A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED PHASE I STUDY TO EVALUATE THE SAFETY AND EFFICACY OF AMPION IN PATIENTS WITH PROLONGED RESPIRATORY SYMPTOMS DUE TO COVID-19 (LONG-COVID)

STUDY PROTOCOL

STUDY NUMBER: AP-018

NCT04880161

15 May 2021

CLINICAL STUDY PROTOCOL TITLE PAGE

Protocol Title:	A Randomized, Double-Blinded, Placebo-Controlled Phase I Study to Evaluate the Safety and Efficacy of Ampion in Patients with Prolonged Respiratory Symptoms due to COVID-19 (Long-COVID)
Study Number:	AP-018
Investigational Product:	Ampion
Drug Development Phase:	Phase I
Indication:	Prolonged Respiratory Symptoms due to COVID-19 (Long-COVID)
Route of Administration:	Inhalation
Regulatory Agency Identifier:	IND 19828
Sponsor:	Ampio Pharmaceuticals, Inc. 373 Inverness Parkway Englewood, CO 80112
Date:	15 May 2021

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-018 dated 15 May 2021 and agree to meet all obligations of Ampio Pharmaceuticals Inc. as detailed in all applicable regulations and guidelines.

Signed By:

1 PROTOCOL SUMMARY

1.1 Protocol Synopsis

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

Title of Study:

A Randomized, Double-Blinded, Placebo-Controlled Phase I Study to Evaluate the Safety and Efficacy of Ampion in Patients with Prolonged Respiratory Symptoms due to COVID-19 (Long-COVID)

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, and has overwhelmed healthcare systems. Increasing numbers of people with COVID-19 are experiencing the lingering effects of COVID-19 and continue to have prolonged respiratory complications months after the onset of the disease, also known as Post-Acute Sequelae of SARS-CoV-2 (PASC), long-COVID, and/or long-hauler patients.

The SARS-CoV-2 virus is transmitted through the respiratory system, which can cause a severe dysregulation of the immune response and damage in the lungs. Chronic, prolonged inflammation of the lungs maybe responsible for a myriad of continuing respiratory signs and symptoms post-infection, including cough, shortness of breath, chest discomfort, low exercise tolerance and low blood oxygen saturation.

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 long-COVID patients.

This study aims to evaluate the safety of Ampion and the clinical outcomes in patients with long-COVID. This is the first study for at-home use of Ampion inhaled treatment for long-COVID patients and will be used to inform decisions for the clinical development of Ampion.

Lead Investigator(s):

Dr. Michael Barber MD

Indication:

Prolonged Respiratory Symptoms due to COVID-19 (Long-COVID)

Number of Sites:

Up to 5 sites

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

Number of Patients:

Sample size is approximately 15 participants per treatment arm

Treatment Groups:

There are two planned treatment arms randomized 1:1, active to control:

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

Objectives and Endpoints:

Objective	Endpoint
Primary	
Assess the effect of Ampion compared to placebo on safety	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 28
Exploratory	
Assess the effect of Ampion compared to placebo on symptom improvement	• Change in FDA Assessment of 14 Common COVID-19-Related Symptoms questionnaire from baseline to Day 7; Day 28; Day 60 • Change in Borg Dyspnoea Scale (mBDS) from baseline to Day 7; Day 28; Day 60 • Change in all-cause mortality
Assess the effect of Ampion compared to placebo on pulmonary function	• Change in blood oxygen saturation from baseline to Day 7; Day 28; Day 60 • Change in six-minute walk test (6MWT) score from baseline to Day 7; Day 28; Day 60
Assess the effect of Ampion compared to placebo on global impression	• Change in Quality-of-Life Assessment using SF-36 from baseline to Day 7; Day 28; Day 60 • Change in Fatigue questionnaire from baseline to Day 7; Day 28; Day 60 • Change in Sleep Disturbance questionnaire from baseline to Day 7; Day 28; Day 60
Assess the effect of Ampion compared to placebo on radiographic findings	• Change in chest imaging on ground-glass opacity, local patchy shadowing, bilateral patchy shadowing, and/or interictal abnormalities from baseline to Day 60

Study Design:

Overall Design

This is a Phase I randomized, double-blinded, placebo-controlled study in adult participants with prolonged respiratory symptoms due to COVID-19 (long-COVID).

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 inclusion/exclusion criteria prior to any study procedures. If the participant is eligible and interested after this review, the participant will sign the appropriate informed consent document(s) prior to completion of any study procedures.

Double-Blind Treatment and Assessment

Participants will be randomized 1:1 to active treatment or placebo control using a random allocation sequence. 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	Ampion Inhalation for 5-day
Control	Placebo Inhalation for 5-days

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) repeated daily for 5 days
- Complete safety monitoring and data collection
- Study visit types are described in the tables below:

Study Day	Visit Description	Visit Type
1	Baseline safety/efficacy measures	Conducted in outpatient clinic
1-5	Ampion inhalation treatment, safety measures	Conducted at home
7, 28, 60	Post-treatment safety/efficacy measures	Conducted in outpatient clinic

Post-Treatment Follow-up

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

Disclosure Statement

This treatment study will be blinded to the investigator(s) and subject(s)

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.

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

Diagnosis and Main Criteria for Inclusion:

1. Male or female adults: ≥ 18 years.
2. Must have a clinical diagnosis of COVID-19 at least 4 weeks prior to the screening date, with at least one of clinical symptoms (e.g., fever ≥ 38°C, fatigue, cough) and a positive result by the reverse-transcription polymerase chain reaction (RT-PCR) testing or equivalent.
3. Experiencing at least two COVID-19 respiratory symptoms with a score of two or higher using the FDA Assessment of 14 Common COVID-19-Related Symptoms questionnaire for at least 4 weeks (28 days) after initial positive COVID-19 diagnosis: cough, sore throat, runny/stuffy nose, shortness of breath (difficulties breathing), tightness of chest, low exercise tolerance.
4. Able to bear weight and ambulate a minimum of 10 meters distance.
5. Women of childbearing potential and their partner must agree to use at least one highly effective method of contraception (e.g., hormonal contraceptives [implants, injectables, combination oral contraceptives, transdermal patches, or contraceptive rings], intrauterine devices, bilateral tubal occlusion, or sexual abstinence) for the duration of the study.
6. Informed consent obtained from the patient.

Main Criteria for Exclusion:

1. Subjects who require hospitalization.
2. Patient has severe chronic obstructive or restrictive pulmonary disease (COPD) as defined by prior pulmonary function tests, chronic renal failure, or significant liver abnormality (e.g., cirrhosis, transplant, etc.).
3. History of Chronic Fatigue Syndrome prior to COVID-19 infection.
4. Patient is on chronic immunosuppressive medication.
5. Patient requires surgery that could be life-threatening within the study window.
6. 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-acetyl tryptophan, sodium caprylate).
7. Patient has known pregnancy or is currently breastfeeding.
8. 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).
9. Clinically significant findings via electrocardiogram (ECG), including acute myocardial infarction, acute ischemic changes, atrial fibrillation, atrial flutter, paced rhythms in individuals who have undergone permanent pacemaker placement, evidence of prior infarction, unchanged stable conduction abnormalities e.g., right bundle branch block, or any other finding which does not significantly impact mortality.
10. Pre-existing co-morbid condition(s) preventing outcome assessments, e.g. disease or condition that would prevent ability to transfer and walk for 6 minutes, prior to confirmed COVID-19 diagnosis (assisted walking devices are acceptable)

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

11. As a result of the medical review and screening investigation, the Principal Investigator considers the patient unfit for the study.

Test Product, Dose, and Mode of Administration:

Participants in the active arm will inhale nebulized Ampion (8 mL) administered four times daily, every four to six hours, for 5 days. Nebulized drug will be delivered using the Aerogen Solo Nebulizer System with the Aerogen Solo Adaptor (FDA 510K K133360) manufactured by Aerogen Limited, Galway, Ireland. The Aerogen Solo Adaptor is a vibrating mesh nebulizer with a drug reservoir used for delivery of respiratory therapy, including the hand-held Aerogen Ultra.

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

Reference Therapy, Dose and Mode of Administration:

Participants in the control arm will inhale nebulized placebo (8 mL) administered four times daily for 5 days. Nebulized placebo will be delivered using the Aerogen Solo Nebulizer System with the Aerogen Solo Adaptor (FDA 510K K133360) manufactured by Aerogen Limited, Galway, Ireland. The Aerogen Solo Adaptor is a vibrating mesh nebulizer with a drug reservoir used for delivery of respiratory therapy, including the hand-held Aerogen Ultra.

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

Study Duration:

Treatment: 5 days

Follow-up: 60 days

1.2 Schedule of Assessments

Visits will be conducted as described:

	Screening	Treatment					Post-Treatment Follow Up		
Study Day[1]	**-3 to 1**	**1**[2]	**2**	**3**[2]	**4**	**5**[2]	**7**	**28**	**60**
Visit Window (+/- # days)	--	--	--	--	--	--	3	3	3
COVID-19 tests	X								
Informed consent	X								
Medical history and pre-existing conditions	X								
Electrocardiogram (ECG)	X								
Pregnancy test	X								
Inclusion/exclusion criteria	X	X							
Demographics	X								
Randomization		X							
Treatment[1, 2]		X	X	X	X	X			
Health check[1, 2]		X		X		X			
Blood oxygen saturation		X					X	X	X
Assessment of 14 Common COVID-19-related symptoms questionnaire		X					X	X	X
Borg Dyspnoea Scale (mBDS)		X					X	X	X
Walk test		X					X	X	X
Chest x-ray imaging		X[3]							X
Concomitant medications[2]		X	X	X	X	X	X	X	X
Quality-of-Life Assessment SF-36		X					X	X	X
PROMIS Fatigue questionnaire		X					X	X	X
PROMIS Sleep Disturbance questionnaire		X					X	X	X
Adverse events[2]		X	X	X	X	X	X	X	X

[1] Day 1, baseline assessments (health check, blood oxygen saturation, assessment of symptoms/questionnaires, mBDS, walk test, and chest x-ray) should occur before the first dose of treatment. These tests and the first dose of treatment will occur at the clinic. Treatment for the remainder of Day 1 and Days 2, 3, 4, 5 will occur at home.

[2] Day 1 post-first dose, Day 3 and Day 5 health check, concomitant medications and adverse event collection will occur via telephone visit.

[3] Baseline chest x-ray may be performed after Screening, up to 3 days prior to Day 1.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6MWT	Six-Minute Walk Test
AE	Adverse event
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
HSA	Human serum albumin
ICH	International conference on harmonization
IRB	Investigational review board
ITT	Intent to treat
IV	Intravenous
mBDS	Modified Borg Dyspnoea Scale
PASC	Post Acute Sequelae SARS-CoV-2
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 resulted in the pandemic spread of coronavirus disease 2019 (COVID-19), which has a high rate of infection, has a high rate of hospitalization, and has overwhelmed healthcare systems. Increasing numbers of people with COVID-19 are experiencing the lingering effects of COVID-19 and continue to have prolonged respiratory complications months after the onset of the disease, now clinically known as Post-Acute Sequelae SARS-CoV-2 (PASC), long-COVID, and/or long-hauler patients. Identifying drugs and therapies that address the prolonged respiratory complications related to COVID-19 infection is critical for patients to resolve the symptoms.

This is a Phase I randomized, double-blinded, placebo-controlled trial to evaluate inhaled Ampion for adults with long-COVID. Ampion is being developed as an immunomodulatory therapy with anti-inflammatory effects that could provide a beneficial treatment for COVID-19 patients with these symptoms, including cough, shortness of breath, chest discomfort, low exercise tolerance and low blood oxygen saturation.

This study aims to investigate the safety and the clinical outcomes in these patients with long-COVID after a COVID-19 infection when treated with Ampion. The data from this study will inform decisions for the clinical development of 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 the continuing, chronic, low level inflammation observed in patients with long-COVID respiratory symptoms.

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

Ampion or placebo (saline) will be delivered by inhalation using a nebulizer as a 32mL daily dose administered four times daily (8mL per treatment) every four to six hours for five days. Ampion or placebo is presented as a sterile liquid in a glass vial. For each use, the contents of are transferred to the drug reservoir in the nebulizer and nebulized continuously until the drug is fully administered.

Ampion is aerosolized for inhalation using the Aerogen Ultra handheld nebulizer (Aerogen Limited, Galway, Ireland, FDA 510K K133360), which consists of the Aerogen Pro-X Controller and the Aerogen Ultra handheld unit. The system is a portable medical device for single patient use indicated for aerosolization of physician-prescribed solutions for inhalation. Ampion is nebulized using the continuous setting on the Aerogen controller, and the study drug is nebulized until it is fully aerosolized.

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 sometimes requiring supplemental oxygen in the more severe cases of the disease.

SARS-CoV-2 is transmitted through the respiratory system and can cause severe dysregulation of the immune response, particularly involving molecular pathways in inflammation (Coperchini 2020). Chronic or prolonged inflammation of the lungs maybe responsible for a myriad of respiratory signs and symptoms experienced by patients after a COVID-19 infection. Chest x-rays and computerized tomography (CT) scans reveal disturbing patterns of perhaps extensive fibrosis and potential loss of elasticity and oxygen diffusion capacity (Alhiyari 2020, Lechowicz 2020, Vasarmidi 2020).

A review of over 3000 patients recovering from COVID-19 found that many still suffered from respiratory complications due to COVID-19, including dyspnoea, cough, and persistent low oxygen (Nalbandian 2021). Furthermore, these complications were accompanied by a decline in quality of life, fatigue, and loss of sleep.

Respiratory symptoms after a COVID-19 infection include shortness of breath, cough, chest discomfort, low exercise tolerance and low oxygen saturation, all of which point to potential inflammation related complication sequalae. Infiltrating or resident cells in the immune system (e.g., macrophages, peripheral blood mononuclear cells, etc.) maybe responsible for the development of these respiratory long-term consequences.

Some treatments (remdesivir, dexamethasone, bamlanivimab) are being used in patients with immediate, short-term COVID-19 symptoms, but no treatments have been indicated for patients with long-COVID. There is a continued need to identify treatments that can interrupt chronic inflammation to address the needs of patients with long-COVID.

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

Ampion has been administered via inhalation to humans with severe COVID-19 in a Phase I clinical trial utilizing inhaled Ampion in treating respiratory distress in patients as a result of COVID-19 (AP-014). The results from this study to date (n=27 subjects) indicate inhaled Ampion may be safe and well-tolerated with no differences in the incidence, frequency, and severity of adverse events for patients treated with Ampion compared to control. Preliminary results from the AP-014 Phase I trial of inhaled Ampion indicate:

- **Ampion demonstrated an improvement in all-cause mortality in COVID-19 patients compared to standard of care (SOC).** A lower all-cause mortality rate of 8% is observed for the Ampion treatment group, compared to 21% in standard of care alone.
- **Patients who received Ampion required less hospitalization time.** The average hospital length of stay was 7 days for the Ampion group compared to 11 days for standard of care patients.
- **Patients who received Ampion required less oxygen** than standard of care alone, and 86% of Ampion patients were stable or had improvement compared to 75% of SOC patients.
- **More patients who received Ampion were stable or had improvement** on a scale of clinical improvement compared to standard of care alone. By day 5, 86% of patients who received Ampion were stable or had improvement compared to 75% of standard of care patients. This trend in improvement with Ampion treatment is noted as early as day 2 and continues to day 5.
- **Adverse events were the same between Ampion and standard of care**, and no drug-related serious adverse events have been reported.

Ampion has also been administered via intravenous (IV) infusion to humans with severe COVID-19 in a Phase I safety study (AP-016). The final results from this study (n=10 subjects) found Ampion to be safe and well-tolerated with no differences in the incidence, frequency, and severity of adverse events for patients treated with Ampion compared to control.

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 noted to date.

Additionally, Ampion is derived from pharmaceutical grade 5% human serum albumin (HSA), a human blood product approved by FDA for IV infusion, that has a safety profile including more than 50 years of use in many settings of perioperative medicine including hypovolemia, shock, burns, surgical blood loss, sepsis, and ARDS (Farag, 2016).

2.4 Study Rationale

2.4.1 Study Design

This protocol has been designed to evaluate the safety and efficacy of inhaled Ampion in adults with prolonged respiratory complications after COVID-19 infection. The data from this study will inform decisions for the clinical development of Ampion.

2.4.2 Study Background

COVID-19 infection is associated with respiratory findings which may be related to inflammation of the 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 complications.

This study focuses on the patients who have long-term symptoms and clinical signs related to continued respiratory illness after the viral infection is cleared. The SARS-Cov-2 virus is transmitted through the respiratory system, which can cause a severe dysregulation of the immune response and damage in the lungs (Coperchini 2020). Chronic, prolonged inflammation of the lungs maybe responsible for a myriad of continuing respiratory signs and symptoms post-infection, including cough, shortness of breath, chest discomfort, low exercise tolerance and low blood oxygen saturation.

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 of patients with continuing symptoms (Thomas 2020). This study evaluates the safety and tolerability of IV Ampion treatment for patients with prolonged respiratory symptoms which continue after the COVID-19 infection has cleared.

2.4.3 Preclinical Data

The proposed treatment regimen uses the smallest amount of Ampion that may provide a positive clinical outcome while minimizing safety risks. The safety of exposure is leveraged using the systemic exposure to Ampion's active ingredients in 5% HSA, which has been given to patients with ARDS and other critical indications (Polito 2013). Ampion is derived from the low-molecular weight filtrate of pharmaceutical grade 5% HSA and consists of small molecules that can move freely between the lung and bloodstream.

The potential for local toxicity of Ampion has been evaluated in an *in vivo* preclinical study using the inhaled route of administration. In the *in vivo* toxicity study, there were no clinical signs observed that were considered related to treatment with Ampion. There were no treatment-related effects on body weight or food consumption during the treatment period. There were no treatment-related changes in hematology, clinical biochemistry or respiratory tract histopathology that could be attributed to treatment with Ampion. The inhalation exposure of Ampion at the highest dose for 5 days was well tolerated and produced no apparent changes in any of the parameters evaluated. The maximum feasible dose achieved in the study was established as the no observable adverse effect level (NOAEL), which corresponded to approximately 15 times the proposed clinical dose of Ampion in this study.

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 (Del Valle 2020, Yang 2017, Channappanavar 2017).

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).

2.4.4 Dosing Rationale

The proposed daily dose of 32mL of Ampion delivered via inhalation is based on Ampion's *in vitro* effect to reduce cytokine (TNFα) combined with results from *vivo* safety study and experience from the Phase I study AP-014. Nebulized Ampion will be administered as an individual 8mL dose four times daily (q.i.d.) every 4-6 hours (q4-q6), which is a daily dose of 32mL. This treatment regimen is repeated for 5 days. This dosing uses a dose of Ampion that may provide a positive clinical outcome while minimizing safety risks.

2.5 Benefit and Risk Assessment

Patients with prolonged respiratory complications after a COVID-19 infection 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 inhaled Ampion treatment are considered low and are based preclinical safety studies, inhalation use in COVID-19 patients, IV use in COVID-19 patients, intra-articular use for another indication (osteoarthritis), and decades of use of the sole starting material of the product, IV HSA, in severely ill patients. 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 patients with prolonged respiratory complications after a COVID-19 infection, the overall benefit-risk assessment of this study is considered favorable. As an immunomodulatory drug, Ampion may interrupt the inflammatory cascade for patients with prolonged respiratory complications after a COVID-19 infection which could improve clinical outcomes.

3 STUDY DESIGN

3.1 Study Design Overview

This is a randomized, double-blinded, placebo-controlled Phase I trial to evaluate the safety and efficacy of a 5-day Ampion inhalation treatment in participants with prolonged respiratory complications after a COVID-19 infection.

Participants with COVID-19 infection will be screened for eligibility and consented. Participants (n=30) will be randomized in one of two groups, active (n=15) or placebo control (n=15) and randomized 1:1 following an equal allocation. The treatment arm will receive Ampion inhalation treatment, and the control arms will receive saline inhalation treatment. Participants will be followed for 60 days.

3.2 Study Objectives

3.2.1 Primary Objective

The primary trial objective is to evaluate the safety and tolerability of inhaled Ampion versus placebo control in adult participants with prolonged respiratory complications after COVID-19 infection.

3.2.2 Secondary Objectives

The secondary trial objectives evaluate the efficacy of inhaled Ampion versus placebo control in improving the clinical course and outcomes of participants with prolonged respiratory complications after a COVID-19 infection.

3.3 Study Endpoints

3.3.1 Primary Endpoint

Adverse events (AEs) will be evaluated from baseline through Day 60. The primary endpoint is incidence and severity of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 28 and Day 60.

3.3.2 Exploratory Endpoints

Exploratory efficacy endpoints assess the effect of inhaled Ampion compared to placebo on the clinical outcomes for participants with prolonged respiratory complications after a COVID-19 infection as follows:

Objective	Endpoint
Exploratory	
Assess the effect of Ampion compared to placebo on symptom improvement	• Change in FDA Assessment of 14 Common COVID-19-Related Symptoms questionnaire from baseline to Day 7; Day 28; Day 60 • Change in Borg Dyspnoea Scale (mBDS) from baseline to Day 7; Day 28; Day 60 • Change in all-cause mortality
Assess the effect of Ampion compared to placebo on pulmonary function	• Change in blood oxygen saturation from baseline to Day 7; Day 28; Day 60 • Change in six-minute walk test (6MWT) score from baseline to Day 7; Day 28; Day 60
Assess the effect of Ampion compared to placebo on global impression	• Change in Quality-of-Life Assessment using SF-36 from baseline to Day 7; Day 28; Day 60 • Change in Fatigue questionnaire from baseline to Day 7; Day 28; Day 60 • Change in Sleep Disturbance questionnaire from baseline to Day 7; Day 28; Day 60
Assess the effect of Ampion compared to placebo on radiographic findings	• Change in chest imaging on ground-glass opacity, local patchy shadowing, bilateral patchy shadowing, and/or interictal abnormalities from baseline to Day 60

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 1:1 to active treatment or placebo control, following an equal allocation to treatment arms as follows:

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

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.

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, as the study progresses, of IV Ampion. 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

This is a Phase I study and is not intended to be powered for the primary endpoint. This trial is designed to enroll up to 30 subjects, randomized to active treatment or placebo control, following an equal allocation to treatment arms (n=15 subjects per arm).

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 prolonged respiratory symptoms consistent with COVID-19. Treatment of COVID-19 depends on the stage of disease with a continued hyperinflammatory state observed that are thought to lead to prolonged clinical complications. Treatment with immunomodulators at this point in the disease would be more effective than anti-viral treatments.

The population of participants with prolonged respiratory complications after an initial COVID-19 infection were selected for this study to evaluate the efficacy of Ampion as an immunomodulatory therapy that may improve the clinical outcome by addressing the chronic, prolonged inflammation in the lungs that maybe responsible for a myriad of continuing respiratory issues, including cough, shortness of breath, chest discomfort, low exercise tolerance and low blood oxygen saturation.

4.4 Inclusion Criteria

Patients should fulfill all the following inclusion criteria:

1. Male or female adults: ≥ 18 years.
2. Must have a clinical diagnosis of COVID-19 at least 4 weeks prior to the screening date, with at least one of clinical symptoms (e.g., fever ≥ 38°C, fatigue, cough) and a positive result by the reverse-transcription polymerase chain reaction (RT-PCR) testing or equivalent.
3. Experiencing at least two COVID-19 respiratory symptoms with a score of two or higher using the FDA Assessment of 14 Common COVID-19-Related Symptoms questionnaire for at least 4 weeks (28 days) after initial positive COVID-19 diagnosis: cough, sore throat, runny/stuffy nose, shortness of breath (difficulties breathing), tightness of chest, low exercise tolerance.
4. Able to bear weight and ambulate a minimum of 10 meters distance.
5. Women of childbearing potential and their partner must agree to use at least one highly effective method of contraception (e.g., hormonal contraceptives [implants, injectables,

combination oral contraceptives, transdermal patches, or contraceptive rings], intrauterine devices, bilateral tubal occlusion, or sexual abstinence) for the duration of the study.

6. Informed consent obtained from the patient.

4.5 Exclusion Criteria

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

1. Subjects who require hospitalization.
2. Patient has severe chronic obstructive or restrictive pulmonary disease (COPD) as defined by prior pulmonary function tests, chronic renal failure, or significant liver abnormality (e.g., cirrhosis, transplant, etc.).
3. History of Chronic Fatigue Syndrome prior to COVID-19 infection.
4. Patient is on chronic immunosuppressive medication.
5. Patient requires surgery that could be life-threatening within the study window.
6. 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-acetyl tryptophan, sodium caprylate).
7. Patient has known pregnancy or is currently breastfeeding.
8. 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).
9. Clinically significant findings via electrocardiogram (ECG), including acute myocardial infarction, acute ischemic changes, atrial fibrillation, atrial flutter, paced rhythms in individuals who have undergone permanent pacemaker placement, evidence of prior infarction, unchanged stable conduction abnormalities e.g., right bundle branch block, or any other finding which does not significantly impact mortality.
10. Pre-existing co-morbid condition(s) preventing outcome assessments, e.g., disease or condition that would prevent ability to transfer and walk for 6 minutes, prior to confirmed COVID-19 diagnosis (assisted walking devices are acceptable)
11. As a result of the medical review and screening investigation, the Principal Investigator considers the patient unfit for the study.

5 STUDY PLAN, PROCEDURES, AND ASSESSMENTS

5.1 Study Plan

Patients with confirmed COVID-19 infection will be screened for up to 72 hours from referral to an outpatient clinic. Patients meeting the selection criteria will be approached for consent in the study. If consented, participants will be randomized in one of two groups, active or control. Within each group, participants are randomized 1:1 following an equal allocation to treatment arms to either active or control arms of the study as follows:

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

Participants who have been randomized into the control arm will receive a daily dose (32mL/day) of placebo (saline) inhalation treatment. Participants who have been randomized into the active treatment arm will receive a daily dose (32mL/day) of inhaled Ampion. Inhaled treatment (Ampion or placebo) will be delivered four times daily, every four to six hours using the nebulizer in equally divided doses of 8mL. This treatment regimen is repeated for 5 days.

The health check vital signs and baseline assessments for blood oxygen saturation, assessment of symptoms/questionnaires, mBDS, walk test, and chest x-ray should occur before the first dose of treatment. These tests and the first dose of treatment will occur at the clinic. Treatment for the remainder of Day 1 and Days 2, 3, 4, 5 will occur at home. Health checks, concomitant medications, and adverse events for Day 1 post-first dose and Days 3 and 5 will be collected via telephone visit.

Safety will be assessed by recording adverse events and with a health check during treatment. Safety is assessed by recording adverse events for the length of the study (60 days).

Efficacy will be assessed by recording the effects of inhalation Ampion compared to placebo on the clinical outcomes for participants with prolonged respiratory complications after a COVID-19 infection using the following clinical outcomes: time to symptom improvement, percentage of participants demonstrating improvement, change in Borg Dyspnoea Scale, time to sustained recovery, blood oxygen saturation, a walk test, and chest x-ray. Assessments for clinical outcomes are performed from baseline to Day 7, Day 28, and Day 60.

Exploratory will be assessed by recording the effects of inhalation Ampion compared to placebo on the clinical outcomes for participants with prolonged respiratory complications after a COVID-19 infection using the following clinical outcomes: Quality of life assessment using SF-36, PROMIS fatigue questionnaire and PROMIS sleep disturbance questionnaire. Assessments for clinical outcomes are performed from baseline to Day 7, Day 28, Day 60.

5.2 Description of Study Visits

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

Study Day	Visit Description	Visit Type
1	Baseline safety/efficacy measures	Conducted in outpatient clinic
1-5	Ampion inhalation treatment, safety measures	Conducted at home
7, 28, 60	Post-treatment safety/efficacy measures	Conducted in outpatient clinic

5.2.1 Screening (-3 Days to Day 1)

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.
- Confirm date of initial positive COVID-19 test.
- Medical history, pre-existing conditions, ECG, and comorbidities. Include the symptom onset date for COVID-19 symptoms.
- Obtain informed consent before the starting any study specific procedures, including COVID testing.
- Demographics (age, sex, race, height and weight)
- Pregnancy test for women of childbearing age.

5.2.2 Baseline (Day 1)

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

- Confirm eligibility (review inclusion/exclusion criteria)
- Randomize patient to study arm. Start inhalation treatment within 24 hours.
- Vital signs
- Blood oxygen saturation
- Assessment of 14 Common COVID-19-related symptoms questionnaire
- Borg Dyspnoea Scale and walk test
- Chest x-ray imaging
- Record concomitant medications/therapies
- Quality-of-life assessment SF-36
- PROMIS fatigue and PROMIS sleep disturbance questionnaires
- Record adverse events

5.2.3 Treatment Period (Day 1 to Day 5)

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

- Begin inhalation treatment of study intervention (active or placebo) daily within 24 hours of randomization. Subjects will administer inhaled treatment (active or placebo) through nebulization daily as described in Section 2.1.

- Subjects will have a general health check conducted via telephone or text message on Day 1, 3, 5.
- Record concomitant medications/therapies
- Record adverse events

5.2.4 Post Treatment Follow Up (Day 7)

The following procedures will be performed at Day 7 (endpoint):

- Blood oxygen saturation
- Assessment of 14 Common COVID-19-related symptoms questionnaire
- Borg Dyspnoea Scale and walk test.
- Record concomitant medications/therapies
- Quality-of-life assessment SF-36
- PROMIS fatigue and PROMIS sleep disturbance questionnaires
- Record adverse events

5.2.5 Post-Treatment Follow-Up (Day 28)

The following procedures will be performed at follow-up visits (Day 28):

- Blood oxygen saturation
- Assessment of 14 Common COVID-19-related symptoms questionnaire
- Borg Dyspnoea Scale and walk test
- Concomitant medications/therapies
- Quality-of-life assessment SF-36
- PROMIS fatigue and PROMIS sleep disturbance questionnaires
- Record adverse events

5.2.6 Post-Treatment Follow-Up (Day 60)

The following procedures will be performed at follow-up visits (Day 60) end of study:

- Blood oxygen saturation
- Assessment of 14 Common COVID-19-related symptoms questionnaire
- Borg Dyspnoea Scale and walk test
- Chest x-ray imaging
- Record Concomitant medications/therapies.
- Quality-of-life assessment SF-36
- PROMIS fatigue and PROMIS sleep disturbance questionnaires
- Record adverse events

5.3 Assessment Methods

Demographic Data

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

Health Check

A health check will be conducted at the clinic at baseline to include the measurement of vital signs (i.e., heart rate, blood pressure, respiratory rate, temperature). A general health check will be conducted Day 1 post-first dose, Day 3, and Day 5 via telephone visit.

Blood Oxygen Saturation (SpO2)

SpO2 will be collected at baseline and Days 7, 28, and 60 using a pulse-oxygen measuring device.

Assessment of 14 Common COVID-19-related symptoms questionnaire

The FDA assessment of 14 Common COVID-19- related symptoms questionnaire will be conducted at baseline, Day 7, Day 28, and Day 60 as follows:

Example items *For items 1–10, sample item wording could be: "What was the severity of your **[insert symptom]** at its worst over the last 24 hours?"*	**Example response options and scoring***
1. Stuffy or runny nose	None = 0 Mild = 1 Moderate = 2 Severe = 3
2. Sore throat	
3. Shortness of breath (difficulty breathing)	
4. Cough	
5. Low energy or tiredness	
6. Muscle or body aches	
7. Headache	
8. Chills or shivering	
9. Feeling hot or feverish	
10. Nausea (feeling like you wanted to throw up)	
11. How many times did you **vomit (throw up)** in the last **24 hours**?**	I did not vomit at all = 0 1–2 times = 1 3–4 times = 2 5 or more times = 3

continued

Table 1, continued

12. How many times did you have **diarrhea (loose or watery stools)** in the last **24 hours**?**	I did not have diarrhea at all = 0 1–2 times = 1 3–4 times = 2 5 or more times = 3
13. Rate your sense of **smell** in the last **24 hours**	My sense of smell is THE SAME AS usual = 0 My sense of smell is LESS THAN usual = 1 I have NO sense of smell = 2
14. Rate your sense of **taste** in the last **24 hours**	My sense of taste is THE SAME AS usual = 0 My sense of taste is LESS THAN usual = 1 I have NO sense of taste = 2

* Note: Score values are included in the table for ease of reference. FDA cautions against including the score values within the response options presented to trial subjects to avoid confusing subjects.
** The response options shown for items 11 and 12 are intended only for use with a 24-hour recall period.

Time to Sustained Recovery

The time to sustained recovery will be evaluated using the FDA Assessment of 14 Common COVID-19-Related Symptoms questionnaire. Time to sustained recovery is defined as the first day on which the patient achieves a state of 'none' or '0' using the FDA assessment.

Borg Dyspnoea Scale

Borg Dyspnoea Scale will be conducted at baseline, Day 7, Day 28, and Day 60. This scale asks you to rate the difficulty of your breathing it starts at number 0 where you are breathing is causing you no difficulty at all and progresses through to number 10 where your breathing difficulty is maximal.

Walk Test

A six-minute walk test will be conducted at baseline, Day 7, Day 28, and Day 60. The walk test is conducted as follows:

- Flat, straight corridor 30 m (100 feet) in length
- Turnaround points marked with a cone
- Patient should wear comfortable clothes and shoes
- Patient rests in chair for at least 10 minutes prior to test (ie, no warm-up period)

- Heart rate and pulse oxygen saturation (SpO_2) should be monitored throughout the test
- If the patient is using supplemental oxygen, record the flow rate and type of device
- Have patient stand and rate baseline dyspnea and overall fatigue using Borg scale*[1]
- Set lap counter to zero and timer to six minutes
- Instruct the patient: Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Pivot briskly around the cone.
- Standardized encouragement statements should be provided at one-minute intervals, such as "You are doing well. You have _ minutes to go" and "Keep up the good work. You have _ minutes to go."
- At the end of the test, mark the spot where the patient stopped on the floor
- If using a pulse oximeter, measure the pulse rate and SpO_2 and record
- After the test record the Borg*[1] dyspnea and fatigue levels
- Ask, "What, if anything, kept you from walking farther?"
- Calculate the distance walked and record

1. *Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982; 14:377.*
2. *American Thoracic Society. ATS statement: Guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166:111.*
3. *Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J 2014; 44:1428.*

Chest X-Ray Imaging

Chest x-ray images will be taken at baseline and Day 60. The standard chest examination consists of a PA (posterior anterior) and lateral chest x-ray.

Concomitant Medications

Concomitant medications will be collected: prior concomitant medications, concomitant medications through study exit.

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.

Quality-of-Life Assessment SF-36

Quality of life assessment SF-36 questionnaire with 36 questions health survey for subject's general health will be conducted at baseline, Day 7, Day 28, and Day 60.

PROMIS Fatigue and Sleep Questionnaires

PROMIS fatigue and PROMIS sleep questionnaires will be conducted at baseline, Day 7, Day 28, and Day 60.

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 nebulization 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

Participants randomized to the active arm will receive inhaled Ampion through nebulization daily for 5 days. Participants randomized to the control arm will receive placebo through nebulized inhalation 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

Study treatment (Ampion or placebo) will be provided as a solution in vials ready for human use and treatment administration. The participant will be trained to self-administer the study treatment. Treatment should start within 24 hours of randomization and should terminate on last treatment day (Day 5).

Study treatment (Ampion or placebo) will be delivered by inhalation using a nebulizer as a 32mL daily dose administered four times daily (8mL per treatment) every four to six hours for five days.

Study treatment (Ampion or placebo) is presented as a sterile liquid in a glass vial. For each use, the contents of are transferred to the drug reservoir in the nebulizer and nebulized continuously until the drug is fully administered.

Study treatment (Ampion or placebo) is aerosolized for inhalation using the Aerogen Ultra handheld nebulizer (Aerogen Limited, Galway, Ireland, FDA 510K K133360), which consists of the Aerogen Pro-X Controller and the Aerogen Ultra handheld unit. The system is a portable medical device for single patient use indicated for aerosolization of physician-prescribed solutions for inhalation. Ampion is nebulized using the continuous setting on the Aerogen controller, and the study drug is nebulized until it is fully aerosolized.

Product administration may be temporarily suspended until resolution if a serious, or sudden side effects are observed. 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) and/or corticosteroids.

As described in Section 6.1, if any 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

Study treatment (Ampion or placebo) should be stored at room temperature (59º – 77ºF or 15º – 25ºC) in a secure area with restricted access while at the clinic. 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. Study drug stored at home should be stored at room temperature.

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 hospitalization or 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 until study exit 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.

This is a Phase 1 study and is not intended to be powered for the primary endpoint. The study will evaluate safety, tolerability, and efficacy of Ampion in improving the clinical course and outcome of adults with prolonged respiratory complications after a COVID-19 infection.

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

An interim analysis for safety and efficacy endpoints will be performed once all subjects have completed their Day 7 assessments.

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 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 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 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 until study exit (or 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 treatment arm (active or control).

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

Adverse events (AEs) will be evaluated from baseline through Day 60. The primary endpoint is incidence and severity of adverse events (AEs) and serious adverse events (SAEs) from baseline to Day 28.

There will be no statistical tests for incidence and severity of AEs. AEs will be tabulated using descriptive statistics and shift tables and presented for safety.

9.4.2 Exploratory Endpoints

Exploratory efficacy endpoints assess the effect of inhaled Ampion compared to placebo on the clinical outcomes for participants with prolonged respiratory complications after a COVID-19 infection per Section 3.3.2.

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

(moderate and severe), and timepoint. Where appropriate, data will be tabulated and presented cumulatively across cohorts and/or timepoints.

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.

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 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, 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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