Clinical Study Protocol

RLF-100 for the Treatment of Critical COVID-19 with Respiratory Failure

Protocol: RLF100-001

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IND Number Phase	**IND 149152** Phase 2b/3 IRB Approved
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1. SYNOPSIS

Name of Investigational Product:	RLF-100
Name of Active Ingredient:	Aviptadil
Reference Product(s):	Vasoactive Intestinal Polypeptide (synthetic)
Title of Study:	RLF-100 for the Treatment of Critical COVID-19 with Respiratory Failure
Phase of Development:	Phase 2b/3
Study Site(s):	Multi Center: University of Miami, UC Irvine, Houston Methodist, and others
Total Participant Number:	198 evaluable (mITT) subjects with Critical COVID-19 on mechanical ventilation (for <8 days), non-invasive ventilation or high flow rate nasal cannula at minimum 20L flow and 50% FIO_2
Study Duration:	60-day total follow-up
Background and Rationale:	The research to be conducted under this protocol references Phase 1 research conducted under IND 52,088 in which Vasoactive Intestinal Polypeptide (VIP) has previously shown suggestions of efficacy in the treatment of ARDS related to sepsis, pulmonary arterial hypertension and other respiratory disorders.
Objectives:	To demonstrate the efficacy of RLF-100 in improving survival and reducing respiratory distress / requirement for mechanical ventilation in patients with Critical (NIH/FDA definition) COVID-19 receiving maximum standard of care other than extracorporeal membrane oxygenation (ECMO).
Endpoint(s):	<u>Primary Outcome Measure:</u> 1) Cumulative distribution of the time to respiratory failure resolution with concurrent survival through Day 28. <u>Key Secondary Measure:</u> 2) NIAID Ordinal Scale for Clinical Improvement (Scores 6-8) through Day 28 <u>Secondary Outcome Measures (through Day 28 unless otherwise specified):</u> 3) Survival lifetable through Day 28 and through Day 60 4) Time to ICU discharge 5) Time on invasive mechanical ventilation 6) Time on non-invasive ventilation 7) Time to discharge alive 8) Multi-organ failure free days 9) NIAID Area under the curve (AUC) <u>Other Outcome Measures (through Day 28)</u>: 10) Respiratory Distress while on mechanical ventilation: Blood PaO2/FiO2 ratio [Time Frame: enrollment through extubation] 11) Oxygenation Index 12) Improvement in chest x-ray 13) IL-6, TNFα and other inflammatory markers

Study Design:	Patients with Critical COVID-19 who are receiving Maximal Standard of Care (SOC) therapy that includes non-invasive high pressure or mechanical ventilation will be randomized to intravenous Aviptadil + Maximal SOC vs. Placebo + Maximal SOC, escalating from 50 pmol/kg/hr to 150 pmol/kg/hr.
Inclusion Criteria:	1) Primary Cohort: Critical COVID-19 (test positive in past 7 days) with respiratory failure by NIH/FDA definition requiring either mechanical ventilation, non-invasive ventilation or high flow rate nasal cannula at minimum 20L flow and 50% FIO_2. No more than 7 days of mechanical ventilation. 2) Physician commitment to maximum Standard of Care as deemed necessary
Exclusion criteria:	1) Pregnancy 2) Age <18 years 3) Mechanical ventilation for more than 7 days in primary cohort. 4) Mean Arterial Pressure < 65 mm Hg with use of pressor per ICU protocol 5) Irreversible condition (other than COVID-19) with projected fatal course 6) ECMO 7) Current or recent (within 30 d) enrollment in another investigational trial of anti-IL6 drug or immunomodulator drug; 8) Active diagnosis of Acquired immune deficiency syndrome; 9) Transplant patients currently immunosuppressed; 10) Chemotherapy-induced neutropenia (granulocyte count <1000/mm3); 11) Cardiogenic shock; congestive heart failure – NYHA Class 3 or 4; 12) Recent myocardial infarction – within last 6 months and troponin > 0.5 13) Anuria (urine output < 50 ml/d) or other signs of multi-organ failure 14) Severe liver disease with portal hypertension or bilirubin 4.0 or higher; 15) Recent stroke or head trauma within last 12 months 16) Increased intracranial pressure, or other serious neurologic disorder; 17) Liquid diarrhea more than 3x/day; defined as more than 3 non-bloody watery stools within a 24-hour period, requiring additional fluid and electrolyte supplementation
Safety Assessments:	A safety analysis was conducted by the Data Monitoring Committee at 21 patients. All patients will be surveilled for Serious Adverse Events (SAE). All SAEs will be reported to independent Data Safety Monitoring Board and to FDA
Dosage, Route of Administration, and Schedule:	Three escalating doses of RLF-100 (Aviptadil): 50, 100, and 150 pmol/kg/hour or placebo administered over 12 hours via IV infusion on successive days. If side effects cannot be managed with ICU protocol, dose reduced or halted.
Statistical Methods:	Primary endpoint (cumulative distribution of the time to respiratory failure resolution) with concurrent survival through Day 28 will be displayed by a lifetable through Day 28 and analyzed using a proportional hazard model analysis containing 4 pre-defined baseline covariates and randomized treatment group (primary) as well as an unstratified log rank test (secondary). Primary endpoint ascertainment requires that recovery from respiratory failure be maintained for at least 7 days without relapse. The cumulative distribution will be constructed through Day 28 with no censoring for death or withdrawal; such patients will be regarded as failures. Other time to event outcomes will be similarly analyzed with the exception of survival which will censor for any withdrawals. NIAID Score will be analyzed by carrying forward any early deaths through Day 28; other ordinal and continuous outcomes will be assessed by mixed model repeated measures through Day 28. The primary analysis will be based on the modified intent-to-treat (mITT) population to be based on all treated patients.

Sample Size Calculation:	For the primary efficacy endpoint, assuming a 40% success percent in the placebo group by Day 28 and a 62% success percent in the RLF-100 group by Day 28, for log rank test with two-sided overall 5% Type 1 error and 81% power, the sample size to detect a 22% absolute improvement in the percent achieving respiratory failure resolution by Day 28 (from 40% to 62%) is 198 subjects in the primary analysis group (2:1 randomization favoring the RLF-100 group). A 20% absolute advantage in the percent achieving respiratory failure resolution by Day 28 is deemed to be clinically meaningful.
Interim Analyses	An interim look for safety and futility will be conducted once the first 21 randomized subjects complete Day 7. A second interim look for safety, futility, and efficacy will be conducted once the next 81 subjects complete Day 28 but the study will not be prematurely stopped for efficacy.
Maintenance of Study Blind	Investigational drug will be administered in identical infusion bags, labeled by the hospital research pharmacist who is the only unblinded member of the study site. ICU personnel are entirely masked as to treatment assignment, as are patients. Even in the event of unintentional unblinding because of differential efficacy or safety of the investigational medicinal products (drug and placebo), the randomization scheme prevents staff from knowing the next treatment assignment. Moreover, the primary and declared secondary endpoints are not subject to interpretation by ICU staff.

2. CONTACT INFORMATION

Role in Study	Name	Telephone / Email
National Co-Chairs	Dushyantha Jayaweera, MD Jihad Georges Youssef, MD	djayawee@med.miami.edu jgyoussef@houstonmethodist.org
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3. PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the conduct of the study according to 21 CFR parts 50, 54, 56 and 812, 45 CFR 46, to GCP, as described in ICH guideline E6 and to hospital Institutional Review Boards.

_____ _____

Clinical Site Investigator Signature Date

_____

Clinical Site Investigator Printed Name

_____ _____

Sponsor Signature Date

Jonathan C. Javitt, MD, MPH

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5. LIST OF ABBREVIATIONS

AA	Amino Acid
ALI	Acute Lung Injury
ARDS	Associated Acute Respiratory Distress Syndrome
COVID-19	Corona Virus Disease 2019
ECMO	Extracorporeal membrane Oxygenation
ED	Erectile Dysfunction
EMR	Electronic Medical Record
Fas	Cell Surface Death Receptor
HCl	Hydrogen Chloride
ICU	Intensive Care Unit
ITT	Intent-to-Treat
IL	Interleukin
IND	Investigational New Drug
IV	Intravenous
SOC	Maximal Intensive Care
MERS	Middle East Respiratory Syndrome
MMRM	Mixed model repeated measures
RDR	Respiratory Distress Ratio
SOC	Standard of Care
SAE	Serious Adverse Events
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	SARS-Coronavirus 2
TNFα	Tumor Necrosis Factor Alpha
VIP	Vasoactive Intestinal Peptide

6. INTRODUCTION

6.1 Executive Summary

No therapeutic agent has demonstrated safety and efficacy in treating COVID-19 related Respiratory Failure. RLF-100™ (aviptadil) is a synthetic form of Vasoactive Intestinal Polypeptide (VIP), a ubiquitous, naturally synthesized human peptide binds to the Alveolar Type II (ATII) cell in the pulmonary epithelium and inhibits SARS-CoV-2 replication, blocks cytokine synthesis, inhibits cytopathy, and upregulates production of surfactant. The Coronavirus effect on the ATII cell causes the profound respiratory failure associated with COVID-19, perhaps precipitated by failure of surfactant production in the alveolus. RLF-100™ has been granted Fast Track designation for the treatment of COVID-19 Respiratory Failure by the FDA and admitted to the CoronaVirus Treatment Acceleration Program.

Aviptadil has a long history of human safety and has previously been granted investigational new drug (IND) status by the US FDA and the EMEA for treatment of Acute Respiratory Distress Syndrome (ARDS), Acute Lung Injury (ALI), Pulmonary Fibrosis, and Sarcoidosis. At least five well-controlled studies have been conducted for the use of aviptadil in chronic lung conditions.

VIP has demonstrated specific binding to pulmonary alveolar type II cells, which are the primary target of the SARS-CoV-2 virus. In the Type II cell, VIP upregulates surfactant production, inhibits SARS virus replication, inhibits cytokine production, and inhibits cytopathy in numerous non-clinical models of SARS infection, ARDS, and ALI. Aviptadil has shown early evidence of efficacy in preserving life in patients with ARDS. A phase 1 safety trial of aviptadil was conducted in eight patients with sepsis-related ARDS, who had an expected 50% mortality rate. Seven patients demonstrated clinical benefit based on improved blood oxygenation and were successfully withdrawn from advanced life support. Six of those patients survived long term and left the hospital, with one succumbing to an unrelated myocardial infarction post ICU discharge. These results demonstrate in a phase 1 study that aviptadil significantly reduced expected mortality in patients with ARDS (mortality reduction of $p<.01$ vs historical controls). We propose RLF-100 (aviptadil) for the treatment of Critical COVID-19.

6.2 Definition of Critical COVID-19

In May 2020, FDA defined Critical COVID-19 to be used in clinical trials and disease staging as follows:

Critical COVID-19	• Positive testing by standard RT-PCR assay or an equivalent test
	• Evidence of critical illness, defined by at least one of the following:
	  ○ Respiratory Failure defined on resource utilization requiring at least one of the following:
	  ▪ Endotracheal intubation and mechanical ventilation, oxygen delivered by high-flow nasal cannula (heated, humidified, oxygen delivered via reinforced nasal cannula at flow rates > 20 L/min with fraction of delivered oxygen ≥ 0.5), noninvasive positive pressure ventilation, ECMO, or clinical diagnosis of respiratory failure (i.e., clinical need for one of the preceding therapies, but preceding therapies not able to be administered in setting of resource limitation)
	  ○ Shock (defined by systolic blood pressure <90 mmHg, or diastolic blood pressure <60 mmHg or requiring vasopressors)
	  ○ Multi-organ dysfunction/failure

Note that treatment with ECMO, mean arterial pressure <65 mmHg on pressors, and Multi-organ failure are exclusion criteria for this protocol

Acute Lung Injury in COVID-19 is characterized by progressive failure of corporeal oxygenation, attributed on large part by SARS-CoV-2 infection of Alveolar Type II cells (Mason 2020). Extensive nonclinical studies document that 70% of VIP in the body binds to $VPAC_1$ receptors on the Alveolar Type II cell, where VIP is known to block cytokine production and upregulate production of surfactant.

The pathologic hallmark of COVID-19 lung injury is diffuse alveolar damage, vascular endothelium damage, and damage to the surfactant-producing type II cells which results in loss of the integrity of the

alveolar-capillary barrier, transudation of protein-rich fluid across the barrier, pulmonary edema, and hypoxemia from intrapulmonary shunting. Typically, patients who have progressed to Critical COVID-19 require care in an intensive care unit (ICU). The mortality rate is approximately 50%. Deaths usually result from multisystem organ failure rather than respiratory failure alone.

6.3 RLF-100 Experimental Therapy in COVID-19

Under this protocol, patients with Respiratory Failure associated with Critical COVID-19 will be randomly allocated to intravenous RLF-100™ (aviptadil), with the aim to support pulmonary alveolar function, combat the cytokine-induced inflammation, improve blood oxygenation, and reduce mortality.

6.4 Aviptadil Background

Aviptadil (vasoactive intestinal peptide; VIP) is an abundant biologically active peptide endogenous in humans as well as in other species. It is produced by neurons in the peripheral and central nervous system, by endocrine cells such as pituitary lactotrophs, cells of the endocrine pancreas as well as T-lymphocytes, and B-lymphocytes. This natural peptide is one of the signal molecules of the neuroendocrine-immune network comprising vasodilation, anti-proliferative, anti-inflammatory, and immuno-suppressive features. Aviptadil is predominantly localized in the lungs and a vast body of experimental, pharmacological as well as clinical evidence suggests Aviptadil to be an attractive candidate as a treatment option for acute pulmonary disorders.

Endogenous aviptadil is synthesized from a precursor molecule which contains 170 amino acids and is processed to its biologically active form via a signal peptidase in the endoplasmic reticulum and finally cleaved by prohormone convertases and by carboxypeptidase-B like enzymes to functional Aviptadil comprising the following 28 amino acids: His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met- Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn.

Aviptadil was first identified in the intestine and then in the central and peripheral nervous systems and has since been recognized as a widely distributed neuropeptide, acting as a neurotransmitter and neuromodulator in many organs and tissues, including heart, lung, thyroid gland, kidney, immune system, urinary tract and genitals.

The widespread distribution of VIP is correlated with its involvement in a wide variety of physiological activities including smooth muscle relaxation, increased cardiac output, bronchodilation, immune-modulation, inhibition of smooth muscle cell proliferation, anti-apoptosis, neurotropic effects, and some differential effects on secretory processes in the gastrointestinal tract and gastric motility.

The rationale for the current application can be summarized as follows: VIP binds to and protects the specific cell (the Alveolar Type II cell) that is attacked by the SARS-CoV-2 virus in COVID-19. Once bound to the cell via the VPAC1 receptor, VIP blocks viral replication, increases surfactant production, blocks cytokine synthesis, and blocks cell death (cytopathy).

6.5 Clinical Rationale

Given by intravenous infusion in appropriate concentrations, aviptadil has been shown in clinical trials to have a manageable safety profile with no observed SAEs to date that would rise to the level of a black box warning. In an administratively-controlled trial of patients with COVID-19 respiratory failure and severe comorbidity (21 aviptadil-treated/24 controls), IV aviptadil dosed in 3 sequential infusions of 50/100/150 pmol/kg/hr was associated with a 9 fold increase in probability of recovery from respiratory failure and a comparable increase in survival over 60 days.

7. OBJECTIVES

7.1 Primary Objective

The primary objective of this study is to test the hypothesis that RLF-100 + maximal standard of care (SOC) shortens the time to respiratory failure resolution (see Section 6.2) in patients with Critical COVID-

19 compared to those treated with placebo + maximal SOC, in both cases not including ECMO.

7.2 Secondary Objective

The key secondary objective is to test the hypothesis that RLF-100 + maximal SOC improves NIAID Score over the first 28 (primary) and over the first 60 (secondary) days (from pre-treatment) in patients with Critical COVID-19 compared to those treated with placebo + maximal SOC, in both cases not including ECMO.

8. STUDY DESIGN

8.1 Overview

This study, RLF100-001, is a multi-center, randomized double-blind, placebo-controlled phase 2 trial, comparing RLF-100 + Maximal SOC to placebo + Maximal SOC. Aviptadil or placebo will be administered as a 12-hour IV infusion on three consecutive days. Patients with Critical COVID-19 infection will be screened. Informed consent will be obtained from the patient or a responsible party. Once entered into the study, participants will be randomized in a 2:1 ratio of IV RLF-100 + maximal SOC vs. placebo + maximal SOC. Once enrolled, patients assigned to receive RLF-100 will be given in three (3) doses of i.v. infusion, each dose given over 12-hour period at the same time on consecutive days, in escalating doses from 50pmol/kg/hr to 150 pmol/kg/hr of RLF-100. The primary outcome to be measured following RLF-100 infusion is the cumulative distribution of the time to respiratory failure resolution by 28 days with concurrent survival through 28 days. The declared secondary outcome to be measured following RLF-100 infusion will be the improvement in baseline for the NIAID Score from pre-treatment baseline to Day 28/exit using the remdesivir criterion (NIAID Score 6-8). Respiratory failure is defined as resource-based according to the FDA May 2020 respiratory failure definition (see Section 6.2). Eight other secondary efficacy endpoints are also to be evaluated in support of multi-dimensional efficacy.

8.2 Design

Randomized, prospective, double-blind, placebo-controlled trial of RLF-100 + maximal SOC vs. Placebo + maximal SOC therapy in patients with Critical COVID-19.

Following informed consent, patients will be randomized and administered either placebo or escalating doses of RLF-100 from 50 pmol/kg/hour IV to 150 pmol/kg/hour as three (3) 12 hour infusions each at the same time of day on subsequent study days 1, 2, and 3. Vital signs and telemetry will be monitored throughout the infusion and for 48 hours after completion of all 3 infusions. Pulmonary, cardiovascular, hepatic, CNS, renal, and coagulation functions will be assessed daily for 5 days, and plasma levels of aviptadil and selected cytokines will be measured.

The primary efficacy outcome is a pre-defined cumulative distribution of the time to respiratory failure resolution during the 28-day follow-up with concurrent survival. In other words, recovery from respiratory failure in the absence of survival to the end of the observation period is not considered treatment success. The cumulative distribution over the 28-day interval is consistent with the recent remdesivir approval. However, because the patients enrolled in this trial are exclusively those in category 6 and 7 of the remdesivir trial (where efficacy was not shown at 28 days), we will evaluate their key secondary efficacy endpoint (the percent achieving a NIAID Score 6-8 by Day 28/exit and by Day 60/exit).

Secondary outcome measures will include the cumulative distribution lifetable through Day 28 with attention paid to the Day 28 estimate from the lifetable. Time to ICU discharge, time on invasive mechanical ventilation, time on non-invasive ventilation, and time to discharge alive will be evaluated through Day 28. NIAID area under the curve (AUC) will be evaluated through Day 28.

Other outcome measures to be evaluated through Day 28 include the blood PaO2/FiO2 ratio (enrollment through extubation), oxygenation index, improvement in chest x-ray, IL-6, TNFα and other inflammatory markers.

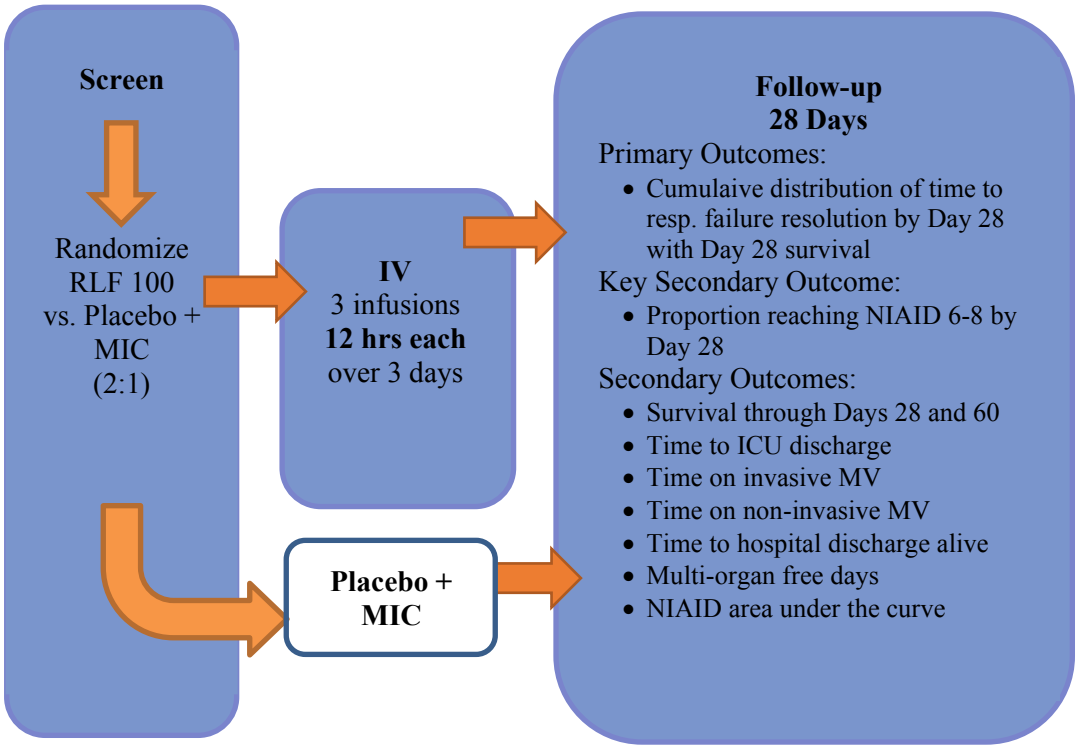
The endpoint rationale is to include the traditional 28-day all-cause mortality plus clinically meaningful

endpoints indicative of multi-dimensional recovery.

8.3 Number of Participants

Initially, randomization will result in 14 participants to receive RLF-100 + SOC and 7 participants to receive placebo + SOC in the primary efficacy cohort comprised of patients who meet enrollment criteria and have been on mechanical ventilation for <8 days (including zero days for those not on mechanical ventilation). The total number of participants may expand to 198 patients (132 drug/66 placebo) if there are acceptable efficacy and safety profiles at the interim analyses. The analysis at 21 patients will be unblinded so this cohort will not be part of the final analysis but will be included in a supportive analysis since results remain confidential. Thus, the sample size for analysis purposes will be 198 patients for the primary analysis.

8.4 Study Design



- Randomized controlled trial of RLF-001 50pmol – 150pmol/kg/hr in 3 ascending doses, each administered over a 12 hr IV infusion on consecutive days
- Check vitals two (2) hours after each dose begins for Side effects (See Flow Chart, Figure 2)
- Endpoint outcomes and assessments for survival, Blood 0_2, organ failure and TNF-α

* MIC = Maximal Intensive Care

Figure 1 shows a graphical schema of the RLF100-001 protocol study design.

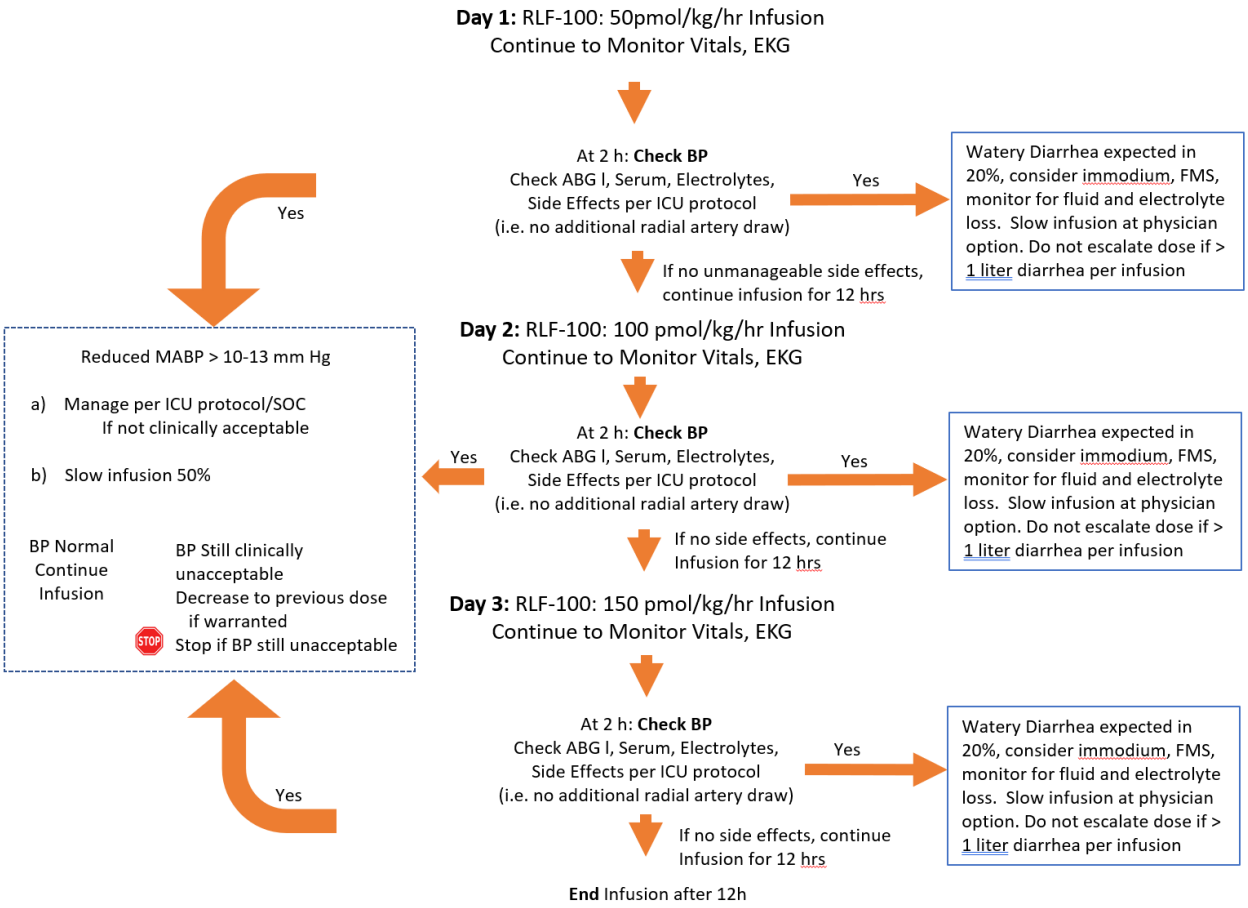


Figure 2: Protocol RLF-100-001 Study Flow Chart

8.5 RLF-100 Treatment Protocol

Figure 2 shows a graphical flow chart of the RLF100-001 protocol study design.

Note: The "check ABG" prompts in Figure 2 apply to ventilated patients with arterial lines only. These ABGs are to be carried out mid-infusion, at the 6 hour timepoint.

Note: If a determination is made to slow down the infusion rate by 50% - the next infusion would still be applied at the next 24-hour period. (E.g. if infusion was started at 8:00 AM and it runs for 18 hours, the next infusion should still take place at 8:00 AM the following day.) If infusion is halted, patient remains in the study. Physician can dose next day at last well-tolerated dose for a maximum of 3 doses.

8.6 Anticipated Risk

Reported adverse events for intravenous aviptadil include hypotension, tachycardia, facial flushing and watery diarrhea. If this were to develop, the infusion would be slowed or stopped until resolved. See Flow Chart (Figure 2 above).

9. SELECTION OF SUBJECTS

9.1 Study Population

The trial will be conducted in hospitalized patients with Critical COVID-19 by FDA/NIH criteria (section 6.2.) Subjects must meet all the inclusion and none of the exclusion criteria to be qualified to participate in this study.

9.2 Inclusion Criteria

All patients entered this trial will have the diagnosis of Critical COVID-19 (test positive in past 7 days). Subjects will be observed for a 1 to 24-hour period, during which time all inclusion criteria must be met. If all criteria are met once (not necessarily simultaneously), the patient will be enrolled and randomized to receive the study drug or placebo within 12 hours of the following entry criteria being fulfilled:

1) Critical COVID-19 with respiratory failure by NIH/FDA definition requiring either mechanical ventilation, non-invasive ventilation, or high flow rate nasal cannula at minimum 20L flow and 50% FIO_2.
2) Physician commitment to maximal Standard of Care treatment – as deemed necessary
3) Primary cohort: patients with less than 8 days of exposure to mechanical ventilation, non-invasive ventilation, or high flow rate nasal cannula at a minimum of 20L flow and 50% FIO_2.

9.3 Exclusion Criteria

1) Pregnancy
2) Age <18 years
3) Mechanical ventilation for more than 7 days in primary cohort.
4) Mean Arterial Pressure < 65 mm Hg with use of pressors per ICU protocol
5) Irreversible condition (other than COVID-19) with projected fatal course
6) ECMO
7) Current or recent (within 30 d) enrollment in another investigational trial of anti-IL6 drug or immunomodulator drug
8) Active diagnosis of Acquired immune deficiency syndrome.
9) Transplant patients currently immunosuppressed.
10) Chemotherapy-induced neutropenia (granulocyte count <1000/mm3).
11) Cardiogenic shock; congestive heart failure – NYHA Class 3 or 4.
12) Recent myocardial infarction – within last 6 months and current troponin > 0.5
13) Anuria (urine output < 50 ml/d) or other signs of multi-organ failure
14) Severe liver disease with portal hypertension; Bilirubin ≥4.0
15) Recent stroke or head trauma within last 12 months
16) Increased intracranial pressure, or other serious neurologic disorder.
17) Liquid diarrhea more than 3x/day; defined as more than 3 non-bloody watery stools within a 24-hour period, requiring additional fluid and electrolyte supplementation

10. RECRUITMENT, CONSENT & ENROLLMENT

10.1 Recruitment & Consent Procedures

All admissions to the intensive care unit will be screened for fulfillment of inclusion criteria by the Principal Investigator. Direct patient consent is always preferred if possible, but patients are sometimes unable to give consent in this setting. The PI or PI's designee will seek consent from the patient's responsible party, after explaining the procedure and consent form, approved by all participating sites. It should be noted that one feature of the COVID-19 pandemic is that non-patients are barred from hospital premises. Therefore, informed consent from responsible parties will generally be obtained telephonically and signature will be obtained via an SMS text message or otherwise compliant consent systems (e.g. REDCAP). Consent will be documented by a witnessed, consent form, to be kept on file together with telephonic signature. This does not preclude a site from obtaining consent via paper, if such arrangements can be made, e.g. fax. Should the patient regain ability to consent, consent will be sought accordingly.

10.2 Obtaining Informed Consent

Direct patient consent is always preferred if possible, but patients are sometimes unable to give consent in this setting. IRB-approved written informed consent forms (ICF) will be obtained from the responsible

party of all subjects before any protocol-specified procedures are carried out. For entry into the study, all inclusion criteria must be met, and none of the exclusion criteria can be met. All signed ICF forms will be logged and kept in locked research cabinets or other restricted electronic system under the supervision of the local Site PI and available for audit upon request.

10.3 Protecting Confidentiality

Any information obtained in this study will be treated as confidential and will be safeguarded. The data will be coded and kept in a secure location. When published, the results of the study will be in a form that does not identify any of the patients. In compliance with federal regulations, the records of the study will be available to representatives of the Food and Drug Administration.

10.4 HIPAA Compliance

The study will be compliant with the Health Insurance Portability and Accountability Act (HIPAA), which protects the privacy and security of healthcare information. All personal identifying information (PII) will be securely stored and only accessible to healthcare providers and authorized personnel only.

10.5 Standard of Care Support

Supportive treatment in the ICU, including antibiotics, oxygen, mechanical ventilation, blood pressure-supporting medications, and all other standard of care interventions as needed, will be continued, regardless of participation in the study.

10.6 Population Bias

For unknown reasons, males are affected by severe COVID-19 symptoms twice (2X) as often as are females. It is likely that the enrollment in this trial will be consistent with that gender difference. There is no evidence to suggest that RLF-100 will be differently tolerated based on race.

10.7 Enrollment

The enrollment will be in accordance with a group sequential design. Up to 12 sites will participate.

The sample size for analysis purposes well be 198 patients (132 drug/66 placebo) for the primary analysis.

10.8 Randomization Procedures

Randomization will be on a 2:1 basis with a random block size of 3 or 6; a centralized randomization will be used. Subjects will be stratified according to RDR ($<$200 mmHg, $\geq$200 mmHg) and previous anti-viral therapy for COVID-19 (none, any).

Using this method, even if site personnel believe they can tell the difference between clinical reaction to RLF-100 vs. placebo, they have no possibility to guess what the next allocation will be.

10.9 Procedure for Maintaining the Blind

Investigational drug will be delivered in identical infusion syringes, labeled by the hospital research pharmacist who is the only unblinded member of the study site. ICU personnel are entirely blinded, as are patients. Even in the event of unintentional unblinding because of differential efficacy of drug and placebo, the randomization scheme prevents staff from knowing the next treatment assignment. Moreover, the primary and declared secondary endpoints are not subject to interpretation by ICU staff.

11. TREATMENT OF SUBJECTS

11.1 Maximal Standard of Care

All patients entered in the trial receive Maximal Standard of Care treatment and support, including fluids, antibiotics, vasoactive agents, non-invasive or mechanical ventilation, hemodialysis, surgery, and other supportive measures excluding extra-corporeal membrane oxygenation (ECMO). Ventilation methods are:

- Invasive mechanical ventilation includes ventilation associated with an endotracheal or tracheostomy tube. Types of mechanical ventilation with this interface include volume-controlled and pressure-controlled ventilation. Examples are assist-control, pressure control and APRV
- Non-invasive mechanical ventilation includes CPAP, BiPAP, NIPPV (non-invasive positive pressure ventilation)
- Oxygen delivery systems include HFNC, nasal cannula, non-rebreather mask, Airvo, oxygen hood, Oxymask, Oxymizer, simple face mask, and the Venturi mask.

The need for ECMO will be considered a treatment failure on the basis that the lung has been deemed incapable of supporting adequate metabolic function. Decisions regarding the use of intravenous fluids, cardiovascular and respiratory support, and surgical intervention are made by each patient's attending physician and are not dictated by the study protocol. At the completion of the three 12-hour infusions, patients are followed up to 60 days. The schedule of events reflects study plans to follow all patients for up to 60 days even after discharge, but it will not be practical during the Corona Pandemic either to bring patients back for post-discharge visits or to make home visits. Death while on life support vs. death following discharge from mechanical ventilation will be tracked separately. Discharge from mechanical ventilation means that the treating physicians have deemed the patient capable of breathing without the need for mechanical ventilation. A death that occurs because life support has been withdrawn in the absence of this determination will be recorded as death while on life support.

11.2 Proper Standard of Care (SOC) Management Principles

Management principles are similar despite different etiologies. Oxygenation must be maintained, and the underlying cause of acute lung injury corrected. Meticulous attention is necessary to prevent nutritional depletion, O_2 toxicity, superinfection, barotrauma, and renal failure, which may be worsened by intravascular volume depletion. While the diagnosis is being considered, life-threatening hypoxemia must be treated with a high FiO_2 and monitored with repeated arterial blood gases or noninvasive oximetry. Prompt endotracheal intubation with mechanical ventilation and PEEP may be needed to deliver O_2 if hypoxemia is refractory to O_2 inhalation by face mask or nasal cannula. Despite the presence of alveolar edema, IV fluids should be given if needed to restore peripheral perfusion, urine output, and BP. Monitoring vascular volume is crucial because both hypovolemia and overhydration are deleterious. Physical findings and central venous pressure may be misleading in critically ill patients undergoing mechanical ventilation, and if severe hypoxemia persists, if skin perfusion is poor, if mentation is impaired, or if urinary output decreases (< 0.5 mL/kg/h), a reliable index of intravascular volume is needed immediately.

ECMO is a lifesaving modality that may be used when mechanical ventilation is unable to support life. However, the availability of ECMO is limited and decisions to allocate ECMO resources to patients are likely to be based on broader triage concerns. Therefore, ECMO is not considered part of SOC for the purposes of this study and the decision to escalate care to ECMO will be considered a treatment failure for the purpose of this study on the grounds that it signifies a determination that the lung is unable to support life even with mechanical ventilation.

11.3 Placebo

A normal saline placebo will be supplied by the hospital pharmacy in a labeled container indistinguishable from active drug.

11.4 Dosing Regimen

The escalating dosing regimen for RLF-100 is shown in Figure 2. Each infusion is planned for 12 hours duration, however, the infusion (either drug or placebo) may be slowed by up to 50% (for an 18-hour total infusion time) at the discretion of the site personnel if side effects cannot be managed to a clinically-acceptable level by the institution's ICU protocol or SOC. Monitoring of vital signs and other safety measurements will be ongoing and decisions relating to dose-reduction or cessation will be based on the evaluation of these parameters.

11.5 Potential Side Effects of Drug Therapy

Signs and symptoms to look for: facial flushing, tachycardia, hypotension, diarrhea. These will be monitored for the duration of the infusion, and for 12 h afterward, by blood pressure and heart telemetry measurements and patient observation. One identified side-effect of Aviptadil infusion is watery diarrhea. As noted above, non-bloody watery diarrhea > 3x/day requiring additional fluids and electrolyte supplementation is an enrollment exclusion. If watery diarrhea is observed during infusion, the infusion rate should be first reduced by 50% to relieve symptoms. If symptoms persist cease IV infusion.

12. ASSESSMENT OF SAFETY

12.1 Serious Adverse Events

Measurement of serious adverse events (SAE) will be the basis for assessing safety of RLF-100. All SAE's will be recoded on an IRB approved SAE Report Form. The SAE Report Form will include the exact nature of the event and the circumstances of the subject at the time of the event, in addition to the usual information required to document AEs or SAEs. Data on all the above will be collected on separate eCRF pages. The latest version of the AE dictionary, MedDRA, will be used for the classification and analysis of AEs entered in the study database. For regulatory reporting, SAEs will be coded using MedDRA.

12.2 Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per ICH). AEs will be classified as drug-related, possibly drug-related, or non-drug related by the study safety monitor.

12.3 Definition of a Serious Adverse Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

1. Death,
2. Life-threatening adverse events,
3. Inpatient hospitalization or prolongation of existing hospitalization,
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life function, or
5. Congenital anomaly or birth defect.

If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study drug and the event (e.g., death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality). In addition, the following variables will be collected for SAEs:

- Date AE met criteria for SAE
- Date investigator became aware of SAE
- Explanation of seriousness of this AE
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to study drug
- Causality assessment in relation to other medication
- Description of AE

12.4 Relationship to Study Drugs

Adverse events and SAEs will be collected from the time of signature of informed consent throughout the treatment period. The investigator will assess the causal relationship (i.e., the relationship to study treatment) between the investigational product and the AEs and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?" For SAEs, causal relationship will also be assessed.

12.5 Recording of Adverse Events

Abnormal and clinically significant lab values will be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the study drug. The principal investigator and the study biostatistician will establish the rules for what will constitute abnormal and clinically significant lab values based on established site-specific lab normal ranges. Adverse events, including abnormal lab values (clinically significant and clinically non-significant), will be reviewed monthly for trends by the principal investigator and the medical monitor. Should any abnormal lab values exceed acceptable rates, the FDA will be notified within regulatory timelines. Wherever possible, the reporting investigator will use the clinical, rather than the laboratory term (e.g., anemia versus low hemoglobin value).

12.6 Reporting of Adverse Events

All relevant AEs and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated Informed Consent Form (ICF) is obtained until completion of the subject's last study-related procedure. All events that meet the definition of a SAE will be reported as SAEs, regardless of whether they are protocol-specific assessments. All AEs, regardless of seriousness, severity, or presumed relationship to study drug, must be recorded using medical terminology in the source document and the eCRF. Whenever possible, diagnoses will be given when signs and symptoms are due to a common etiology. The sponsor assumes responsibility for appropriate reporting of AEs to the regulatory authorities.

12.7 Reporting of Serious Adverse Events

Reportable SAEs will be sent to the IRB and FDA by the sponsor. When a SAE is discovered, it will be reported immediately (within 24 business hours) to the Medical Monitor. Serious adverse events will be reported within 24 business hours of the site's knowledge of the event to the Sponsor, Medical Monitor, and study site investigators.

13. ASSESSMENT OF EFFICACY

13.1 Data Collection

Data will be collected via the electronic medical record (EMR) or other hospital information system and ICU telemetry systems. Once collected, data will be transferred by study personnel to the Target Health

electronic data capture system, which has been audited by FDA in connection with multiple drug approvals. GCP will be observed throughout. Extensive data not already defined in this protocol will be available for analysis via the EMR and telemetry systems.

13.2 Maintenance of Blinding

Investigational drug will be delivered by infusion and labeled by the hospital research pharmacist who is the only unblinded member of the study site. ICU personnel are entirely masked as to treatment assignment, as are patients. Even in the event of unintentional blinding because of differential efficacy of drug and placebo, the randomization scheme prevents staff from knowing the next treatment assignment. Moreover, the primary and declared secondary endpoints are not subject to interpretation by ICU staff.

13.3 Intercurrent Events

Given that this is an ICU study, the standard practice is to use the ICH E9 (R1) "treatment policy strategy" where all additional treatments given to the patient are considered to be part of care in both treatment groups. Thus, the analysis does not control for them but rather ignores them. Both remdesivir and dexamethasone will be considered in this way since there are no approved indications for Critical COVID-19. The treatment policy strategy is based on the ICH E9 ITT Principle. Thus, the occurrence of an intercurrent event is considered irrelevant in defining the treatment effect of interest; the value for the variable of interest is used regardless of whether or not the intercurrent event occurs. For example, when specifying how to address use of additional medication as an intercurrent event, the values of the variable of interest are used whether or not the patient takes additional medication. The study further benefits in that study treatment is limited to three days so any subsequent intercurrent event is considered to be part of the treatments being compared. One situation where the treatment policy strategy cannot be implemented for intercurrent events that are terminal events, e.g. going on ECMO or dying since values for the variable after the intercurrent event do not exist; then the estimand based on this strategy cannot be constructed with respect to a variable that cannot be measured due to death or being put on ECMO. Thus, dying or going on ECMO will impose a failure on all time to event outcomes other than survival.

13.4 Dropouts

Dropouts are not expected given that this is an ICU study. In the event of dropouts (including early withdrawals), these events will be reviewed at a Biostatistics Data Review Meeting (BDRM) to determine (in a blinded manner) the impact on each efficacy endpoint as well as an alternative impact to be applied in sensitivity analyses. The general approach will be to consider early withdrawals from a best practices perspective; for example, a patient going home on Day 10 who refuses follow-up at Day 28 and Day 60 is likely alive where a patient being transferred to a hospice is to be assumed as dead at Day 28 and Day 60.

13.5 Primary Efficacy Endpoint

The original primary efficacy endpoint has changed from a binary composite endpoint originally suggested by the original FDA division to a cumulative distribution of respiratory failure resolution at the suggestion of the study Data Monitoring Committee held July 14, 2020. The DMC counseled the use of a primary endpoint that would use “all the data” available to establish a difference between drug and placebo. The DMC recommended a time series approach, e.g. cumulative distribution, for the time to resolution of respiratory failure, originally a secondary endpoint but elevated to the primary endpoint.

The new primary efficacy endpoint is thus consistent with the recent FDA approval of remdesivir for a less ill study population as informed by recent FDA input (Reference ID: 4613030 NeuroRx, Inc.) in which FDA recommended the cumulative distribution of the time to resolution through Day 28 with concurrent Day 28 survival. The endpoint is further informed by recent FDA guidance not released at the time of study inception.[1]

[1] Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency June 2020

13.6 Key Secondary Endpoint

Given the recent remdesivir approval and publication, the percents achieving a NIAID Score 6-8 by Day 28/exit (primary) and by Day 60/exit (secondary) will be the key secondary endpoint which was the remdesivir primary efficacy endpoint.

13.7 Assessments of Endpoints

Cumulative Distribution: Resolution of Respiratory Failure

The cumulative distribution will use all randomized and treated patients. Resolution of respiratory failure is defined as no mechanical or non-mechanical ventilation or high-flow nasal O_2. Primary endpoint ascertainment requires that recovery from respiratory failure be maintained for at least 7 days without relapse. Patients must survive through Day 28 to be counted as having resolved. Any deaths irrespective of respiratory failure resolution or withdrawals without respiratory failure resolution will be counted as failures at Day 28; only documented resolution events will be counted as success in the distribution construction; this conservative approach is what was done for remdesivir. Imputing Day 28 failure aligns the cumulative distribution curve with the cumulative respiratory failure free days. A 20% absolute advantage is considered to be clinically meaningful.

NIAID 8-point scale

The NIAID 8-point scale will be used as a measure of recovery from Critical COVID-19. NIAID Scores will be collected daily through hospital discharge and at Day 28 and at Day 60. All on-study deaths (NIAID Score=1) will be carried through Day 28/exit and Day 60/exit. The distribution of the improvement will be assessed relative to the pre-treatment baseline. The percents achieving a NIAID Score 6-8 by Day 28/exit (primary) and by Day 60/exit (secondary) will be of interest from a regulatory and clinical perspective to be consistent with the remdesivir approval.

Survival

Survival lifetables will be constructed through Day 28 and then through Day 60. Patient death is defined as asystole. Data will be collected on whether the death occurred after withdrawal of care and, if so, the reason for withdrawal of care. Patient death is most likely to occur where the patient was hospitalized. Data will be collected on whether the death occurred after withdrawal of care and, if so, the care withdrawal reason.

Time to Events

Secondary time to event outcome measures include time to ICU discharge, time on invasive mechanical ventilation (subgroup analysis), time on non-invasive ventilation (subgroup analysis), time to discharge alive, and time to resolution of organ failure through Day 28. In addition, the Day 28 estimate from each lifetable will be analyzed. These endpoints will be displayed and analyzed in the same way as the time to respiratory failure resolution. Any deaths irrespective of an event (all favorable) or withdrawals without the event will be counted as failures at Day 28; only documented events will be counted as success in the distribution construction; this conservative approach is consistent with what was done for remdesivir. Imputing Day 28 failure aligns the cumulative distribution curve with the cumulative event days.

Index of Respiratory Distress

The PaO_2/FiO_2 ratio shall be calculated from the continual telemetry data obtained from the ICU information system and arterial blood gas (ABG) for patients while on mechanical ventilation. This variable is not useful after O_2 on mechanical ventilation has been reduced because the ratio is reduced even though oxygenation is already improving. In the absence of an arterial line, ABG's will be collected only when medically indicated.

Oxygenation Index

The Oxygenation index is defined as:

$$OI = \frac{FiO_2 \times M_{PAW}}{PaO_2}$$

Inflammatory Markers

TNFα and other cytokine studies will be performed by the central lab. Note that these are not declared efficacy or safety endpoints.

Improvement in Chest x-ray
Chest x-ray images will be collected (1) pre-treatment, (2) 48-96 hours post start of infusion, (3) 1 week ±2 days post start of infusion, and (4) two weeks ± 2 days post start of infusion, provided the patient is still hospitalized. All x-rays will be read by a blinded radiologist panel and scored according to the Rapid Assessment of Lung Edema (RALE) score (Warren 2017).

Clinical decision to terminate mechanical ventilation
Study research personnel will collect all available data regarding the clinical decision-making that guides removal mechanical ventilation in relation to clinical deterioration or lack of clinical response (e.g. change in status to comfort measures, poor prognosis for recovery, allocation of resources in a pandemic setting, etc.).

Additional data to be collected
Because of the unique nature of this study and the circumstances under which it is being conducted, at FDA's request, sponsor will collect all data regarding the use of experimental therapeutic agents (e.g. hydroxychloroquine, IL-6 inhibitors, and other topics of interest) and deliver those data to FDA for further analysis. Sponsor will make this analysis data set available to the broader scientific community as well.

13.8 Patient Follow-up Strategies

This study begins with the patients in the ICU and could end with the subject still in the ICU, in another hospital, at home, or deceased. Every possible effort has been taken to minimize dropouts and losses to follow-up. The following actions have been taken to ensure follow-up:

1. encourage sites to establish reliable follow-up networks with patients for use post discharge.
2. follow an algorithm that includes multiple communication/information gathering strategies to starting with 3 documented telephone calls, followed by fit for purpose use of text messages, email, next of kin/contact person outreach, review of system medical records as appropriate, primary physician follow up, public records and, again if appropriate, locator services.

Thus, we expect the loss to follow-up in this study to be quite low.

14. STATISTICAL ANALYSIS

14.1 Statistical Design and Analyses

All analyses will be pre-specified in a Statistical Analysis Plan to be finalized with FDA prior to database lock.

A group sequential design will be employed with one interim analyses for safety and futility (n=21) and a second interim analysis for safety and futility (n=81). At the request of FDA, the efficacy look was dropped prior to conducting the n=81 evaluation.

All calculations will be performed using SAS statistical software, version 9.3 or later, and StatXact, version 10 or later. All efficacy and safety data will be displayed in tables and listings separately for each treatment group. Post-Text TLFs will be provided in collated electronic MS Word .rtf files (i.e. table columns and rows appear in MS Word Table format).

The statistical analyses of the study will be conducted in a GCP environment (ICH E6).

There will be an independent statistical reporting group (ISRG) responsible for conducting all analyses; there will be separate unblinded biostatistician supporting the DSMB and a blinded biostatistician

responsible for any protocol modifications.

All patient data will be collected via the electronic medical record and in-house ICU telemetry systems. Dedicated research personnel will extract data to a de-identified study database for statistical analysis.

14.2 Sample Size Calculation

The sample size calculation of 198 subjects (2:1 allocation favoring the RLF-100 group) is required to detect a 22% absolute increase in the percent achieving respiratory failure resolution favoring the RLF-100 group (assuming 50% success) and the SOC group (assuming 40% success) with 81% power and two-sided overall 5% Type 1 error accounting for the group sequential design using the method in Schoenfeld. This degree of efficacy corresponds to a 0.52 hazard ratio.

14.3 Study Populations

The intent-to-treat (ITT) population will be based on all randomized patients receiving any study treatment. ITT will be analyzed as randomized.

The safety population will be based on all randomized patients receiving any study treatment. Safety will be analyzed as treated.

The modified intent-to-treat (mITT) population will exclude untreated patients as well as ineligible patients; this will be the primary efficacy population. The primary analysis set will not condition on any post-randomization outcomes.

In the event that >5% of the mITT population experience a major protocol deviation that impacts the primary efficacy endpoint, such cases will be excluded and a Per Protocol (PP) population will be constructed and the primary efficacy endpoint analyses will be repeated for the PP population. A BDRM will be held to review and to determine such major deviations which would make it difficult to evaluate the study therapy, e.g. going on an approved Critical COVID-19 therapy (none currently exist) or premature withdrawal from study. The BDRM will be conducted at study completion to determine final mITT and PP population membership.

14.4 Statistical Methods

The primary analyses will be based on the modified intent-to-treat (mITT) population.

All analyses will be conducted according to the treatment policy strategy to handle intercurrent events. In the event of any dropouts, the BDRM will discuss how to best count these patients in the primary analysis and then in a sensitivity analysis.

Evaluation of the primary efficacy endpoint (cumulative distribution through Day 28) will be analyzed by a pre-defined proportional hazard model to include age (<65, ≥65 years), baseline NIAID score (2, >2), RDR (<150 mmHg, ≥150 mmHg), previous anti-viral therapy for COVID-19 (none, any), and study treatment as covariates (primary); the proportional hazard model will be used to estimate unbiased treatment effects while controlling for these four baseline covariates. To count in the accruing numerator, the event must be confirmed by the site to last for at least 7 days with Day 28 survival. SAS PROC PHREG will be used for these analyses. See Sections 13.3-4 for rules to count respiratory failure resolution and the handling of intercurrent events and dropouts.

The key secondary efficacy endpoint will be the percent achieving a NIAID Score 6-8 by Day 28/exit (primary) and by Day 60 (secondary) and will be analyzed by comparing success percents. Patients who die or withdraw will not be counted as achieving success (event). The primary analysis will be a logistic regression model containing the same covariates as the primary efficacy endpoint. The percents achieving success by Day 28/exit will also be compared using a two-sided Fisher Exact test.

The survival curve will be displayed using Kaplan-Meier lifetables to account for censoring in the estimation of the Day 28 and Day 60 survival outcomes; Greenwood's formula will be used to estimate Day 28 outcomes with two-sided 95% confidence intervals constructed to compare the treatment group differences. The same proportional hazard model as for the primary efficacy endpoint will be used to analyze survival; in addition, an unstratified log rank test will be performed.

The other time to (favorable) event outcomes will also be assessed using cumulative distributions through Day 28 with an additional evaluation at Day 28; these outcomes include the time to ICU discharge, time on invasive mechanical ventilation, time on non-invasive ventilation, time to discharge alive, and time to resolution of organ failure. These endpoints will be analyzed and displayed the same way as the time to respiratory failure resolution with the exception of just through Day 28. Two-sided 95% confidence intervals will be displayed for the Day 28 success rate differences in the same manner as for the key secondary efficacy endpoint. Any deaths or placement on ECMO on or before Day 28, irrespective of an earlier favorable event, or withdrawals without the favorable event ever occurring will be counted as failures at Day 28; only documented favorable events will be counted as success in the distribution construction and the denominator will include all mITT patient per treatment group. The Day 28 failure imputation allows the cumulative distribution to align with the calculation of mean days saved; thus, patients withdrawing earlier than Day 28 or not reaching a favorable event by Day 28 will be counted as failures at Day 28 in the construction of the time to event lifetables; this is consistent with how favorable events were handled for remdesivir. See Sections 13.3-4 for the handling of intercurrent events and dropouts.

The mean NIAID score will further be displayed over time. The area under the curve will also be calculated and compared using an unpaired t-test. Patients dying prior to Day 28 (then Day 60) will be carried through Day 28 (then Day 60) as being dead (NIAID=1).

The NIAID score will further be assessed for the change from baseline to Day 28/exit and then to Day 60/exit. A mixed model repeated measures (MMRM) containing the same covariates as the primary efficacy endpoint will be performed to explore the impact of modeling serial data; an unstructured covariance symmetry will be assumed; in the event that the unstructured covariance structure does not converge, then an autoregressive (1) structure will be tried, and if that does not work, then a Toeplitz structure will be tried.

Other continuous variables, e.g., blood pressure, respiratory rate, tidal volume, organ function measurements will be summarized by mean ± standard deviation, median, and range. Categorical variables, e.g., lung injury score, chest X-ray findings, will be summarized by frequency and corresponding % incidence.

14.5 Interim Analysis, Futility/Safety

Two pre-planned interim analyses will be conducted (n=21 initially, then an additional n=81). There will be 81% power and two-sided 5% overall Type I error to assess the primary endpoint with a total of 198 patients. The DSMB recommended that the sample size be increased to detect a smaller clinically meaningful difference for the primary efficacy endpoint (reduced from 25% to 22%).

The first interim analysis will be reviewed by the independent DSMB based on unblinded, unmasked data upon completion of 7-day data collection in the first 21 patients. DSMB meeting minutes will be recorded and provided to FDA. An unmasked/unblinded analysis will be performed for the independent DSMB in order to assess safety and efficacy. Safety determinations will entail a review of AEs and SAEs by the independent Data Safety and Monitoring Board in consultation with FDA. Based on this evaluation, the Sponsor will decide whether to permit adding additional study sites.

If the decision is made to continue the study, the next interim analysis will be performed once the next 81 patients complete the Day 28 evaluation for a safety and futility determination. As per FDA, the study will continue enrollment to 198 patients.

The details of the futility analysis follow. The futility analysis was conducted when 81 of the original 144 planned patients completed the study. If the conditional power, under the alternative hypothesis is less

than 10%, then the study will stop for futility. $CP = \Phi(\mu\sqrt{1-t} + z_{1-p}\sqrt{\frac{t}{1-t}} - \frac{z_{0.975}}{\sqrt{1-t}})$, where $t = 81/144$ and p is the current corresponding one-sided p-value. The one-sided p-value to stop if the interim analysis occurs at exactly 81 patients will be 0.5949. This stop is non-binding and the DSMB will have the ability to continue if they see a positive signal in a secondary endpoint. This futility assessment will not spend any Type 1 error which will remain at 0.05.

The final sample size of 198 subjects (2:1 allocation favoring the RLF-100 group) is required to detect a 22% absolute increase in the percent with respiratory failure resolution by Day 28 for the RLF-100 group (assuming 62% success) and the SOC group (assuming 40% success) with 81% power and two-sided overall 5% Type 1 error accounting for the group sequential design using the Schoenfeld method.

The DSMB will advise regarding the favorable benefit/risk associated with early efficacy with safety.

15. HYPOTHESIS TESTS

15.1 Treatment Comparisons

Hierarchical testing will be used to preserve the Type 1 error associated with multiple endpoints (per FDA guidance) in pursuit of additional labeling claims beyond the primary efficacy endpoint. Hierarchical testing will not apply to the interim analyses; it will only apply to the final analysis. The testing order follows with two-sided p=0.05 required to continue testing for additional label claims:

Primary Outcome Measure:

1) Cumulative distribution of the time to respiratory failure resolution with concurrent survival through Day 28 (primary) using all mITT patients.

Key Secondary Measure:

2) NIAID Ordinal Scale for Clinical Improvement reaches NIAID Score 6-8 by Day 28/exit (primary); percent achieving a NIAID Score 6-8 will be evaluated using all mITT patients; Day 60/exit (confirmatory)

Secondary Outcome Measures (through Day 28 except for survival through both Day 28 and Day 60)

3) Time to ICU discharge using all mITT patients
4) Time on invasive mechanical ventilation using all mITT patients initially on invasive mechanical ventilation
5) Time on non-invasive ventilation using all mITT patients initially on non-invasive ventilation
6) Time to hospital discharge using all mITT patients
7) Overall survival using all mITT patients
8) NIAID area under the curve using all mITT patients
9) Multi-system organ failure using all mITT patients.

15.2 Primary Hypothesis

The null hypothesis (H_0) is that there is no difference in the cumulative distribution (CD) for the time to respiratory failure resolution by Day 28 with Day 28 survival as primary while the alternative hypothesis (H_a) is that there is a RLF-100 advantage in the CD:

H_0: $CD_{RLF\text{-}100} < CD_{PLA}$

H_a: $CD_{RLF\text{-}100} > CD_{PLA}$

15.3 Primary Efficacy Endpoint: Time to Respiratory Failure Resolution

The primary outcome measurement is the cumulative distribution which shows when resolution occurs for

each treatment group. Resolution must last for at least 7 days and start by Day 28; patients must be alive on Day 28. In accordance with the treatment policy strategy, deaths and going on ECMO during the first 28 days are counted as failures; they are counted in the Day 28 denominator to permit the area under the curve to correspond to event-days saved. The mITT patients per treatment group are the respective denominators. The data will be analyzed using a pre-defined proportional hazards model (primary) and unstratified log rank test (secondary); the hazard ratio and two-sided 95% confidence interval will be the critical statistics; in addition, a two-sided 95% confidence interval will be computed for the Day 28 outcome. A 20% absolute improvement in the Day 28 success rate is deemed to be clinically meaningful. A 40% success rate is expected for Placebo + Maximal SOC while a >50% success rate is expected for RLF-100 + Maximal SOC.

Sensitivity analyses are also planned; see Section 13.4. The BDRM will consider the most likely outcome on a patient-by-patient basis. However, a tipping point analysis using the proportional hazard model will be performed where all RLF100-1 patients dropping out will initially be considered as failures while all placebo patients will initially be considered to be successes. The two-dimensional tipping point analysis will shift the balance over the spectrum of all independently being successes and all being failures.

The mITT population will be used for the analysis.

15.4 Key Secondary Efficacy Endpoint: Exit NIAID Score Reaches 6-8

The key secondary outcome measurement is the change in NIAID Score from pre-treatment baseline through Day 28/exit (then Day 60/exit). Deaths are carried forward using NIAID Score=1 while, for withdrawals, the last value before Day 28 (60) is used for the Day 28 (60)/exit outcome. The denominator is fixed per treatment group. The data will be analyzed using a pre-defined logistic regression model (primary) and Fisher Exact test (secondary); the proportion achieving NIAID Score 6-8 by Day 28/exit (primary) and by Day 60/exit (confirmatory) will be presented as the key summary measure to be estimated from the lifetable. A 20% absolute improvement in the Day 28 (and Day 60) percent is deemed to be clinically meaningful. A 40% success rate is expected for Placebo + Maximal SOC while a >60% success rate is expected for RLF-100 + Maximal SOC.

The same two-dimensional tipping point analysis will be conducted as for the primary efficacy analysis except using a logistic regression model.

In addition, a MMRM model using the same covariates will be run for the change from pre-treatment baseline to Day 28/exit using the same baseline covariates for the primary efficacy endpoint. The mean change from pre-treatment baseline to Day 28/exit will be the endpoint of interest. The analyses will be repeated at Day 60/exit (confirmatory). The MMRM model will use an unstructured covariance symmetry will be assumed; in the event that the unstructured covariance structure does not converge, then an autoregressive (1) structure will be tried, and if that does not work, then a Toeplitz structure will be tried. The mean change from pre-treatment baseline to Day 28/exit (primary) and Day 60/exit (confirmatory) will also be of interest.

The mITT population will be used for these analyses.

15.5 Secondary Efficacy Endpoints: Time to Favorable Events

These secondary efficacy endpoints will be analyzed in the same manner as the primary efficacy endpoint. For each such endpoint, the outcome measurement is the cumulative distribution which is a figure that shows when the favorable event occurs for each treatment group. Any deaths or placement on ECMO on or before Day 28, irrespective of an earlier favorable event, or withdrawals without the favorable event ever occurring will be counted as failures at Day 28; only documented favorable events will be counted as success in the distribution construction and the denominator will include all mITT patient per treatment group. The Day 28 failure imputation permits the area under the curve to correspond to event-days. The denominator is fixed per mITT treatment group. To count in the accruing numerator, the event must be confirmed by the site to last for at least 7 days. The data will be analyzed using the same pre-defined

proportional hazards model (primary) and an unstratified log rank test (secondary); the hazard ratio will be the critical statistic.

The mITT population will be used for the analysis.

15.6 Secondary Efficacy Endpoint: Survival

Kaplan-Meier lifetables for survival will be constructed through Day 28 (primary) and through Day 60 (confirmatory). Survival is defined as not having died as ascertained from the medical record. If death occurs post discharge and prior to Day 28 (or Day 60), study personnel will attempt to obtain a death certificate but may not succeed in the current emergency. A lifetable will be generated; a proportional hazard model will be run to include the four previously stated endpoint covariates (primary) and an unstratified log rank test (secondary) will also be run; the hazard ratio will be the critical statistic. All deaths from any cause are included in the survival analysis as events; the 28-day and 60-day all-cause mortality percents will be estimated from the respective Kaplan-Meier lifetables. The Day 28 and Day 60 estimates from the lifetables will be compared using two-sided 95% confidence intervals.

The mITT population will be used for the analysis.

15.7 Secondary Efficacy Endpoint: NIAID 8-point Scale Area Under the Curve

The NIAID 8-point scale will be used to construct the area under the curve. Deaths will be counted as NIAID Score =1 while the NIAID Score will be scaled by multiplying the AUC times 27/D where D is the number of days on study minus 1. The AUC will be computed for each patient to perform the AUC comparison using an unpaired t-test for the mean difference (and average difference per day).

The mITT population will be used for the analysis.

15.8 Secondary Efficacy Endpoint: Multi-system Organ Failure

The days free of multi-system organ failure will be computed; organs include sepsis, blood, brain, heart, liver, and kidneys. Any such indication constitutes organ failure. The days free of multi-system organ failure will be assessed using the Schoenfeld method. The mean difference is the critical statistic.

The mITT population will be used for the analysis.

15.9 Other Efficacy Endpoint: Blood PaO2/FiO2 Ratio (while on MV)

Blood PaO2/FiO2 ratio will be analyzed in the same manner as the NIAID Score MMRM model except that data collection stops when invasive mechanical ventilation ends. In addition, the percent improving by at least 100 points will be presented as a descriptive statistic.

The mITT population will be used for the analysis.

15.10 Exploratory Efficacy Endpoint: Oxygenation Index

The Oxygenation Index is considered an exploratory variable because it is inconsistently measured across sites. Ideally, it is measured at every 24 hours and 28 days post intubation with additional endpoint determinations through cessation of mechanical ventilation. Time to normalization (last SaO2 value >95%) will be evaluated; this endpoint will also be analyzed in the same manner as the primary efficacy endpoint for the time to the successful event with the requirement to not relapse within 7 days.

The mITT population will be used for the analysis.

15.11 Other Exploratory Efficacy Endpoint: Improvement in Chest x-ray

The RALES score will be ascertained by a blinded radiologist panel. The percent with improvement in chest x-ray over time will be independently determined by the panel. Percent improving will be compared

using a two-sided Fisher Exact test while the RALES score distribution over time will be displayed descriptively.

The population with available data will be used for the analysis.

15.12 Laboratory Endpoints: VIP and Inflammatory Markers

The secondary laboratory outcome measures are VIP, TNFα, IL6, and other inflammatory markers as measured in the central laboratory. Mean VIP per day will be compared using a paired t-test within treatment group and using an unpaired t-test between treatment groups per day. The inflammatory markers will be evaluated for time to normalization (to be determined per endpoint prior to database lock); these endpoints will be analyzed in the same manner as VIP when treated as a continuous measure.

The mITT population will be used for the analysis. VIP and inflammatory markers are not required beyond the 165 patients enrolled mark.

15.13 Overall Safety Endpoints

Standard safety analyses are planned. Adverse events will be coded in MedDRA. The incidence, severity, relationship, and worst degree will be assessed with attention paid to AEs occurring during the first three days when infusion is to be administered. The incidence of SAEs and deaths/withdrawals due to treatment will also be assessed. The following additional adverse events are of special interest:

- Diarrhea that leads to treatment discontinuation
- Renal failure
- Bleeding events
- Liver toxicity
- Hypotension/shock.

All safety results will be presented separately for each treatment group. Safety endpoints will include adverse events, vital signs (VS), and relevant clinical chemistries and hematology parameters. Safety and tolerability will be assessed using two-sided Fisher Exact tests to compare RLF-100 vs. Placebo. For each treatment group as well as for the difference, two-sided 95% confidence intervals will be computed for each system organ class and for any preferred terms with >10% incidence for either study treatment.

Mean changes from randomization baseline will be displayed for each serum chemistry and hematology parameter as well as the shift; unpaired t-tests will be used to compare RLF -100 vs. Placebo at designated study times. In addition, paired t-tests will be performed for the change from randomization baseline within each study treatment group.

The safety population will be used for these analyses.

16. SOURCE DATA & DOCUMENTS

16.1 Sources of Research Materials

The following source data and documents will be collected. All blood samples, except those taken for cytokine and arachidonate metabolite level monitoring, which would be part of this investigation, would be obtained in the normal course of clinical care of these patients as described above.

16.2 Case Report Form

The Case Report Form (CRF) is an integral part of the study and subsequent reports. The CRF must be used to capture all study data recorded in the patient's medical record. The CRF must be kept current to reflect patient status during the study. Only a patient screening and randomization number will be used to identify the patient.

16.3 Patient Identification

Patient name, medical record number, age, gender, height, weight, ethnic background, residence address with zip code will be collected.

16.4 General Clinical Data

Primary diagnosis, coexisting diagnoses / conditions, hospital length of stay and outcome will be collected.

16.5 Lung Injury / ARDS Data

PaO_2/FiO_2 ratio; PEEP/CPAP; peak, mean & plateau pressures; compliance (dynamic and quasi-static); tidal volume; respiratory rate; minute ventilation, lung injury score, chest X-ray findings will be collected.

16.6 Laboratory Data

Arterial blood pH, PCO_2, PO_2; electrolytes (Na, K, Cl, HCO3); renal function (BUN, serum creatinine); glucose, albumin & total plasma proteins; liver function tests; blood cell count; coagulation profile; lactic acid and D-Dimer will be collected.

16.7 Vital Signs, Respiratory and Hemodynamic Data

Blood pressure (systolic, diastolic, mean), heart rate, respiratory rate, cardiac output/cardiac index, pulmonary artery (PA) wedge pressure, systolic, diastolic & mean pressures (if PA catheter is in place), right atrial/central venous pressure, systemic and pulmonary vascular resistance, systemic 0_2 delivery, daily urine output, daily fluid intake will be collected.

16.8 Medical Therapy Data

The therapeutic milieu for RLF100-001 – ICU-type settings during the COVID-19 pandemic – requires a thoughtful and practical approach to the capture of concomitant medications in clinical studies. Specifically, the capture of relevant concomitant medications is required under three circumstances:

1. As part of a relevant medical history entry, where relevant medical history is defined as relating to an inclusion or exclusion criteria;
2. As part of an AE entry, for agents administered in response to the AE; and
3. Agents of interest, namely Biotin, IL6, antiviral therapies, steroids, nitric oxide, epoprostenol (Flolan), pressors (e.g., norepinephrine, epinephrine, Vasopressin), paralytics, anticoagulants, convalescent plasma, and tocilizumab (Actemra) and other immunomodulators.

Please note there is a unique dedicated CRF page for capturing concomitant use of convalescent plasma – no need for a duplicate entry on a concomitant medication form.

Furthermore, sites will be asked to report unusual circumstances in their ICU that require medical therapy that can negatively affect the prognosis of a patient, e.g. emergence of treatment resistant bacteria leading to high mortality.

16.9 Pharmacokinetic Sampling

The pharmacokinetics of intravenous Aviptadil are well understood and do not require revalidation in this study.

16.10 Blood Sampling

Blood samples are obtained for biochemical, electrolyte, and hematologic profiles per ICU protocol. Blood samples for cytokine (TNF-alpha, IL-6, which correlate with the inflammatory response are obtained before infusion, during infusion, and at 12 hours, and then daily on day 5, day 7 and day 28 or day of

discharge. Leukotrine E4 (urine) and Plasma VIP levels are checked at the same intervals to ensure the delivery of the peptide into the circulation. Plasma is separated within 30 min, frozen and stored at -70 C until the assays are performed, by specific radio-immunoassays or ELISA. Additionally, D-Dimer may be added when available. The procedures for blood samples for cytokines, leukotrine E4 urine and VIP plasma are not required after enrollment of the 165th evaluable patient.

LABORATORY VISIT SCHEDULE FOR PROTOCOL RLF100-001

NeuroRx, Inc.

	VISIT NAME	DAY1/DAY2/DAY3	DAY5/DAY7/EOS
	VISIT TYPE (RQ=Required, Opt=Optional, U=Unscheduled)	U	U
	OCCURRENCE	-	-
	KIT TYPE	T-1	T-2
Group Name(s)	*Specimen Type*		
CYTOKINES	Serum		X
CYTOKINES PRE	Serum	X	
CYTOKINES 2HR	Serum	X	
CYTOKINES 12HR	Serum	X	
Service(s)			
VASOACTIVE INTSTNL POLYPEPTIDE	Plasma	X	X
LEUKOTRIENE E4 URINE	Urine	X	X

X Mandatory testing

17. STUDY MONITORING

17.1 Study Monitoring

The study sites have been evaluated by the sponsor in conjunction with the CRO to determine suitability for the proposed study. Information reviewed included, but was not be limited to, facility details and site capabilities, past performance in similar studies, investigator, and staff experience, ongoing studies at the site, projected enrollment in this study, and FDA or other agency audit findings. Prior to subject enrollment, a virtual study initiation visit will be completed by video conference to ensure the following: IRB approval has been obtained and documented prior to subject screening, the investigators and study personnel are appropriately trained and clearly understand the study, the investigators and study personnel accept the obligations incurred in undertaking this clinical investigation.

Periodic remote monitoring visits will be made in accordance with the approved monitoring plan at all active study sites throughout the clinical study to assure that the investigator obligations are fulfilled, and all applicable regulations and guidelines are being followed. These visits will assure that the facilities are still compliant with study requirements, the protocol and investigational plan are being followed, the IRB has been notified of approved protocol changes as required, complete records are being maintained, appropriate and timely reports have been made to the sponsor and the IRB, and the site Investigator is executing all agreed activities. Given the public health crisis, these visits will be done via virtual means and specific monitoring arrangements with each site.

17.2 Data Safety and Monitoring

All results from this study will be reviewed by a Data Safety and Monitoring Committee, chaired by Prof. Alfred Sommer, MD, MHS, Dean Emeritus of the Bloomberg School of Public Health. Membership of this committee will include: a clinician specialist in Critical Care Medicine, a biostatistician, and a lay patient

advocate. The committee will critique the progress of the trial, carefully examine any incidents of adverse reactions, and may suggest appropriate revisions in the protocol. DSMB functions include, among others, ongoing assessment of unblinded data to determine whether any of the treatment arms show increased risk for AEs.

17.3 Medical Monitor

The Medical Monitor will review and approve the eligibility of all screened subjects, review all AEs, assess the benefits and risks of protocols on an ongoing basis, and work in collaboration with the IRB and DMC to identify safety signals and trends. In addition, the Medical Monitor will be available for site questions regarding inclusion/exclusion criteria, protocol conduct, and safety. Trained and qualified physicians will be available to provide coverage during times when the medical monitor is unavailable. Sites will be provided with the Medical Monitor's cell phone number for emergency situations. Otherwise, sites are instructed to contact the Medical Monitor through email. All critical conversations with sites will be documented by the Medical Monitor and reviewed periodically by the sponsor. Each month the Medical Monitor will receive a listing of protocol violations for review and identification of possible trends.

17.4 Audits and Inspections

Contracts with study sites will specify that sponsor or its representatives will have direct access to source data and documents for study monitoring, which may be done through virtual/electronic means. Additionally, the IRB and FDA may review source data following appropriate guidelines for this process.

17.5 Institutional Review Board

The study protocol and any amendments will be reviewed by the Advarra Institutional Review Board (IRB). The IRB will review the informed consent form, their updates (if any), and any written materials given to the participants. IRB approval will be obtained and documented prior to subject enrollment and screening. Before study initiation, the investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigators will provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, and Administrative Letters) according to regulatory requirements and Institution procedures. A detailed list of required regulatory documents, to be submitted to the sponsor, will be sent upon final approval of the protocol.

18. ETHICAL CONSIDERATIONS

18.1 Ethical Considerations

Anticipated benefits to participants and others: There are at present no satisfactory specific treatments for ARDS, or multiple organ dysfunction. Based on a large body of experimental work, there is reason to expect that RLF-100 (aviptadil) may prove effective in increasing survival rates in COVID-19 patients with ARDS, as well as increasing blood 0_2, reducing organ failure and reducing cytokine cascades.

Anticipated risks to participants and others: The only anticipated risks are due to the side-effects noted above of hypotension, tachycardia and watery diarrhea. Aviptadil has been evaluated in 4 species toxicology studies and the LD50 is more than 50x the exposure contemplated in this trial.

18.2 Ethical Conduct of the Study

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

18.3 Informed Consent

Direct patient consent is always preferred if possible, but patients are sometimes unable to give consent in this setting. Under these circumstances, the patient's legally authorized representative (LAR) must be capable of understanding the nature of this study and its potential risks, discomforts, and benefits. Study physicians or their delegate will obtain consent after they have fully explained the study purpose and procedures, and the LAR has demonstrated an understanding of the protocol, willingness to participate, and competency to consent. The investigator or their delegate must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the site-specific consent form is the responsibility of the site Principal investigator and must include all elements required by CFR 21 Part 50.25 and the IRB.

19. DATA HANDLING & RECORD KEEPING

19.1 Data Capture

An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on everyone treated with the investigational product or entered as a control in the investigation.

19.2 Data Collection

No manual data collection is anticipated in the ICU. Data will be extracted from the EMR and other sources by hospital clinical research staff in a HIPAA-compliant, de-identified manner and transferred to the study database.

19.3 Retention of Records

The investigator must retain investigational product disposition records, case report forms, and source documents for the maximum period required by applicable regulations and guidelines, or in accordance with institution procedures, and at least for 10 years. This is study practice in the study sites.

19.4 Use of Information and Publication Policy

All publications will be reviewed by the sponsor for accuracy before submission to peer-reviewed journals or scientific meetings. Abstracts will be submitted for review at least 10 days before submission, and publications should be submitted for review at least 30 days before submission. The study will be posted to Clinicaltrials.gov, and results will be reported in accordance with Clinicaltrials.gov guidelines.

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21. APPENDIX A; SCHEDULE OF EVENTS

Schedule of Events: Appendix A		Day 1		Day 2		Day 3		Day 4	Day 5	Day 6	Day 7	Day 8-Day 27	Day 28		Day 60
Procedures	**Screening**	**1st Dose[a]**	**During infusion**	**2nd Dose[a]**	**During infusion**	**3rd Dose[a]**	**During infusion**	**Follow-Up**	**Follow-Up**	**Follow-Up**	**Follow-Up**	**Follow-Up**	**Follow-Up D28 or day of discharge**	**Day 28 Follow-up Phone Call**	**Day 60 Follow-up Phone Call**
Informed Consent Process	X														
Evaluation of Exclusion & Inclusion Criteria	X														
Medical History/Demographics	X														
Confirm Eligibility and Randomization		X													
Vital Signs (Pulse, Respirations)[b]	X	X	X[b]	X	X[b]	X	X[b]								
Blood Pressure[c]	X	X		X		X									
Weight	X	X		X		X		X	X	X	X		X		
CNS Assessment	X	X		X		X		X	X	X	X		X		
Urine Output and Fluid Intake		X		X		X		X	X	X	X				
IV Administration of Study Drug		X	X	X	X	X	X								
ICU Telemetry: ECG		X		X		X		X	X						
PaO2/FiO2 Ratio	X	X		X		X		X	X	X	X		X		
CBC (Complete Blood Count with Differential & Platelets)	X	X		X		X		X	X	X	X		X		
CMP (Complete Metabolic Panel)	X	X		X		X		X	X	X	X		X		
ABG - Non-ventilated subjects (for PaO2/FiO2 ratio): pH, pCO2, pO2, CO2, HCO3 per ICU protocol	X								X			X[g]			

Schedule of Events: Appendix A		Day 1		Day 2		Day 3		Day 4	Day 5	Day 6	Day 7	Day 8-Day 27	Day 28		Day 60
Procedures	Screening	1st Dose[a]	During infusion	2nd Dose[a]	During infusion	3rd Dose[a]	During infusion	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up D28 or day of discharge	Day 28 Follow-up Phone Call	Day 60 Follow-up Phone Call
ABG - Ventilated subjects (for PaO2/FiO2 ratio): pH, pCO2, pO2, CO2, HCO3 per ICU protocol[h]	X	X	X	X	X	X	X	X	X	X	X	X	X		
Murray Score	X	X		X		X		X	X	X	X		X		
D-Dimer	X	X		X		X		X	X	X	X		X		
PT/INR/aPTT	X														
Lactic Acid	X	X		X		X		X	X	X	X		X		
Serum/Urine Pregnancy Test (Either Serum or Urine, not both)	X														
HIV (based on history, no HIV testing is required)	X														
Cytokines: Circulating IL6[e, j]		X	X[e]	X	X[e]	X	X[e]		X		X		X		
Cytokines: TNFa[e, j]		X	X[e]	X	X[e]	X	X[e]		X		X		X		
Urine Leukotriene[f, j]		X		X		X			X		X		X		
Plasma: VIP (Vasoactive Intestinal Polypeptide)[f,j]		X		X		X			X		X		X		
Study Drug Pharmacy Dispensing		X		X		X									
Assess for Adverse Events[d]		X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X	X		
Assess Concomitant Medications	X	X		X		X		X	X	X	X	X	X		
NIAID Ordinal Scale for Clinical Improvement		X		X		X		X	X	X	X	X	X	X	X
Chest X-ray (sub-study sites only)[i]	TBD =>														

Footnotes:

a	Each dose is administered over 12 hours at the same time each day. Date and time of start and end of infusion will be recorded.
b	Pulse and respirations will be continuously monitored but will be recorded prior to and at 2, 4, 6, 8, 10 and 12-hour timepoints after each dose escalation.
c	Blood pressure will be recorded prior to infusion and at 2, 4, 6, 8 and 12-hour timepoints throughout the infusion.
d	Infusion reactions/adverse events check at 2 hours, and then continuously throughout the 12-hour infusion period.
e	Collected pre-dose, 2 hours and 12 hours after the start of each infusion over 3 days, then on Day 5, Day 7 and Day 28 / or day of discharge if sooner.
f	Plasma VIP and urine leukotriene E4 should be collected prior to initiating each dose escalation, on days 1, 2,3; then at day 5, day 7 and day 28 or day of discharge if sooner.
g	Only on Day 10.
h	Ventilated Patients only - ABGs as follows: Days 1, 2 and 3 - Pre-Dose and at 6 hours after infusion begins (± 1 hour). Day 4 through discharge - once daily.
i	Chest X-ray images to be captured via a sub-study at a subset of identified sites only, specifics ox X-ray timings and number TBD.
j	Procedures for blood samples for cytokines; urine leukotriene; and plasma VIP are not required after 165 evaluable patients have been enrolled.