A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED PHASE II STUDY TO EVALUATE THE SAFETY AND EFFICACY OF INTRAVENOUS AMPION IN ADULT COVID-19 PATIENTS REQUIRING OXYGEN SUPPLEMENTATION

STUDY PROTOCOL

STUDY NUMBER: AP-017

NCT04839965

21 JANUARY 2022

CLINICAL STUDY PROTOCOL TITLE PAGE

Protocol Title:	A Randomized, Double-Blinded, Placebo-Controlled Phase II Study to Evaluate the Safety and Efficacy of Intravenous Ampion in Adult COVID-19 Patients Requiring Oxygen Supplementation
Study Number:	AP-017
Investigational Product:	Ampion
Drug Development Phase:	Phase II
Indication:	Adult COVID-19 patients on supplemental oxygen
Route of Administration:	Intravenous (IV) infusion
Regulatory Agency Identifier:	IND 22628
Sponsor:	Ampio Pharmaceuticals, Inc. 373 Inverness Parkway Englewood, CO 80112
Date:	21 January 2022

Study Conduct: The study is conducted in accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP) and regulatory requirements as applicable.

PROTOCOL ATTESTATION

I have read and understand the contents of this clinical protocol for Study Number AP-017 dated 21January 2022 and agree to meet all obligations of Ampio Pharmaceuticals Inc. as detailed in all applicable regulations and guidelines.

Signed By:

<Study personnel signature> **<Enter date>**

1 PROTOCOL SUMMARY

1.1 Protocol Synopsis

Sponsor:	**Investigational Product:**
Ampio Pharmaceuticals, Inc.	Ampion™

Title of Study:

A Randomized, Double-Blinded, Placebo-Controlled Phase II Study to Evaluate the Safety and Efficacy of Intravenous Ampion in Adult COVID-19 Patients Requiring Oxygen Supplementation

Rationale:

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in the pandemic spread of coronavirus disease 2019 (COVID-19), which has a high rate of infection, has a high rate of hospitalization, has overwhelmed healthcare systems, and can be fatal.

Ampion is the low molecular weight filtrate of human serum albumin with the *in vitro* ability to modulate inflammatory cytokine levels. Ampion has the potential to improve clinical outcomes for COVID-19 patients by reducing inflammatory cytokines correlated with the disease.

This study aims to evaluate Ampion and clinical outcomes in patients with COVID-19 who require supplemental oxygen. The data from this study will inform decisions for the Ampion clinical development.

Lead Investigators:

Michael Roshon, MD, PhD, USA

Number of Sites:

Approximately 10 sites

Indication:

Adult COVID-19 patients on supplemental oxygen

Number of Participants:

Sample size is approximately 200 patients, randomized 1:1 for Ampion or control.

An interim analysis will be conducted at 30 participants, randomized 1:1 for Ampion or control to allow for sample size re-estimation as needed.

Treatment Groups:

There are two planned treatment arms:

Treatment Arm	Investigational Treatment
Active	IV Ampion for 5-days
Control	IV Placebo for 5-days

Sponsor:	**Investigational Product:**
Ampio Pharmaceuticals, Inc.	Ampion™

Objectives and Endpoints:

Objective	Endpoint
Primary	
Assess the effect of Ampion compared to placebo on prevention of need for mechanical ventilation or death	• Occurrence of mechanical ventilation or death by Day 28.
Secondary	
Assess the effect of Ampion compared to placebo on safety	• Incidence of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 60.
Assess the effect of Ampion compared to placebo on hospital stay	• Hospital length of stay (LOS) from admission to discharge.
Assess the effect of Ampion compared to placebo on oxygen use	• Change from baseline in oxygen use, blood oxygen saturation, and oxygen flow rate from baseline through Day 5.
Assess the effect of Ampion compared to placebo on duration of respiratory failure to ventilator-free days	• Percentage of participants who progress to respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation) by Day 28.
Assess the effect of Ampion compared to placebo on intensive care	• Percentage of participants who require Intensive Care Unit (ICU) admission by Day 28. • ICU-free days through Day 28. • ICU LOS from ICU admission to discharge.
Assess the effect of Ampion compared to placebo on clinical improvement	• Change in ordinal scale from baseline through Day 7. • Change in ordinal scale from baseline to hospital discharge or Day 14 (whichever occurs first). • Change in ordinal scale from baseline to hospital discharge or Day 28 (whichever occurs first).
Assess the effect of Ampion compared to placebo on clinical health	• Change in NEWS2 score from baseline through Day 5. • Change in NEWS2 score from baseline to hospital discharge.
Assess the effect of Ampion compared to placebo on cytokine profile	• Modulation of cytokine levels from baseline to Day 5

Study Design:

Overall Design

This is a Phase II randomized, double-blinded, placebo-controlled study in adult participants with COVID-19 who require supplemental oxygen.

Screening

Interested participants will sign the appropriate informed consent document(s) prior to completion of any study procedures. The investigator will review symptoms, risk factors and other non-invasive inclusion, including COVID testing, and exclusion criteria prior to any study procedures. If the participant is eligible after this review, then the site will perform the necessary, if any, study procedures to confirm eligibility.

Treatment and Assessment

Participants will be randomized to active treatment or control, following an equal allocation to treatment arms. Given the changing nature of the pandemic, periodic adjustments to the allocation ratio may be made in an effort to achieve equal allocation across the treatment arms at the end of the enrollment. There are two planned treatment arms:

Treatment Arm	Investigational Treatment
Active	IV Ampion for 5-days
Control	IV Placebo for 5-days

The general sequence of events during the treatment and assessment period:

- Complete baseline procedures and sample collection
- Participants are randomized to receive either Ampion or a placebo.
- Participants receive study intervention (active or placebo) as an IV infusion for 5 days.
- Complete safety monitoring and post-infusion sample collection.

The Schedule of Assessments is shown in Section 1.2. Visit types are described as follows:

Study Day	Visit Description	Visit Type
0-5	Treatment	Site
28, 60	Follow-up	Telephone
Early discontinuation	ED	May be conducted at site or via telephone

Post-Treatment Follow-up

Post-treatment follow-up will occur after Day 5 to assess clinical status and for adverse events.

Disclosure Statement

Assigned treatment, active or placebo, will be blinded to the patients, clinicians, and the investigators conducting the study.

Sponsor:	**Investigational Product:**
Ampio Pharmaceuticals, Inc.	Ampion™

Data Safety Monitoring Board

There will be a Data Safety Monitoring Board (DSMB). Safety, including incidence of AEs/SAEs will be evaluated by a DSMB throughout the study.

Diagnosis and Main Criteria for Inclusion:

1. Male or female, ≥ 18 years old
2. Diagnosed with COVID-19, as evaluated by laboratory diagnostic test or diagnosis based on radiological clinical findings.
3. Baseline severity categorization of severe or critical COVID-19 infection per FDA Guidance for developing drugs and biological products for COVID-19 (February 2021):
   a) Severe COVID-19:
      - Symptoms suggestive of severe systemic illness with COVID-19, which could include shortness of breath or respiratory distress
      - Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, SpO2 ≤ 93% on room air at sea level (SpO2 ≤ 90% at altitude) or PaO2/FiO2 ≤ 300

   b) Critical COVID-19:
      - Oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced cannula at flow rates > 20 L/min with fraction of oxygen ≥ 0.5) or
      - Non-invasive mechanical or endotracheal mechanical ventilation
4. Informed consent obtained from the patient or the patient's legal representative.

Main Criteria for Exclusion:

1. As a result of the medical review and screening investigation, the Principal Investigator considers the patient unfit for the study and/or progression to death is imminent and inevitable irrespective of the provision of treatments.
2. Clinical diagnosis of respiratory failure (therapy not able to be administered in setting of resource limitation)
3. Shock defined by systolic blood pressure <90 mm Hg, or diastolic blood pressure <60 mm Hg or requiring vasopressors. Vasopressor therapy is exclusionary if used to treat shock. Subjects receiving low dose vasopressors to maintain renal perfusion are eligible.
4. Multi-organ dysfunction/failure
5. Patient has severe chronic obstructive or restrictive pulmonary disease (COPD) (as defined by prior pulmonary function tests), chronic renal failure (only exclusionary if subject is currently on renal replacement therapy, i.e., subjects receiving peritoneal dialysis, hemodialysis, ultrafiltration are ineligible), or significant liver abnormality (e.g., cirrhosis, transplant, etc.).

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6. Patient has chronic conditions requiring chemotherapy or immunosuppressive medication (only exclusionary if subject is currently receiving a high dose or therapy for transplantation, e.g., subjects receiving low dose inhaled or oral steroids for asthma are eligible).
7. A history of allergic reactions to human albumin (reaction to non-human albumin such as egg albumin is not an exclusion criterion) or ingredients in 5% human albumin (N-acetyltryptophan, sodium caprylate).
8. Prolonged QT interval.
9. Patient has known pregnancy or is currently breastfeeding.
10. Patient planning to become pregnant, or father a child, during the treatment and follow-up period and/or is not willing to remain abstinent or use contraception.
11. Participation in another clinical trial (not including treatments for COVID-19 as approved by the FDA through expanded access, emergency, or compassionate use), or participation in a trial such that enrollment in this study would fall within the time frame of the half-life of the other investigational product(s).

Test Product, Dose, and Mode of Administration:

Participants in the active arm will receive a 250cc daily dose of Ampion as an IV infusion for 5 days. The daily dose of Ampion will be delivered by IV twice daily (125cc/dose), every 12 hours, for 5 days.

All subjects receiving test product will also receive the standard of care (SOC) for COVID-19, which includes:

- Oxygen administration to maintain oxygen saturation of 90% or greater.
- Nursing physical that may include review of neurological, pulmonary, cardiac gastrointestinal, and urinary assessment at least daily.
- Vital monitoring (heart rate, blood pressure, temperature, respiratory rate, SpO2) daily
- Telemetry monitoring to evaluate heart rhythm and rate.
- Diet as tolerated to satisfy nutritional needs.
- Treatments for COVID-19 symptoms including antibiotics, cough suppressants/ expectorants, anti-coagulants, fever reducers/pain killers, anti-nausea drugs, and/or bronchodilators.
- Treatments for COVID-19 as approved by the FDA including expanded access, emergency, or compassionate use (i.e., remdesivir, dexamethasone, convalescent plasma).

Medications will be recorded as concomitant medication, tabulated, and compared among groups.

Reference Therapy, Dose and Mode of Administration:

Sponsor:	**Investigational Product:**
Ampio Pharmaceuticals, Inc.	Ampion™
Participants in the control arm will receive a 250cc daily dose of Placebo as an IV infusion for 5 days. The daily dose of Placebo will be delivered by IV twice daily (125cc/dose), every 12 hours, for 5 days. All subjects receiving placebo will also receive the SOC for COVID-19 as described previously.	
Study Duration: Treatment: 5 days Follow-up: 60 days	

1.2 Schedule of Assessments (SoA)

Visits will be conducted as described:

	Screen	Treatment						Hospitalization/ Continued Care	Follow-Up	
Study Day	**-3 to 0**	**0**	**1**	**2**	**3**	**4**	**5**	**Every day until Day 7**	**28**	**60**
Visit Window (± days)	**--**	**--**	**--**	**--**	**--**	**--**	**--**	**--**	**3**	**3**
COVID-19 diagnosis	X									
Informed consent	X									
Medical history and pre-existing conditions	X									
Inclusion/exclusion criteria	X	X								
Urine pregnancy test [1]		X								
Demographics		X								
Randomization		X								
IV treatment			X	X	X	X	X			
Vital signs		X	X	X	X	X	X	X		
Blood oxygen saturation		X	X	X	X	X	X	X		
ECG monitoring (telemetry)[2]		X	X	X	X	X	X	X [2]		
Hematology[3]		X		X		X	X	X [3]		
Biochemistry[2]		X		X		X	X	X [3]		
Cytokine/chemokine assay[4]		X					X	X [4]		
Mortality								X	X	X
Ordinal scale for clinical status		X	X	X	X	X	X	X	X	X
NEWS2 score for clinical status		X	X	X	X	X	X	X	X	X
Hospital LOS and ICU LOS			X	X	X	X	X	X	X	X
Date of intubation/ extubation, days on ventilation			X	X	X	X	X	X	X	X
Concomitant medications		X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

[1] As applicable

[2] Telemetry monitored as needed for patients who have events or abnormal readings requiring measurements.

[3] Hematology and biochemistry tests are performed at baseline and every other day through treatment and inpatient stay unless an abnormal value is observed. In the case of an abnormal lab result, continue to collect and test those samples to follow subject through resolution.

[4] Cytokine and chemokine assays are collected at baseline before treatment, at Day 5 after treatment, and at discharge.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
BP	Blood pressure
CDC	Centers for Disease Control and Prevention
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 19
CRO	Contract research organization
DSMB	Data and safety monitoring board
EC	Ethics committee
ECMO	Extracorporeal membrane oxygenation
eCRF	Electronic case report form
EDC	Electronic data capture
EMR	Electronic medical record
EUA	Emergency use authorization
HSA	Human serum albumin
ICH	International conference on harmonization
ICU	Intensive care unit
IRB	Investigational review board
ITT	Intent to treat
IV	Intravenous
LAR	Legally authorized representative
LOS	Length of Stay
NEWS	National Early Warning Score
q12	Every 12 hours
RCT	Randomized controlled trial
SAE	Serious adverse event
SOP	Standard operating procedure
SpO2	Blood oxygen saturation
TNFα	Tumor necrosis factor alpha
WHO	World Health Organization

2 INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the pandemic spread of coronavirus disease 2019 (COVID-19). The COVID-19 virus has a high rate of infection and complications due to the virus has resulted in a high rate of hospitalization, has overwhelmed healthcare systems, and can be fatal. Approximately 20% of patients with COVID-19 will progress to severe disease (CDC.gov). Identifying drugs and therapies that address the full spectrum of symptoms related to COVID-19 infection is critical.

This is a phase II randomized controlled trial (RCT) to evaluate intravenous (IV) Ampion for adults with COVID-19 who require supplemental oxygen. Ampion is being developed as an immunomodulatory therapy with anti-inflammatory effects that could provide a beneficial treatment for COVID-19 patients with symptoms, like difficulties breathing and the need for supplemental oxygen. This study aims to investigate the safety and the clinical outcomes in these patients with COVID-19 on supplemental oxygen when treated with IV Ampion.

2.1 Study Drug

Ampion is the low molecular weight filtrate of 5% Human Serum Albumin (HSA), USP, derived from human blood. It is a homogenous solution containing three main active components: Aspartyl-Alanyl Diketopiperazine (DA-DKP), N-Acetyl-Tryptophan (NAT), and Sodium Caprylate (Caprylate). These ingredients have the *in vitro* ability to decrease inflammatory cytokine levels. The modulation of these inflammatory cytokines is expected to mitigate respiratory complications in patients with COVID-19 as these cytokines are correlated with the respiratory symptoms and disease severity. Ampion is packaged as a sterile solution (125cc) ready for IV infusion.

The study control arm will use a saline placebo solution packaged in the same configuration as Ampion to protect blinding.

Ampion or placebo will be delivered intravenously as a 125cc dose, delivered twice a day, for a 250cc daily dose administered for five days. Ampion or placebo will be administered using an infusion pump through an existing intravenous access (central line, PICC, peripheral, or saline lock). Ampion will be administered as an intravenous piggyback/secondary if the patient at the time of the administration of Ampion has an existing primary infusion. If there are no intravenous fluids being administered at the time of the Ampion infusion, Ampion treatment will be infused as an intermittent intravenous infusion. Ampion will be administered undiluted at a flow rate of 100cc/hour.

2.2 Background to the Disease

COVID-19 infection is a respiratory illness caused by a novel coronavirus (SARS-COV-2) and has been classified as a pandemic with no known cure to date. COVID-19 is detected and diagnosed with a laboratory test. The primary symptoms of COVID-19 infection include mild symptoms such as fever, cough, chills, muscle pain, headache, gastrointestinal symptoms, and the loss of taste or smell. Once infected, the virus moves down a patient's respiratory tract,

where the lungs may become inflamed, making breathing difficult and requiring supplemental oxygen, resulting in more severe cases of the disease.

Inflammation associated with COVID-19 may trigger even more severe complications including pneumonia, acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS), which is a leading cause of mortality in COVID-19 (Zhou 2020). ALI/ARDS is a rapidly progressive disease characterized by widespread inflammation in the lungs. Under normal circumstances, there is approximately a 40% mortality rate for patients with ALI/ARDS (Bellani 2016). However, ARDS secondary to COVID-19 infection might prove to be more lethal than ARDS due to other causes. A study of 191 patients in Wuhan, China reported an 85% (50/59) case mortality rate of ARDS secondary to COVID-19 infection (Zhou 2020).

Inflammation is initiated when a patient experiences moderate to severe COVID-19 symptoms (Gandhi 2020) and the continuing destructive cycle of inflammation impact the clinical course of COVID-19 patients in several significant and potentially fatal ways. Patients with severe COVID-19 require supplemental oxygen and careful monitoring as inflammation continues. Remdesivir and dexamethasone have demonstrated benefits in hospitalized patients with severe COVID-19, but in patients with moderate disease, dexamethasone is not efficacious (and may be harmful) and data are insufficient to recommend for or against routine use of remdesivir (Gandhi 2020). There is a continued need for a treatment that can interrupt and/or prevent this inflammatory cascade to address the severe and critical COVID-19 patient population who require oxygen supplementation.

As an immunomodulatory agent, Ampion may be effective in interrupting the inflammation associated with COVID-19 and improving the clinical course and outcome of patients.

2.3 Previous Human Experience

Human serum albumin (HSA, 5%) is a human blood product approved by FDA for intravenous (IV) infusion to treat hypovolemia and other serious conditions, including ARDS (Polito 2013, Farag, 2016). Albumin and large molecules are removed to manufacture Ampion, the low molecular weight (< 5 kDa) ultrafiltrate of 5% HSA.

Ampion has been investigated for clinical use as an IV treatment compared to standard of care for adults with COVID-19 infection requiring supplemental oxygen in a Phase 1, randomized, open label study in 10 adults with severe COVID-19. The study met its primary endpoint for the safety and tolerability of IV Ampion treatment, with no remarkable difference in the incidence, frequency, and severity of adverse events between IV Ampion and standard of care. At hospital discharge, Ampion-treated patients showed greater clinical improvement than standard of care alone using the WHO ordinal scale and NEWS2 scale for clinical health.

Ampion is also in human clinical development for a separate inflammatory indication (arthritis). In that program more than 1,000 patients have been exposed to a localized 4cc injection of Ampion with no treatment-related adverse events.

2.4 Study Rationale

This study aims to evaluate the impact of IV Ampion on the occurrence of mechanical ventilation or death and clinical outcomes in adults with COVID-19 who require supplemental oxygen. The data from this study will inform decisions for the clinical development of Ampion.

2.4.1 Study Background

COVID-19 infection is associated with pathological findings including pneumonia and inflammation of lung tissue. The triggering insult to the tissue has been associated with a hyper innate inflammatory response in which cytokines and related proteins, such as tumor necrosis factor alpha (TNFα), are excessively increased with the severity of COVID-19 and respiratory failure. This inflammatory response commonly results in ALI or its more severe form, ARDS, in which leads to pulmonary edema and fibrosis with poor prognosis. ALI/ARDS is believed to be a major cause of mortality in COVID-19 patients.

In vitro nonclinical studies show Ampion modulates cytokine levels in various immune cell models where it decreases the levels of inflammatory cytokines, including TNFα. Due to its mode of action, Ampion may be a viable treatment option for those infected with COVID-19 in effort to improve clinical outcomes, slow the progression and severity with associated critical COVID-19 inflammatory conditions (e.g., progression to respiratory failure, the need for assisted breathing, and ultimately mortality) (Thomas, 2020). This study evaluates the safety and tolerability of IV Ampion treatment for COVID-19 patients who require supplemental oxygen before they progress to these critical cases.

2.4.2 Preclinical Data

Ampion is a filtrate of a human blood product, 5% human serum albumin (HSA), approved by FDA for IV infusion to treat critically ill patients including patients with compromised respiration with ALI and ARDS (HSA prescribing information; Farag, 2016; Polito 2013). The IV administration of HSA has exposed humans, including the indicated population with respiratory inflammation, to the components in Ampion with no remarkable safety concerns.

The Ampion preclinical testing program is designed to support clinical development for the treatment of inflammatory diseases and the dysregulation of proteins responsible for modulating the immune response. COVID-19 infection is associated with a hyper innate inflammatory response, where increased levels of cytokines and related proteins (e.g., TNFα) are correlated with the severity of COVID-19 illness (Channappanavar 2017, Feldman 2020).

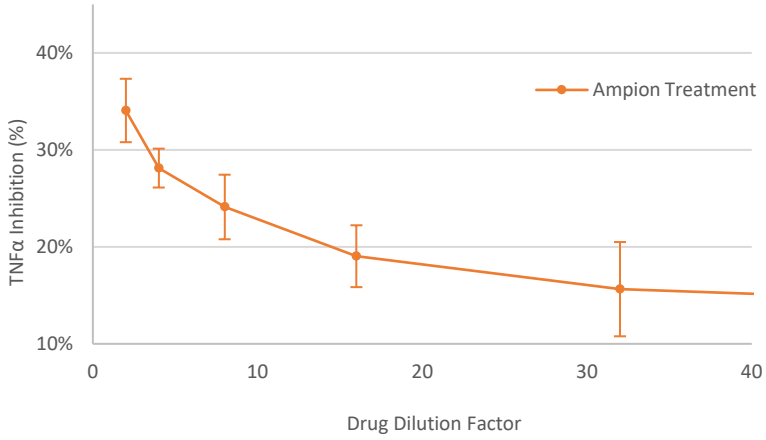
Preclinical pharmacology studies of Ampion using human *in vitro* models of immunology (e.g., peripheral blood mononuclear cells and macrophages) indicate that Ampion reduces inflammatory cytokines (e.g., TNFα) responsible for the inflammation and tissue damage initiated by viral diseases like COVID-19 (Channappanavar, 2017) and in respiratory distress syndromes (Yang, 2017), while promoting the production of prostaglandins responsible for resolving inflammation in respiratory disease (Loynes 2018).

2.4.3 Dosing Rationale

Human serum albumin, including 5% HSA, has been administered safely via IV for many years at volumes exceeding 500cc in a day (5% HSA prescribing information). A comparison of the active components in Ampion against those in the starting material HSA demonstrates that the proposed daily dose (250cc) of Ampion can be safely administered via IV.

The proposed daily dose of 250cc is based on Ampion's *in vitro* biological activity and the amount of Ampion required to achieve its *in vitro* anti-inflammatory effect. The biological activity of Ampion is measured using a bioassay which tests the release of pro-inflammatory cytokine (TNFα) in activated immune cells. In the bioassay, human peripheral blood mononuclear cells (PBMC) are stimulated to release TNFα. The cells are treated with either Ampion or saline, and Ampion activity is reported as inhibition (%) of TNFα in cells treated with Ampion compared to the cells treated with saline. In the bioassay, the treatment effect is achieved by adding 50 µL of Ampion to 100,000 cells. A dose-dependent effect is observed (a representative dose curve is shown in Figure 1).

Figure 1: TNFα Inhibition and Dose Response in Human Immune Cells Treated with Ampion



To model efficacy of an *in vivo* clinical dose of Ampion, the number blood monocytes in the bloodstream provides a means of estimating the concentration of immune cells in circulation. Based on the average monocyte density in an adult human bloodstream (Sharma, 2018, Healthline.com), and the ratio of cells used in the Ampion TNFα bioassay, a therapeutic dose range of Ampion (250 – 1187cc) would be required to achieve the anti-inflammatory effect. The proposed dose for this study is the lowest possible effective dose of 250cc a day and is the dose used in a previous phase I study.

2.5 Benefit and Risk Assessment

Patients with COVID-19 may be at a high risk of progressing to life-threatening, critical disease. Ampion may provide a safe and effective treatment option for these patients.

The anticipated risks of IV Ampion treatment are considered low and are based on IV use in COVID-19 patients, decades of use of IV HSA in severely ill patients, and other indications (arthritis). Theoretical risks come from historical use of IV HSA, including rare allergic reactions and facial flushing. The product is a derivative of human plasma, however based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob Disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have been identified for 5% HSA or Ampion.

Given the well-describe safety profile of 5% HSA and the lack of therapeutic options targeted for severe and critical COVID-19 patients, the overall benefit-risk assessment of this study is considered favorable. As an immunomodulatory drug, Ampion may interrupt the inflammatory cascade for COVID-19 patients with respiratory distress which could improve clinical outcomes.

3 STUDY DESIGN

3.1 Study Design Overview

This is a Phase II randomized, double-blinded, placebo-controlled study to evaluate the safety and efficacy of IV Ampion in participants with COVID-19 who require supplemental oxygen.

Interested COVID-19 patients will sign the appropriate informed consent document(s) prior to completion of any procedures. The investigator will review inclusion and exclusion criteria prior to any invasive procedures. If the patient is eligible after this review, the site will perform the necessary, if any, study procedures to confirm eligibility.

Participants will be randomized 1:1 to active or placebo using a random allocation sequence stratified by disease severity. The general sequence of events during the treatment and assessment period:

- Complete baseline procedures and sample collection.
- Participants are randomized to Ampion or placebo.
- Participants receive study intervention (active or placebo) through IV infusion for 5 days.
- Complete safety monitoring, study procedures, and sample collection through Day 60.

All subjects receiving test product will also receive the standard of care (SOC) for COVID-19, which includes:

- Oxygen administration to maintain oxygen saturation of 90% or greater.
- Nursing physical that may include review of neurological; pulmonary; cardiac; gastrointestinal; and urinary assessment at least daily during treatment.
- Vital monitoring (heart rate, blood pressure, temperature, respiratory rate, SpO2) at least daily during treatment.
- Telemetry monitoring to evaluate heart rhythm and rate.
- Diet as tolerated to satisfy nutritional needs.
- Treatments for COVID-19 symptoms including antibiotics, cough suppressants/ expectorants, anti-coagulants, fever reducers/pain killers, anti-nausea drugs, and/or bronchodilators.
- Treatments for COVID-19 as approved by the FDA including expanded access, emergency, or compassionate use (i.e., remdesivir, dexamethasone, convalescent plasma).
- Medications will be recorded as concomitant medication, tabulated, and compared among groups.

The clinical effects, safety, and tolerability of treatment will be evaluated from baseline through Day 60. Evaluations at screening and during the 5-day treatment occur in person. Evaluations after the 5-day treatment to the end of the study (days 7 to 60) will typically occur via telephone contact unless the patient is in an inpatient setting.

3.2 Study Objectives

3.2.1 Primary Objective

The primary trial objective is to evaluate the effect of Ampion on the prevention of need for mechanical ventilation or death in adult participants with respiratory distress due to COVID-19.

3.2.2 Secondary Objectives

The secondary trial objectives evaluate the efficacy of IV Ampion versus control in improving the clinical course and outcomes of participants with COVID-19 who require supplemental oxygen.

3.3 Study Endpoints

3.3.1 Primary Endpoint

The primary endpoint assesses the effect of Ampion compared to placebo on the prevention of need for mechanical ventilation or death. This is measured as the occurrence of participants on mechanical ventilation or death by Day 28.

3.3.2 Secondary Endpoints

Secondary efficacy endpoints assess the effect of IV Ampion compared to SOC on the clinical outcomes for participants with COVID-19 who require supplemental oxygen as follows:

Objective	Endpoint
Secondary Endpoints	
Assess the effect of Ampion compared to placebo on safety	• Incidence of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 60.
Assess the effect of Ampion compared to placebo on hospital stay	• Hospital length of stay (LOS) from admission to discharge.
Assess the effect of Ampion compared to placebo on oxygen use	• Duration of oxygen use, blood oxygen saturation, and oxygen flow rate from baseline through Day 5.
Assess the effect of Ampion compared to placebo on duration of respiratory failure to ventilator-free days	• Percentage of participants who progress to respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation) by Day 28.
Assess the effect of Ampion compared to placebo on intensive care	• Percentage of participants who require Intensive Care Unit (ICU) admission by Day 28. • ICU-free days through Day 28. • ICU LOS from ICU admission to discharge.
Assess the effect of Ampion compared to placebo on clinical improvement	• Change in ordinal scale from baseline through Day 7. • Change in ordinal scale from baseline to hospital discharge or Day 14 (whichever occurs first).

Objective	Endpoint
	• Change in ordinal scale from baseline to hospital discharge or Day 28 (whichever occurs first).
Assess the effect of Ampion compared to placebo on clinical health	• Change in NEWS2 score from baseline through Day 5. • Change in NEWS2 score from baseline to hospital discharge.
Assess the effect of Ampion compared to placebo on oxygen use	• Duration of oxygen use, blood oxygen saturation, and oxygen flow rate from baseline through Day 5.

3.4 Blinding and Randomization

The treatment in this study will be blinded to the subjects, investigators, any individual conducting the study (e.g., nursing and pharmacy staff) and clinical study personnel. Participants will be assigned to treatment by a randomization schedule developed and maintained by an independent statistician.

Participants are randomized to active or control, following an equal allocation to treatment arms, stratified by COVID-19 disease severity (severe or critical as defined in Section 4.4).

Study drug and placebo will be provided as blinded investigational product (IP) with appropriate labeling to link to the randomization code. Where required, safety personnel and/or investigator may be unblinded to a particular subject's treatment assignment to meet reporting requirements to Regulators.

A data management plan and statistical analysis plan will be approved by the sponsor prior to unblinding study data.

3.5 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be established to review the safety of IV Ampion as the study progresses. The DSMB will be primarily responsible for reviewing any serious Adverse Event (SAE) and other clinically important safety findings (e.g., discontinuations due to AEs) that may occur during the study.

3.6 Stopping rules

The entire study may be stopped under defined circumstances as outlined in Section 6.

4 SELECTION OF PARTICIPANTS

4.1 Number of Participants

The trial is designed to enroll up to approximately 200 patients across two treatment arms (active or control), with a 100 subjects per arm, randomized 1:1 using a random allocation sequence stratified by COVID-19 disease severity (severe or critical). An interim analysis of the data is planned after 30 subjects (n=15 subjects per arm) to evaluate the relative effect. At the interim analysis, the sample size may be re-estimated to determine the additional number of patients for adequate power up to the 200 subjects.

The number of participants is selected based on the effects observed for the primary endpoint. A study recently reported in Lancet Respiratory Medicine with 243 hospitalized patients receiving the placebo for COVID-19 found 22% did not achieve day 28 ventilation-free survival (Lancet Respir Med 2021; https://doi.org/10.1016/S2213-2600(21)00494-X). In the AP-014 study, a lower rate (8%) was observed for subjects receiving inhaled Ampion. Assuming the same proportions (8% for Ampion and 22% for control) by Day 28, with 100 subjects per group, a two-sided 5% significance level, and using a z-test with unpooled variances, then under binomial enumeration the power to detect this difference is about 81.3%.

4.2 Recruitment Methods

Subjects will be recruited from the population being seen by Investigators at the clinical sites participating in the study.

4.3 Participant Characteristics

The participant population are those infected with SARS-CoV-2 that have developed severe or critical symptoms consistent with COVID-19 and require supplemental oxygen to maintain blood oxygen saturation levels. Treatment of COVID-19 depends on the stage and severity of disease with a hyperinflammatory state observed in the moderate to severe stages that are thought to lead to clinical respiratory complications and critical COVID-19 (Gandhi 2020). Treatment with immunomodulators at this point in the disease may be more effective than anti-viral treatments.

The population of participants with COVID-19 who require supplemental oxygen were selected for this study to evaluate the efficacy of Ampion as an immunomodulatory therapy that may improve the clinical outcome of the disease and may reduce mortality.

4.4 Inclusion Criteria

Patients should fulfill all the following inclusion criteria:

1. Male or female, ≥ 18 years old.
2. Diagnosed with COVID-19, as evaluated by laboratory diagnostic test or diagnosis based on radiological clinical findings.
3. Baseline severity categorization of severe or critical COVID-19 infection per FDA Guidance for developing drugs and biological products for COVID-19 (February 2021):
   a) Severe COVID-19:
      - Symptoms suggestive of severe systemic illness with COVID-19, which could include shortness of breath or respiratory distress
      - Clinical signs indicative of severe systemic illness with COVID-19, such as respiratory rate ≥ 30 per minute, heart rate ≥ 125 per minute, SpO2 ≤ 93% on room air at sea level (SpO2 ≤ 90% at altitude) or PaO2/FiO2 ≤ 300
   b) Critical COVID-19:
      - Oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced cannula at flow rates > 20 L/min with fraction of oxygen ≥ 0.5) or
      - Non-invasive mechanical or endotracheal mechanical ventilation
4. Informed consent obtained from the patient or the patient's legal representative.

4.5 Exclusion Criteria

Patients fulfilling one or more of the following criteria may not be enrolled in the study:

1. As a result of the medical review and screening investigation, the Principal Investigator considers the patient unfit for the study and/or progression to death is imminent and inevitable irrespective of the provision of treatments.
2. Clinical diagnosis of respiratory failure (therapy not able to be administered in setting of resource limitation).
3. Shock defined by systolic blood pressure <90 mm Hg, or diastolic blood pressure <60 mm Hg or requiring vasopressors. Vasopressor therapy is exclusionary if used to treat shock. Subjects receiving low dose vasopressors to maintain renal perfusion are eligible.
4. Multi-organ dysfunction/failure.
5. Patient has severe chronic obstructive or restrictive pulmonary disease (COPD) (as defined by prior pulmonary function tests), chronic renal failure (only exclusionary if subject is currently on renal replacement therapy, i.e., subjects receiving peritoneal dialysis, hemodialysis, ultrafiltration are ineligible), or significant liver abnormality (e.g., cirrhosis, transplant, etc.).
6. Patient has chronic conditions requiring chemotherapy or immunosuppressive medication (only exclusionary if subject is currently receiving a high dose or therapy for transplantation, e.g., subjects receiving low dose inhaled or oral steroids for asthma are eligible).

7. A history of allergic reactions to human albumin (reaction to non-human albumin such as egg albumin is not an exclusion criterion) or ingredients in 5% human albumin (N-acetyltryptophan, sodium caprylate).
8. Prolonged QT interval.
9. Patient has known pregnancy or is currently breastfeeding.
10. Patient planning to become pregnant, or father a child, during the treatment and follow-up period and/or is not willing to remain abstinent or use contraception.
11. Participation in another clinical trial (not including treatments for COVID-19 as approved by the FDA through expanded access, emergency, or compassionate use), or participation in a trial such that enrollment in this study would fall within the time frame of the half-life of the other investigational product(s).

4.6 Inclusion of Patients Incapable of Giving Informed Consent

Patients who are not sedated or otherwise cognitively impaired may be capable of giving informed consent. In the remaining cases, patients will be enrolled in the study via their legally authorized representative (LAR) by telephone or in person. Enrolled via LAR by telephone requires confirmation of enrollment by two clinicians (e.g., registered nurse or physician).

5 STUDY PLAN, PROCEDURES, AND ASSESSMENTS

5.1 Description of Study Visits

The mortality, clinical effects, safety, and tolerability of treatment will be evaluated from baseline through Day 60. Evaluations at screening and during the 5-day treatment occur in person. Evaluations after the 5-day treatment to the end of the study (days 6 to 60) will typically occur via telephone. The Schedule of Assessments is shown in Section 1.2.

5.1.1 Screening (-3 Days to Day 0)

The following procedures will be performed at Screening:

- Evaluate all inclusion and exclusion criteria to ensure that patients meet all inclusion criteria and none of the exclusion criteria.
- Medical history, pre-existing conditions, and comorbidities. Include the symptom onset date for COVID-19 symptoms and date of COVID-19 test.
- Diphenhydramine, hydroxychloroquine, and azithromycin all prolong the cardiac QT interval, increasing risk of fatal cardiac arrhythmia. Therefore, severely-ill subjects receiving multiple drugs that prolong QT intervals will be reviewed carefully by the P.I. on a case-by-case adjudication for benefit-risk ratio and close cardiovascular monitoring. Note, subjects who have baseline QT prolongation are excluded from this study.
- Obtain informed consent before the starting any study specific procedures, including COVID testing.

5.1.2 Baseline (Day 0)

The following procedures will be performed at Baseline (first assessment prior to treatment):

- Confirm eligibility (review inclusion/exclusion criteria).
- Randomize patient to study arm.
- Demographics (age, sex, race, height and weight)
- Concomitant medications/therapies
- Vital signs: heart rate, systolic and diastolic BP, body temperature, respiratory rate
- SpO2 and supplementation oxygen mode/flow rate
- ECG monitoring (telemetry) or 12-lead ECG: record aberrant changes in waves/ intervals
- Hematology, biochemistry tests
- Cytokine and chemokine assays
- Ordinal scale and NEWS2 assessments
- AEs

5.1.3 Treatment Period (Day 1 to Day 5)

The following procedures will be performed during the Treatment Period (Day 1 to Day 5):

- Administer Ampion or placebo as a daily IV infusion. Begin treatment within 6 hours of randomization.
- Concomitant medications/therapies

- Vital signs: heart rate, systolic and diastolic BP, body temperature, respiratory rate – vital signs are collected shortly after (within 30 minutes) treatment.
- SpO2 and supplementation oxygen mode/flow rate
- ECG monitoring (telemetry) or 12-lead ECG: record aberrant changes in waves/ intervals – monitored as needed for patients who have abnormal readings or events requiring measurements.
- Hematology, biochemistry tests – these tests are performed at baseline and every other day through treatment and through hospital stay unless an abnormal value is observed. In the case of an abnormal lab result, continue to collect and test those samples to follow subject through resolution.
- Cytokine and chemokine assays – these tests are performed at baseline and at Day 5.
- Ordinal and NEWS2 assessments
- AEs

5.1.4 Hospitalization Period, as applicable (Day 6 through Day 7)

Hospitalization refers to hospital and/or in-patient settings. The following procedures will be performed for the remainder of the hospitalization, as applicable:

- Concomitant medications/therapies
- Vital signs: heart rate, systolic and diastolic BP, body temperature, respiratory rate
- SpO2 and supplementation oxygen mode/flow rate
- ECG monitoring (telemetry) or 12-lead ECG: record aberrant changes in waves/ intervals – monitored as needed for patients who have abnormal readings or events requiring measurements.
- Hematology, biochemistry tests – these tests are performed at baseline and every other day through treatment and through hospital stay unless an abnormal value is observed. In the case of an abnormal lab result, continue to collect and test those samples to follow subject through resolution.
- Cytokine and chemokine assays – these tests are performed at hospital discharge.
- Ordinal scale and NEWS2 assessments
- AEs

5.1.5 Post-Treatment Follow-Up (Days 28, 60)

The following procedures will be performed at all follow-up visits (Days 28, 60):

- Mortality
- Concomitant medications/therapies
- Hospital LOS
- ICU LOS
- Ordinal scale and NEWS2 assessments
- AEs

5.2 Assessment Methods

Demographic Data

Demographic data will be collected from medical records: age, gender, race, height and weight, comorbidities.

Medical History

Medical history and pre-existing conditions will be collected from medical records.

Concomitant Medications

Concomitant medications will be collected from the medical records: prior (pre-hospitalization) concomitant medications, in-patient concomitant medications.

Mortality

All-cause mortality will be recorded at hospital discharge, Days 28 and 60, as applicable. Cause of mortality will be assessed and documented. All-cause mortality is calculated for the primary endpoint as the percentage of participants with a successful outcome (life) or unsuccessful outcome (death).

Length of Stay (LOS)

Due to the COVID-19 health emergency, the Centers for Medicare and Medicaid is temporarily allowing long-term care facilities not normally used as a resident's room to accommodate beds and residents for care in emergencies/situations needed to help with surge capacity. Therefore, hospitalization refers to hospital and/or inpatient care during the COVID-19 health emergency. Inpatient care may include hospitals treating COVID-19 patients; dedicated COVID-19 treatment facilities with 24/7 care; and/or dedicated research facilities with 24/7 care.

Dates of hospitalization and ICU admission as well as discharge dates will be recorded at hospital discharge, Days 28 and 60, as applicable. The hospital LOS and ICU LOS will be calculated as follows:

- ICU admission: defined (in days) as the first study day when ICU status is changed to "yes" minus baseline date + 1
- Hospital LOS: is defined (in days) as the date of hospital discharge minus date of hospital admission + 1
- ICU LOS: defined (in days) as the date moved out of ICU minus first study date when ICU admission changed to "yes" + 1

Oxygen Use

Oxygen use measured as blood oxygen saturation (SpO2) and oxygen flow rate (liters per minute, lpm) will be recorded at every visit from baseline to Day 5.

Intubation/Extubation

Date and time of intubation/extubation and days on ventilator will be recorded at hospital discharge, Days 28 and 60, as applicable. Proportion of participants who progress to respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation, or high-flow nasal cannula oxygen) will be evaluated.

Vital Signs

Vital signs will be collected daily during the treatment period and at follow-up visits from medical records as follows: heart rate (or pulse rate), systolic BP, diastolic BP, body temperature (ºF in the US, ºC out of the US), respiratory rate. Vital signs are collected shortly after (within 30 minutes) treatment.

Hematology

Hematology lab tests will be collected from the medical records at baseline and every other day through treatment and hospital stay (as applicable) unless an abnormal value is observed. In the case of an abnormal lab result, continue to collect and test those samples to follow subject through resolution.

The follow hematology labs are tested: white cell count, red blood cell count, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Serum Biochemistry

Biochemistry lab tests will be collected from the medical records at baseline and every other day through treatment and hospital stay (as applicable) unless an abnormal value is observed. In the case of an abnormal lab result, continue to collect and test those samples to follow subject through resolution.

The following biochemistry lab tests are tested: sodium, potassium, chloride, bicarbonate, urea, creatinine, glucose, total calcium, phosphate, ferritin, high-sensitivity C-reactive protein (hs-CRP), protein, albumin, globulins, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

Cytokine and Chemokine Assays

Cytokine and chemokine assay lab test results will be collected and recorded before and after treatment (at baseline and again at Day 5 and at hospital discharge). A cytokine panel is tested to include, but not limited to: Tumor Necrosis Factor alpha (TNFα), interferon gamma (IFNγ), Interleukin 1 beta (IL-1β), interleukins (IL-6, IL-8, IL-10, IL-12). Data is reported for research purposes, and the sample is destroyed after data is collected.

Ordinal Scale for Clinical Improvement

The clinical status will be recorded at every visit from baseline to Day 60 using the World Health Organization (WHO) “Ordinal Scale for Clinical Improvement”. This 8-point ordinal

scale was created to be responsive to the eligible patient population, intervention, and course of illness of COVID-19. The following is scores are collected:

Score	Descriptor
0	No clinical or virological evidence of infection
1	No limitation of activities
2	Limitation of activities
3	Hospitalized, no oxygen
4	Hospitalized, oxygen by mask or nasal prongs
5	Hospitalized, non-invasive ventilation or high-flow oxygen
6	Hospitalized, mechanical ventilation
7	Hospitalized, ventilation + additional organ support – pressors, RRT, ECMO
8	Death

NEWS2 Score for Determining the Degree of Illness

The NEWS2 will be calculated using the oxygenation requirement (room air or supplemental oxygen), SpO2 level, and vitals (body temperature, respiratory rate, heart rate and blood pressure) collected during study visits. The NEWS2 score is recommended by the National Institute for Health and Care Excellence ("NICE") for managing COVID-19 patients.

Adverse Events

Any documented adverse event. These include but are not limited to the following: cardiac injury, arrhythmia, septic shock, liver dysfunction, acute kidney injury, and multi-organ failure.

6 DISCONTINUATION CRITERIA

6.1 Early Discontinuation of the Study

Discontinuation or temporary suspension is allowed for any reason. The Sponsor may suspend or terminate the study due to the development of any new or unexpected life threatening or adverse events, prolonged hospitalization, or other potential grounds for stopping the study. The number of subjects, as well as the types and the grade severity of the adverse events (according to Common Terminology Criteria for Adverse Events (CTCAE)) may trigger the temporary suspension of study product administration pending a safety investigation as follows:

If any infusion reactions of grade 3 and above (as defined using the CTCAE grading scale version 5.0 or newer) are observed in any patient within 24 hours of product administration, a safety investigation will occur, which may trigger the temporary suspension of study product administration.

It is agreed that for reasonable cause, either the investigator or the Sponsor may terminate this study, provided written notice is submitted at a reasonable time in advance of intended termination; if by the investigator notice is to be submitted to Ampio Pharmaceuticals, Inc., and if by the Sponsor, notice will be provided to each investigator.

If a severe local reaction or drug-related SAE occurs at any time during the study, the DSMB will review the case immediately.

The study will be immediately suspended and no additional Ampion treatments administered pending review and discussion of all appropriate study data by the DSMB if one or more participants develop any of the following adverse events deemed to be possibly, probably, or definitely related to Ampion by the Investigator and/or Medical Monitor, based upon close temporal relationship or other factors:

- Death
- Respiratory deterioration requiring extracorporeal membrane oxygenation (ECMO)
- Anaphylaxis
- Acute adverse reaction at administration of treatment (i.e., sudden change in vital signs)

The study will not be restarted until all parties have agreed to the course of action to be taken and the IRB/EC has been notified.

6.2 Early Discontinuation of Individual Participants

Discontinuation of individual participants is allowed for any reason. Evaluations of participants who discontinue the study early are described in the Schedule of Assessments in Section 1.2.

7 TREATMENT

All participants will receive the standard of care for COVID-19. Participants randomized to the active arm will receive Ampion as an IV infusion daily for five days. Participants randomized to the control arm will receive placebo as an IV infusion daily for 5 days.

Patients will be allocated to a sequentially numbered treatment in accordance with the randomization schedule following confirmation of eligibility and before treatment.

7.1 Dosing and Administration of Study Medication

Ampion treatment will be provided as a solution in IV bags ready for human use and treatment administration. Appropriately trained site personnel will administer the study treatment. Treatment should start within 6 hours of randomization and should terminate on the 5-day treatment period.

Participants will receive a daily dose (250cc/day) of study intervention (active or placebo) through IV delivered in two equally divided doses of 125cc, every 12 hours, for 5 days. Treatment should commence within 6 hours of randomization. Treatment should terminate at the end of the 5-day treatment period, or when a patient is discharged, whichever comes first.

Treatment (active or control) will be administered on an infusion pump through an existing intravenous access (central line, PICC, peripheral, or saline lock). Treatment may be administered as an intravenous piggy-back/secondary if the subject has an existing primary infusion. If there are no IV fluids being administered at the time of the treatment infusion, Treatment will be infused as an intermittent intravenous infusion. Ampion will be administered undiluted at an infusion rate of 100cc/hour.

Participants will be monitored for any respiratory and/or cardiac distress via vital sign monitoring including respiratory rate, heart rate, and blood pressure shortly after (defined as within 30 minutes from the end of the infusion) study product administration.

Product administration may be temporarily suspended until resolution of any of the following criteria: acute change in vital signs that require intervention and/or support; sudden decrease in oxygen until stabilization occurs and/or consideration of mechanical ventilation depending on severity; and/or infusion reaction. The nature of the event will be evaluated (i.e., allergic; localized; systemic or otherwise) and appropriate measures applied, which may include symptomatic treatment with antihistamines and antiallergic measures, paracetamol (acetaminophen), corticosteroids, pressor, and/or removal and replacement of IV to another location to manage reactions if clinically indicated.

As described in Section 6.1, if any infusion reactions of grade 3 and above (as defined using the CTCAE grading scale version 5.0 or newer) are observed in any patient within 24 hours of product administration, a safety investigation will occur, which may trigger the temporary suspension of study product administration.

7.2 Study Medication Storage and Accountability

Investigational product should be stored at room temperature (59º – 77ºF or 15º – 25ºC) in a secure area with restricted access. The Investigator, the clinical site pharmacist, or other personnel authorized to store and dispense investigational product is responsible for ensuring that the investigational product used in the clinical study is securely maintained as specified by the Sponsor and in accordance with the applicable regulatory requirements.

All investigational product is to be dispensed in accordance with the Investigator's prescription.

It is the Investigator's responsibility to ensure that an accurate record is maintained of investigational product issued. All investigational product not used during the study must be returned to Ampio Pharmaceuticals Inc., or designated representative after study completion.

If any quality issue is noticed upon the receipt or use of an investigational product (i.e., deficiencies in condition, packaging, appearance, associated documentation, labeling, expiry date, etc.), Ampio Pharmaceuticals, Inc. must be promptly notified.

Under no circumstances may the Investigator supply investigational product to a third party, allow the investigational product to be used other than as directed by this clinical study protocol, or dispose of investigational product in any other manner.

7.3 Concomitant Treatments

Any medication used during the study should be recorded. The start and stop dates, total daily dose, route of administration, and indication for all concomitant medications should be recorded.

7.4 Treatment Compliance

Compliance with the investigational product use will be documented.

8 ADVERSE EVENTS

8.1 Definition of an Adverse Event

An adverse event (AE) is defined as any undesired medical occurrence in a patient or clinical investigation patient receiving a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable sign and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a study drug, whether or not related to the study drug.

Assessment of severity of an AE will be rated according to the following categories:

Grade 1 (MILD): The symptom is barely noticeable to the study patient and does not influence performance or functioning. Concomitant medication is not ordinarily indicated for relief of mild AEs.
Grade 2 (MODERATE): The symptom is of sufficient severity to make the study patient uncomfortable and to influence performance of daily activities. Concomitant medication may be indicated for relief of moderate AEs.
Grade 3 (SEVERE): The symptom causes severe discomfort, sometimes of such severity that the study patient cannot continue in the study. Daily activities are significantly impaired or prevented by the symptom. Concomitant medication may be indicated for relief of severe AEs.

Determination of the relationship between the AE and the study drug will be made using the following guidelines:

Unrelated	The adverse event is unlikely to have been caused by study drug.
Possibly related	It is unclear whether the adverse event may have been caused by the study drug.
Related	The adverse event is likely to have been caused by study drug.

8.2 Definition of a Serious Adverse Event

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

- Results in death
- Is life-threatening (patient is at immediate risk of death from the event as it occurred)
- Requires prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in any congenital anomaly/birth defect

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment or blood dyscrasias or convulsions that do not result in in-patient hospitalization.

Hospitalizations for elective surgery or other medical procedures that are not related to a treatment-emergent AE are not considered SAEs.

8.3 Recording of Adverse Events and Serious Adverse Events

Recording and reporting of adverse events should be in accordance with the FDA's final "Guidance for Industry and Investigators Safety Reporting Requirements for INDs and BA/BE Studies" of December 2012.

Any AE is to be recorded in the eCRF. In order to avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the patient's own words. Whenever possible, the investigator should combine signs and symptoms that constitute a single diagnosis.

The existence of an AE may be concluded from a spontaneous report of the patient; from the physical examination; or from special tests e.g., laboratory assessments, where applicable, or other study-specified tests (source of AE).

The reporting period begins from the time that the patient is randomized through index hospitalization until discharge or death, whichever should occur first. Any events continuing at study exit will be followed for 30 days or to resolution, or until no improvement is expected, whichever comes first. Any SAE occurring after the reporting period must be promptly reported if a causal relationship to the investigational drug is suspected. If the patient begins a new therapy, the safety reporting period ends at the time the new treatment is started, however, death must always be reported when it occurs during the study period irrespective of intervening treatment.

Each AE is to be evaluated for duration, severity, seriousness, and causal relationship to the investigational drug. The action taken and the outcome must also be recorded.

8.3.1 AE Follow up

All AEs occurring during the study are to be followed up in accordance with good medical practice until they are resolved, stabilized or judged no longer clinically significant or, if a chronic condition, until fully characterized. Any AEs that are considered drug-related (possibly related, definitely related) must be followed for 30 days, or to resolution, or until no improvement is expected, whichever occurs first.

8.3.2 Overdose

No information on treatment of overdose of Ampion is currently available. In the case of overdose the patient should be followed as for an AE and appropriate supportive medical treatment instigated.

8.4 Serious Adverse Event Reporting

8.4.1 Reporting Requirements

Unexpected serious suspected adverse reactions are subject to expedited reporting to FDA. All SAEs must be entered into the eCRF within 24 hours of first knowledge of the event by study personnel. The investigator must provide his/her assessment of the relationship to study drug at the time of the initial report. The entry of an SAE into the eCRF triggers an automatic alert to the clinical research organization (CRO) safety team. The following information must be reported:

- Protocol number
- Site and/or Investigator number
- Patient number
- Demographic data
- Brief description of the event
- Onset date and time
- Resolution date and time, if the event resolved
- Current status, if event not yet resolved
- Any concomitant treatment and medication
- Investigator's assessment of whether the SAE was related to Investigative product or not.

The CRO Safety Associate will contact the site for clarification of data entered onto the eCRF, or to obtain missing information. In the event of questions regarding SAE reporting, the site may contact the appropriate individual as in Section 8.4.2.

8.4.2 SAE Contact Information

Ampio Pharmaceuticals Inc, or their designee CRO, is responsible for submitting reports of AEs associated with the use of the drug that are both serious and unexpected to FDA according to 21 CFR 312.32 and the final guidance (2012). All investigators participating in ongoing clinical studies with the study medication will receive copies of these reports for prompt submission to their Institutional Review Board (IRB) or Ethics committee (EC).

9 STATISTICAL METHODS

9.1 General Considerations

This section describes the rules and conventions to be used in the presentation and analysis of the data. The details of all these analyses will be presented in the Statistical Analysis Plan.

This trial is designed for approximately 200 patients across two treatment arms (active or control), with a 100 subjects per arm, randomized 1:1 using a random allocation sequence equally stratified by COVID-19 disease severity.

The number of participants is selected based on the effects observed for the primary endpoint, need for mechanical ventilation or death prior to day 28. A study recently reported in Lancet Respiratory Medicine with 243 hospitalized patients receiving the placebo for COVID-19 found 22% did not achieve day 28 ventilation-free survival (Lancet Respir Med 2021; https://doi.org/10.1016/S2213-2600(21)00494-X). In the AP-014 study, a lower rate (8%) was observed for subjects receiving inhaled Ampion. Assuming the same proportions (8% for Ampion and 22% for control) by Day 28, with 100 subjects per group, a two-sided 5% significance level, and using a z-test with unpooled variances, then under binomial enumeration the power to detect this difference is about 81.3%.

9.2 Analysis Populations

9.2.1 Safety Analysis Population:

The safety analysis population is defined as all patients who are randomized. Participants will be analyzed as treated.

9.2.2 Intent-to-treat Population:

The intent-to-treat (ITT) analysis population is defined as all randomized patients. All efficacy analyses will be performed in the ITT population. Patients will be analyzed as randomized.

9.2.3 Interim Analysis

This trial is designed to enroll up to approximately 200 subjects, randomized to active or control, following an equal allocation to treatment arms. An interim analysis of the data is planned after 30 subjects (n=15 subjects per arm) to evaluate the relative effect. At the interim analysis, the sample size may be re-estimated to include additional subjects to attain adequate power up to a 200 total subjects. This will be conducted at an alpha level of 0.001 and thus the overall alpha level for the final test will be performed at $\alpha=0.047$ instead of $\alpha=0.05$. The sponsor also reserves the right to alter the final sample size if the assumptions of the death rates are not satisfied at this interim analysis.

9.3 Data presentation

9.3.1.1 Demographic and Baseline Characteristics:

Demographic (e.g., age, sex, race, ethnicity) and baseline characteristics (e.g., weight, height, comorbidities) summarized using descriptive statistics, overall and by treatment group for the ITT and safety analysis population.

9.3.1.2 Medical History and Physical Examination:

The number and percent of participants with past and current medical disorders at the time of randomization will be presented overall and by treatment group for the ITT and safety analysis population.

9.3.1.3 Concomitant Medications or Treatments:

The number and percent of subjects receiving concomitant medications or therapies prior to and during the study and at the final visit will be tabulated and presented overall and by treatment group for the ITT analysis population.

Concomitant medications/treatments will be summarized using descriptive statistics and will be presented by type of drug (WHO DRUG classification) overall and by treatment group for the safety and ITT analysis populations. There should be no significant differences in the use of concomitant treatments between groups. All concomitant therapies will be recorded to be able to compare any inadvertent imbalances between the groups.

9.3.1.4 Safety Data:

Safety data will be presented by treatment arm. Safety data will be evaluated by changes in vital sign measurements, laboratory assessments, and the frequency and severity of AEs. Concomitant medication will be recorded for safety. AEs will be collected from baseline to Day 60. The severity of AEs (mild, moderate, severe), relatedness (related, possibly related, unrelated) along with the duration, action taken, and outcome (e.g., study withdrawal) will also be recorded.

All data collected under this study protocol will be included in the assessment of patient safety. Missing or incomplete AE data will assume greatest relationship to study drug and/or severity.

Remaining safety data will be collected from enrollment though index hospitalization until hospital discharge (or in-hospital death). Safety data will be tabulated and presented overall and by treatment group for the safety analysis population.

9.3.1.5 Efficacy Data:

The efficacy of treatment on the clinical course and outcome of COVID-19 will be evaluated by treatment arm. Summaries will be performed by severity (severe or critical), individually, and combined.

Unless otherwise specified, continuous variables will be summarized with the number of non-missing observations, mean, standard deviation, median, minimum, maximum, and 95% confidence intervals displayed. Categorical data will be summarized as counts, percentages, and 95% confidence intervals.

9.4 Study Endpoints

9.4.1 Primary Endpoint

The primary endpoint assesses the effect of Ampion compared to placebo on the prevention of need for mechanical ventilation or death. This is measured as the occurrence of mechanical ventilation or death by Day 28.

Let π_A and π_S represent the rate of this outcome for Ampion and control during the first 28 days after randomization. Then it is desired to demonstrate that the mechanical ventilation and death rate for Ampion is different than the corresponding rate for the control. Formally, this is presented as:

$H_0: \pi_A = \pi_S$ versus $H_A: \pi_A \neq \pi_S$

This will be tested utilizing the appropriate chi-square test or Fisher's exact test utilizing PROC FREQ with the EXACT option, if necessary.

9.4.2 Secondary Endpoints

Secondary efficacy endpoints assess the effect of IV Ampion compared to SOC on the clinical outcomes for participants with COVID-19 on supplemental oxygen. The secondary endpoints will be tested in a hierarchical manner in the order presented, and thus no adjustment to the alpha level is necessary:

Objective	Endpoint
Secondary Endpoints	
Assess the effect of Ampion compared to placebo on safety	• Incidence of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 60.
Assess the effect of Ampion compared to placebo on clinical improvement	• Change in ordinal scale from baseline through Day 7. • Change in ordinal scale from baseline to hospital discharge or day 14 (whichever occurs first). • Change in ordinal scale from baseline to hospital discharge or day 28 (whichever occurs first). • Change in NEWS2 score from baseline through Day 5. • Change in NEWS2 score from baseline to hospital discharge. • Modulation of cytokine levels from baseline to Day 5.
Assess the effect of Ampion compared to placebo on duration of respiratory failure to ventilator-free days.	• Percentage of participants who progress to respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation) by Day 28.

Objective	Endpoint
Assess the effect of Ampion compared to placebo on intensive care	• Percentage of participants who require Intensive Care Unit (ICU) admission by Day 28. • ICU-free days through Day 28. • ICU LOS from ICU admission to discharge.
Assess the effect of Ampion compared to placebo on hospital stay	• Hospital length of stay (LOS) from admission to discharge. • Hospital-free days through Day 28.
Assess the effect of Ampion compared to placebo on oxygen use	• Duration of oxygen use, blood oxygen saturation, and oxygen flow rate from baseline through Day 5.

The efficacy endpoints will be evaluated to estimate the treatment effect and use descriptive statistics including mean, standard deviation, percentages, minimum/maximum, and 95% confidence intervals. All endpoints will be tabulated and presented by treatment arm, cohort (severe and critical), and timepoint. Where appropriate, data will be tabulated and presented cumulatively across cohorts and/or timepoints.

All binary outcomes are single incident percentages and thus the differences between the treatment groups will be tested using Fisher's exact test. The length of stay endpoints are also single incident endpoints the differences between the treatment groups will be tested using two sample t-test. All the other change from baseline variables are repeated assessments and will be analyzed utilizing a repeated measures analysis of variance.

9.5 Missing and Spurious Data

All data collected under this study protocol will be included in the assessment of patient safety. Missing or incomplete AE data will assume greatest relationship to study drug and/or severity.

For the effectiveness endpoint analysis of all-cause mortality at Day 28 and 60 a sensitivity analysis will be performed for all participants that have been discharged from the hospital, cannot be reached via telephone contact, and their death status is not known. The sensitivity analysis will be performed where missing mortality status at Day 28 and 60 will be replaced as having died.

10 REGULATORY, ETHICAL AND LEGAL OBLIGATIONS

10.1 Declaration of Helsinki

The Principal Investigator will ensure that this Study is conducted in accordance with the most recent revision of the Declaration of Helsinki.

10.2 Good Clinical Practice

The Study will be conducted according to the study protocol and to Standard Operating Procedures (SOPs) that meet the guidelines provided by the International Conference on Harmonization (ICH) for Good Clinical Practice in clinical studies.

10.3 Institutional Review Boards/Ethics Committees

Before implementing this study, the protocol, the proposed patient informed consent forms and other information for the patients must be reviewed by a properly constituted committee or committees responsible for approving clinical studies. The IRB/IEC written signed approval letter/form must contain approval of the designated investigator, the protocol (identifying protocol title, date, and version number), and of the patient informed consent form (date, version).

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor, the IRB/IEC, and the Health Authorities.

10.4 Regulatory Authority Approval

Before this study is implemented, the protocol must be approved by the relevant regulatory authority.

10.5 Informed Consent

The investigator must fully inform the patient or patient’s legally authorized representative of all pertinent aspects of the trial including the written information approved/favorably assessed by the IRB/IEC.

Prior to the start of the pre-study examination, the written informed consent form must be signed and personally dated by the patient, or the patient’s legally authorized representative, and by the physician who conducted the informed consent discussion. One copy of the written information and signed consent form must be given to each patient or the patient’s legally authorized representative, and one (1) copy must be retained in the investigator's study records.

10.6 Patient Confidentiality and Disclosure

Data on patients collected on eCRFs during the trial will be documented in an anonymous fashion and the patient will only be identified by the patient number, and by his/her initials. If, as an exception, it is necessary for safety or regulatory reasons to identify the patient, all parties are bound to keep this information confidential.

The investigator will guarantee that all persons involved will respect the confidentiality of any information concerning the trial patients. All parties involved in the study will maintain strict confidentiality to assure that neither the person nor the family privacy of a patient participating in the trial is violated. Likewise, the appropriate measures shall be taken to prevent access of non-authorized persons to the trial data.

10.7 Collection, Monitoring and Auditing Study Documentation, and Data Storage

10.7.1 Collection of Data and Monitoring Procedures

This study will use a 21 CFR Part 11 compliant electronic data capture system (EDC). An electronic case report form (eCRF) is used for data recording. All data requested on the eCRF must be entered and all missing data must be accounted for.

The data will be checked for completeness and correctness as it is entered by the real-time online checks applied by the EDC system. Off-line checks will also be run to perform any additional data review required. Discrepancy reports will be generated accordingly and transferred to the study center for resolution by the investigator or his/her designee.

Accurate and reliable data collection will be assured by verification and cross–check of the eCRF against the investigator's records by the study monitor (source document verification), and the maintenance of a study drug–dispensing log by the investigator.

Before study initiation, at a site initiation visit or at an investigator's meeting, a Sponsor representative will review the protocol and case report forms with the investigators and their staff. During the study a monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the case report forms, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment. The monitor will ensure during on-site visits that study medication is being stored, dispensed and accounted for according to specifications. Key trial personnel must be available to assist the monitors during these visits.

The investigator must give the monitor access to relevant hospital or clinical records, to confirm their consistency with the case report form entries. No information in these records about the identity of the patients will leave the study center. Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of primary efficacy and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan.

10.7.2 Auditing Procedure

In addition to the routine monitoring procedures, the Sponsor or the regulatory authority can conduct an audit or an inspection (during the study or after its completion) to evaluate compliance with the protocol and the principles of Good Clinical Practice.

The investigator agrees that representatives of the Sponsor and Regulatory Authorities will have direct access, both during and after the course of this study, to audit and review all study-relevant medical records.

10.7.3 Retention of Documents

The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, including laboratory data, and keep a copy of the signed informed consent form. All information on case report forms must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before trial start and will be recorded directly on the case report forms, which will be documented as being the source data.

10.8 Disclosure of Information

All information provided to the investigator by Ampio Pharmaceuticals, Inc. or their designee, will be kept strictly confidential. No disclosure shall be made except in accordance with a right of publication granted to the investigator.

No information about this study or its progress will be provided to anyone not involved in the study other than to Ampio Pharmaceuticals, Inc or its authorized representatives, or in confidence to the IRB, or similar committee, except if required by law.

10.9 Discontinuation of the Study

Ampio Pharmaceuticals, Inc., may terminate the study at any time upon immediate notice from the Sponsor to all investigators for any reason, including the Sponsor’s belief that discontinuation of the study is necessary for the safety of patients.

10.10 Study Report, Publication Policy and Archiving of Study Documentation

10.10.1 Study Report and Publication Policy

Depending on the outcome of the study, an ICH-compliant integrated clinical and statistical report may be prepared upon completion of the study and data analysis. The results of the study may also be published in a relevant peer-reviewed journal, with authorship status and ranking designated according to the acknowledged contributions of participating investigators, institutions and the Sponsor.

10.10.2 Study Documents

The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, questionnaires, including laboratory data, etc., and keep a copy of the signed informed consent form. All information on the e-case report forms must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before trial start and will be recorded directly on the e-case report forms, which will be documented as being the source data.

10.10.3 Archiving of Documents

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations. The Sponsor will notify the investigator(s)/institution(s) when the study-related records are no longer required. The investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

- IRB/IEC/REB approvals for the study protocol and all amendments
- All source documents and laboratory records
- CRF copies (electronic copies on a CDROM)
- Patients' informed consent forms (with study number and title of trial)
- FDA form 1572
- Any other pertinent study document.

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