*. 2016;3. doi: 10.3389/fmed.2016.-00064.

Pais FM, Sinha P, Liu KD, Matthay MA. Influence of clinical factors and exclusion criteria on mortality in ARDS observational studies and randomized controlled trials. *Respir Care*. 2018;62(8):1060-9.

Panitchote A, Mehkri O, Hastings A, *et al*. Factor associated with acute kidney injury in acute respiratory distress syndrome. *Ann Intensive Care*. 2019;9;74. https://doi:10.1186/s13613-019-0552-5.

Panka BA, de Grooth H-J, Spoelstra-de Man A, Looney M, Tuinman P-R. Prevention of treatment of ARDS with aspirin: a review of preclinical models and meta-analysis of clinical studies. *Shock*. 2017;47(1):13-21.

Parsons PE, Eisner MD, Thompson BT, *et al.* Lower tidal volume ventilation and plasma cytokine markers of inflammation patients with acute lung injury. *Crit Care Med*. 2005;33:1-6.

Peek GJ, Mugford M, Tiruvoipati R, *et al.* Randomised controlled trial and parallel economic evaluation of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR). *Lancet*. 2009;374(9698):1351-63. doi: 1016/-S0140-6736(09)61069-2.

Pham T, Rubenfeld GD. Fifty years of research in ARDS. The epidemiology of acute respiratory distress syndrome. A 50th birthday review. *Am J Respir Crit Care Med*. 2017;195(7):860-70.

Phua J, Stewart TE, Ferguson ND. Acute respiratory distress syndrome 40 years later: time to revisit its definition. *Crit Care Med*. 2008;36(10):2912-21.

Porras G, Ayuso MS, González-Manchón C. Leukocyte-endothelial cell interaction is enhanced in podocalyxin-deficient mice. *Biology*. 2018;99:72-9.

Poston JT, Patel BK, Davis AM. Management of the critically ill adults with COVID-19. *JAMA*. March 26, 2020. doi:10.1001/jama.2020.4914.

Potey PMD, Rossi AG, Lucas CD, Dorward DA. Neutrophils in the initiation and resolution of acute pulmonary inflammation: understanding biological function and therapeutic potential. *J Pathol*. 2019;247:672-85.

Pottecher J, Noll E, Borel M, *et al.* Protocol for TRAUMADORNASE: A prospective, randomized, multicentre, double-blinded, placebo-controlled clinical trial of aerosolized dornase alfa to reduce the incidence of moderate-to-severe hypoxaemia in ventilated trauma patients. *Trials*. 2020;21:274. https://doi.org/10.1186/s13063-020-4141-6.

Raghavendran K, Napolitano LM. ALI and ARDS: Challenges and advances. *Crit Care Clin*. 2011;27(3):-429-37. doi: 10.1016/j.ccc.2011.05.006.

Rana R, Sharma R, Kumar A. Repurposing of existing statin drugs for treatment of microbial infections: How much promising? *Infect Disorders – Drug Targets*. 2019;19(3):224-37.

Ranieri VM, De Tullio R, Dayer JM, *et al.* Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA*. 1999;282:54-61.

Ranieri VM, Rubenfeld GD, Thompson BT, *et al*. Acute respiratory distress syndrome: The Berlin definition. ARDS Definition Task Force. *JAMA*. 2012;307(23):2526-33.

Rawal G, Yadav S, Kumar R. Acute respiratory distress syndrome: An update and review. *J Transl Intern Med*. 2018;6(2):74-7.

Roselló S, Blasco I, García Fabregat L, *et al.* Management of infusion reactions to systemic anticancer therapy: ESMO Clinical Practice Guidelines. *Ann Oncol*. 2017;28(Suppl 4):iv100-18. doi: 10.1093/annonc/mdx216.

Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020. https://doi.org/10.1007/s00134-020-05991-x.

Saguil A, Fargo M. Acute respiratory distress syndrome: Diagnosis and management. *Am Fam Physician*. 2012;85(4):352-8.

Sauaia A, Moore EE, Johnson JL, Ciesla DJ, Biffl WL, Anirban B. Validation of postinjury multiple organ failure scores. *Shock*. 2009;31(5):438-47.

Shi S, Qin M, Shen B, *et al.* Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. 2020 Mar 25. https://www.doi.org/10.1001/jamacardio.2020.0950.

Soo A, Zuege DJ, Fick GH, *et al*. Describing organ dysfunction in the intensive care unit: A cohort study of 20,000 patients. *Crit Care.* 2019;23:186. https://doi.org/10.1186/s13054-019-2459-9.

Stokes EK, Zambrano LD, Anderson KN, *et al*. Coronavirus disease 2019 case surveillance—United States, January 22–May 30, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69. https://www.-cdc.gov/mmwr/volumes/69/wr/pdfs/mm6924e2-H.pdf. Accessed 2-August-2020.

Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140:805-20.

Thomas ME, Blaine C, Dawnay A, *et al.* The definition of acute kidney injury and its use in practice. *Kidney Int.* 2015;87:62-73. https://www.doi.org/10.1038/ki.2014.328.

Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. *N Engl J Med*. 2017;377:562-72.

Tignanelli CJ, Wiktor AJ, Vatsaas CJ, *et al.* Outcomes of acute kidney injury in patients with severe ARDS due to influenza A(H1N1) pdm09 virus. *Am J Crit Care*. 2018;27(1):67-73.

Twaddell SH, Baines KJ, Grainge C, Gibson PG. The emerging role of neutrophil extracellular traps in respiratory disease. *Chest*. 2019;156(4). https://doi.org/10.1016/j.chest.2019.06.012.

Ulvik A, Kvåle R, Wentzel-Larsen T, Flaatten H. Multiple organ failure after trauma affects even long-term survival and functional status. *Crit Care*. 2007;11:R95. doi: 10.1186/cc6111.

Varga Z, Flammer AJ, Steiger P, *et al.* Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020; https://doi.org/10.1016/S0140-6736(20)30937-5.

Versluys AB, Boelens JJ. Morbidity and mortality associated with respiratory virus infections in allogeneic hematopoietic cell transplant: Too little defense or harmful immunity. *Front Microbiol*. 2018;9. https://doi.org/10.3389/fmicb.2018.02795.

Vincent J-L, Moreno R, Takala J, *et al*. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med*. 1996;22:707-10.

Vogelmeier CF, Criner GJ, Martinez FJ, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 Report. GOLD executive summary. *Am J Respir Crit Care Med*. 2017;195(5):557-82.

Wang D, Hu B, Hu C, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020. doi:10.1001/jama.2020.1585.

Williams AE, Chambers RC. The mercurial nature of neutrophils: still an enigma in ARDS? *Am J Physiol Lung Cell Mol Physiol*. 2014;306(3):L217-30.

Wongrakpanich S, Susantitaphong P, Isaranuwatchai S, Chenbhanich J, Eiam-Ong S, Jaber BL. Dialysis therapy and conservative management of advanced chronic kidney disease in the elderly: A systematic review. *Nephron*. 2017;137:178-89.

Wu C, Chen X, Cai Y, *et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus 2019 pneumonia in Wuhan, China. *JAMA Intern Med.* 2020. https://doi.org/10.1001/jamainternmed.2020.0994.

Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China. Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239-42.

Xu L, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. *Liver Int.* 2020;40:998-1004.

Xu Z, Shi L, Wang Y, *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020; published online Feb 17. http://dx.doi.org/10.1016/-S2213-2600(20)30076-X.

Yang M, Zhao J, Zhang Z. More than pneumonia, the potential occurrence of multiple organ failure in 2019 novel coronavirus infection. Available at *SSRN*: https://ssrn.com/abstract=-3532272 or http://dx.doi.org/10.2139/ssrn.3532272.

Yang Y, Shen C, Li J, *et al.* Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. Unpublished but available at: https://doi.org/10.1101/2020.03.02.20029975.

Ye Q, Wang B, Mao J. Cytokine storm in COVID-19 and treatment. *J Infect*. 2020. https://doi.org/-10.1016/j.jinf.2020.03.037.

Zhai Z, Li C, Chen Y, *et al*. Prevention and treatment of venous thromboembolism associated with coronavirus disease 2019 infection: A consensus statement before guidelines. *Thromb Haemost.* 2020; Apr 21. http://www.sah.org.ar/pdf/covid-19/s-0040-1710019.pdf.

Zhang B, Zhou X, Zhu C, *et al.* Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. Preprint at *medRxiv* 2020. https://doi.org/10.1101/2020.03.12.20035048.

Zhou, Z., Ren L, Zhang L *et al*. Heightened innate immune responses in the respiratory tract of COVID19 patients. *Cell Host Microbe* 2020 May 4;S1931-3128(20)30244-4. doi: 10.1016/-j.chom.2020.04.017.

Zhu Y, Yalavarthi S, Shi H, *et al*. Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19. Preprint at *medRxiv* https://doi.org/10.1101/2020.04.09.20059626.

13 APPENDICES

13.1 Appendix 1. Bottle Label

Sample labels for the study drug are illustrated below in **Figure 6 and Figure 7**. Labels will be adapted in accordance with local regulatory and language requirements.

Figure 6: Vial Labels

Study #AB002 LSALT Peptide – 4.95 mg
0.9 mg/mL (5.5 mL fill in 10 mL Clear Vial)
Mfg. Date: 21SEP2018 Lot Number: LE550C02R1
Store at (-15°C to -25°C)
LSALT Peptide Solution for IV Administration –
Use as per sponsor instructions
Sponsor: Arch Biopartners Inc.
(Toronto. ON, M4T 2M5, Canada)
Caution: New Drug – Limited by Federal (or United States) law to investigational use only
Trial Subject ID#: _____

VIAL LABEL: TURKISH TRANSLATION

Klinik çalışma #AB002 LSALT Peptide – 4.95 mg
0.9 mg/mL (5.5 mL dolum miktarı, 10 mL şeffaf flakonda)
Üretim tarihi: 21EYL2018 Seri no: LE550C02R1
-15°C ila -25°C arasındaki ısılarda saklanmalıdır
LSALT Peptid solüsyonu; Sadece IV uygulama için –
Çalışma destekleyicisinin yönergeleri doğrultusunda uygulanmalıdır
Destekleyici : Arch Biopartners Inc.
(Toronto. ON, M4T 2M5, Canada)
Dikkat: Araştırma İlacıdır - Sadece klinik araştırma için kullanılmalıdır
Çalışma hasta numarası #: _____

Figure 7: Outside Carton Labels

Study #AB002
LSALT Peptide – 4.95mg/vial
0.9mg/mL (5.5mL fill in 10mL Clear Vial)
Mfg. Date: 21SEP2018
Lot Number: LE550C02R1
Store at (-15°C to -25°C)
LSALT Peptide Solution for IV Administration - Use as per sponsor instructions
Sponsor: Arch Biopartners Inc.
(Toronto. ON, M4T 2M5, Canada,)
Caution: New Drug – Limited by Federal
(or United States) law to investigational use only
Number of vials per carton: 100
Carton #:_____

CARTON LABEL: TURKISH TRANSLATION

Klinik çalışma #AB002
LSALT Peptid – 4.95mg/flakon
0.9mg/mL (5.5mL dolum miktarı, 10mL şeffaf flakonda)
Üretim tarihi: 21EYL2018
Seri no: LE550C02R1
-15°C ila -25°C arasındaki ısılarda saklanmalıdır
LSALT Peptid solüsyonu; Sadece IV uygulama için –
Çalışma destekleyicisinin yönergeleri doğrultusunda uygulanmalıdır
Destekleyici: Arch Biopartners Inc.
(Toronto. ON, M4T 2M5, Canada,)
Dikkat: Araştırma İlacıdır - Sadece klinik araştırma için kullanılmalıdır
Her karton kutuda 100 flakon vardır
Karton kutu #:_____

13.2 Appendix 2. Clinical Laboratory Tests

Clinical Chemistry	Hematology	Coagulation	Urinalysis
Total bilirubin	Hemoglobin	Activated clotting time*	pH*
AST (SGOT)	Hematocrit	aPTT	Color*
ALT (SGPT)	Erythrocytes	Prothrombin time	Transparency
BUN	Leukocytes + Diff	INR	Specific gravity
Glucose	Thrombocytes		Urobilinogen
Potassium			Ketones
Sodium			Protein
Calcium			Glucose
Alkaline phosphatase			Hemoglobin
Chloride			
Creatinine & eGFR (calc)			
Gamma-glutamyl transferase			
Total protein			
Albumin			
CO_2			

ALT = alanine transferase; AST = aspartate transferase; BUN = blood urea nitrogen; aPTT = activated partial prothrombin time; Diff = differential; INR = International normalized ratio; CO_2 = carbon dioxide; eGFR (calc) = estimated glomerular filtration rate

*Only if SOC

13.3 Appendix 3. Berlin Definition

	Acute Respiratory Distress Syndrome (ARDS)
Timing	Within 1 week of a known clinical insult or new/worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities – not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (*e.g.*, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm H_2O[c]
Moderate	100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg with PEEP ≥ 5 cm H_2O
Severe	PaO_2/FiO_2 ≤ 100 mmHg with PEEP ≥ 5 cm H_2O

[a]Chest radiograph or computed tomography scan
[b]If altitude is >1000 m, the correction factor should be calculated as follows: [PaO_2/FiO_2 x (barometric pressure/760)]
[c]This may be delivered noninvasively in the mild acute respiratory distress syndrome group

13.4 Appendix 4. Sequential Organ Failure Assessment (SOFA) score

Organ System, Measurement	SOFA score				
	0	1	2	3	4
Respiration PaO_2/FiO_2	NL	< 400	< 300	< 200[a]	< 100[a]
Coagulation Platelets $x10^3/mm^3$	NL	< 150	< 100	< 50	< 20
Liver Bilirubin, mg/dL (µmol/L)	NL	1.2 – 1.9 (20 – 32)	2.0 – 5.9 (33 – 101)	6.0 – 119	> 12.0 > 204
Cardiovascular Hypotension	NL	MAP < 70 mmHg	Dopamine ≤5 or dobutamine (any dose)[b]	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Central Nervous System Glasgow Coma Scale	NL	13 – 14	10 – 12	6 – 9	< 6
Renal Creatinine mg/dL (µmol/L) or Urine output	NL	1.2 – 1.9 (110 – 170)	2.0 – 3.4 (171 – 299)	3.5 – 4.9 (300 – 440) or <500 mL/day	>5.0 (>440) or <200 mL/day

*Source: Vincent *et al*, 1996
NL = Normal
[a]With respiratory support (*e.g.,* ventilator)
[b]Adrenergic agents administered for at least 1 hour (doses given are µg/kg/min)

13.5 Appendix 5. Modified Medical Research Council (mMRC) Dyspnea Scale

ID NUMBER: ☐☐☐☐☐☐☐☐ FORM CODE: **MRC** VERSION: **1.0** 10/26/10 Visit Number ☐☐ SEQ # ☐☐☐

0a) Form Date ..... ☐☐/☐☐/☐☐☐☐ 0b) Initials..... ☐☐☐

Instructions: *This form should be completed during the participant's visit. Choose the one best response.*

Please choose the one best response to describe your shortness of breath.

Grade

0 "I only get breathless with strenuous exercise"

1 "I get short of breath when hurrying on the level or walking up a slight hill"

2 "I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level"

3 "I stop for breath after walking about 100 yards or after a few minutes on the level"

4 "I am too breathless to leave the house" or "I am breathless when dressing"

1. Grade ..... ☐

13.6 Appendix 6. Acute Physiology and Chronic Health Evaluation II score

APACHE II SCORE

AGE Points	
≤ 44y	0
45-54y	2
55-64y	3
65-74y	5
≥75y	6

CHRONIC HEALTH Points	
Non-operative, or emergency post-op & any conditions below*	5
Elective operation & any conditions below*	2

*Cirrhosis w/ portal Hypertension or encephalopathy; class IV angina, chronic hypoxia, ↑CO2 or polycytemia; chronic dialysis; immunocompromised

TOTAL APACHE SCORE = AP + CHP + APS

Sum Age Points (AP) + Chronic Health Points (CHP) + Acute Physiologic Score (APS) points.

*1 Sum all variables 1-12 for Acute Physiologic Score (APS) (use one variable each for 5 and 9).

Use the worst value from the preceding 24h.

APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818-29.

ACUTE PHYSIOLOGIC SCORE*1 (APS)

	Physiologic Variable	Points 4	3	2	1	0	1	2	3	4
1	Temp °F	≤85.9	86.0-89.5	89.6-93.1	93.2-96.7	96.8-101.2	101.3-102.1		102.2-105.7	≥105.8
	°C	≤29.9	30-31.9	32-33.9	34-35.9	36 - 38.4	38.5-38.9		39-40.9	≥41
2	HR, bpm	≤39	40-54	55-69		70-109		110-139	140-179	≥180
3	MAP, mmHg	≤49		50-69		70-109		110-129	130-159	≥160
4	RR, bpm	≤5		6-9	10-11	12-24	25-34		35-49	≥50
5	Oxygenation: Use A-a Gradient (5a) if FiO2 ≥0.5 or use PaO2 (5b) if FiO2 <0.5 (*see page 17*)									
5a	A-a Gradient					<200		200-349	350-499	≥500
5b	PaO2	≤54	55-60		61-70	>70				
6	Na+ (S, mmol/L)	≤110	111-119	120-129		130-139	150-154	155-159	160-179	≥180
7	K+ (S, mmol/L)	≤2.4		2.5-2.9	3.0-3.4	3.5-5.4	5.5-5.9		6.0-6.9	≥7.0
8	Cr (S, mg/dL)			<0.6		0.6-1.4		1.5-1.9	2.0-3.4	≥3.5
9	Arterial pH is preferred. Use venous HCO3 if no ABGs.									
9a	pH (arterial)	≤7.14	7.15-7.24	7.25-7.32		7.33-7.49	7.5-7.59		7.6-7.69	≥7.7
9b	HCO3 (venous)	≤14	15-17.9	18-21.9		22-31.9	32-40.9		41-51.9	≥52
10	WBC, cells/uL	≤1.0		1.0-2.9		3.0-14.9	15-19.9	20-39.9		≥40
11	Hct, %	≤20		20-29.9		30-45.9	46-49.9	50-59.9		≥60
12	GCS coma	Score = 15 – GCS Score (see below, Record e.g.: "GCS 9 = E2 V4 M3 at 17:35h".)								

Score	Mortality
0 - 4	4%
5 - 9	4%
10 - 14	15%
15 - 19	25%
20 - 24	40%
25 - 29	55%
30 - 34	75%
> 34	85%

GLASGOW COMA SCALE (GCS)

*Teasdale G, Jennett B. Lancet 1974,2:81-84.

EYE Opening	Best VERBAL	Best MOTOR	Points
		follows commands	6
	oriented	localizes pain	5
spontaneous	confused	withdraws to pain	4
to command	inappropriate words	flexor response	3
to painful stimuli	incomprehensible	extension (abnl)	2
no response	no response	no response	1

SCORE: *Sum Points (eye+verbal+ motor categ).*

Severe ≤ 8.
Mod = 9-12.
Minor ≥ 13.