A Multicenter, Adaptive, Randomized, Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for Hospitalized Patients with Acute Respiratory Distress Syndrome Associated with COVID-19

Protocol Appendix H1: Aviptadil

Version 3.0
08 March 2022

NCT04843761

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

The content of this appendix is confidential and should only be viewed by persons covered by the relevant CDA between NIAID and the collaborating companies.

This appendix provides detailed information pertaining to the study of this investigational agents. If not stated otherwise in this appendix, the text in the TESICO master protocol provides the approach that will be taken to study this agent.

The principal difference of the study of this agent with the master protocol is that it will be studied, in part, using a 2x2 factorial design with remdesivir. Study objectives, randomization and data analyses take this factorialization into account and are described in Appendix H2 for remdesivir.

At the outset of this study, there will not be a shared placebo with another investigational agent.

1. Introduction and rationale for studying aviptadil

Vasoactive Intestinal Peptide (VIP; aviptadil is the generic name for the synthetic peptide) is a 28-amino acid signaling peptide that belongs to the glucagon-secretin superfamily. 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. VIP is an inhibitory neurotransmitter that binds G-protein coupled receptors named VPAC1 and VPAC2, generally leading to an increase in cAMP in target cells. Originally described in the intestinal tract,[1] it is expressed widely in the body, with multiple functions. The lung is the primary location of binding of VIP, as evidenced by radiolabeled VIP perfusion experiments (within 30 minutes, 45% of all infused VIP is bound in the lung, with minimal binding in other organs[2]). Cells expressing VIP receptors in the lung include vascular and bronchial smooth muscle cells as well as alveolar type 2 cells (ATII).[3] Critically, ATII cells are also a primary target for SARS-CoV2, the virus causing COVID-19.

The effects of aviptadil are pleiotropic, with key effects being (1) antiviral effects, (2) immune modulation, (3) increase in ATII surfactant production, (4) ATII cell protection, (5) smooth muscle relaxation (leading to bronchodilation and vasodilation), (6) decrease in platelet activation.

Antiviral effects. VIP is known to decrease HIV production within monocytes,[4,5] which drove interest in evaluating antiviral properties for SARS-CoV2. In a series of experiments, Temerozo and colleagues established that VIP decreased viral replication within infected Calu-3 cells (an immortalized lung cancer cell line), plus increased monocyte and Calu-3 viability after SARS-CoV2 infection.[6] These experiments also established that VIP treatment decreased the production of inflammatory cytokines within SARS-CoV2-infected monocytes.[6]

Immune modulation. VIP has multiple immune-modulatory effects.[7] In the lung, VIP decreases inflammation through multiple interdependent mechanisms, including inhibition of effector T cells and supplementation of regulatory T cells, with an associated decrease in local cytokines, as observed in sarcoid.[8] In a rat ATII cell model of smoke-associated lung inflammation, VIP decreased inflammation and proteinase activity.[3] Similar pre-clinical data in sepsis demonstrated decreases in TNFa and TGFb with VIP administration.[9-11] In terms of post-inflammatory injury, VIP has been shown to decrease myofibroblast proliferation in cell models.[12]

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

Surfactant production. In a lung explant model, VIP directly increased phosphatidylcholine production via PKC and C-Fos mechanisms.[13,14] In a similar model, VIP increased surfactant protein A production in ATII cells.[15]

ATII cell protection. VIP prevents apoptosis of ATII cells via multiple mechanisms including Granzyme and Fas-ligand.[16,17] In multiple animal models of ARDS, VIP is protective against acute lung injury.[17-21]

Smooth muscle relaxation. VIP is a non-adrenergic pulmonary and systemic vasodilator that in ex vivo pulmonary artery is substantially more potent at muscle relaxation than prostacyclin.[22] The increases in muscle relaxation are independent of the endothelium. VIP is also a direct bronchodilator based on relaxation of bronchial smooth muscle.[23] In a cat bronchoconstriction model, intravenous (but not inhaled) VIP resulted in significant bronchodilation.[24]

Platelet effects. VIP inhibits pro-inflammatory platelet activation via inhibition of platelet activating factor.[25]

These mechanistic observations in cell and animal models have been corroborated in various human observations in a variety of conditions, including ARDS and COVID-19.

Clinical experience with aviptadil

Non-randomized data in other disease states

Sarcoidosis. Twenty patients with chronic sarcoidosis were treated with nebulized aviptadil, which was associated with increases in regulatory T cells and decreases in macrophage activation.[8] There were no important safety concerns.

Checkpoint inhibitor pneumonitis. Inhaled VIP was used successfully to treat pneumonitis caused by checkpoint inhibitor therapy in a patient with advanced melanoma. The pneumonitis had recurred after an initial course of steroid therapy, and VIP was used in hopes of avoiding a second course of steroids.[26] The patient recovered from the pneumonitis, and no safety concerns were identified.

Pulmonary hypertension. Twenty patients with pulmonary hypertension (PH) of various etiologies received 100mcg of inhaled VIP during right heart catheterization, with an immediate decrease in vascular resistance. Among patients with lung disease as the cause of PH, increases in oxygen saturation were observed.[27] Similar results were observed in a smaller cohort of PH patients.[28] No important safety concerns were identified.

Non-randomized data in ARDS and COVID-19

Currently, there are multiple case reports and case series of patients with either septic ARDS or COVID-19 ARDS who have been treated with intravenous VIP or in whom biological samples have been collected.

In the mid-2000s, Youssef, Said and colleagues treated 8 patients with septic ARDS with VIP. They used 50 pmol/kg/hr in 5 patients, of whom one had hypotension requiring decrease to 25 pmol/kg/hr. The other three patients received 100 pmol/kg/hr, in whom one patient required temporary reduction (to 85 pmol/kg/hr) for hypotension. The target dosing duration was 6 or 12 hours. (An intended increase to 150 pmol/kg/hr was not undertaken because the senior author retired.) All but 2 patients

survived their ARDS.[29] VIP infusion appeared safe and feasible, and mortality appeared to be on the low end for septic ARDS, suggesting possible clinical efficacy.

During the COVID-19 pandemic, Youssef and colleagues studied 21 patients receiving intravenous aviptadil under an expanded access program (EAP). The patients receiving aviptadil were compared to non-randomized concurrent controls who were either admitted by physicians who were not investigators on the VIP trial or in the two weeks before and after this cohort was assembled. Four-week survival in the EAP cohort (primarily but not exclusively patients with immune suppression or undergoing ECMO therapy who were excluded from a concurrent randomized trial) was 90%; 4 of 5 ECMO patients were “successfully decannulated.” All patients were treated with glucocorticoids, 18 of 21 patients were treated with tocilizumab, and 6 of 21 were treated with remdesivir before VIP infusion. Hypotension occurred in 5 of 21 (24%) of patients receiving VIP infusion, primarily among those on ECMO and/or receiving vasopressors. In the other 16 patients, blood pressure was stable or improved during aviptadil infusion. Diarrhea was present in 4 of 21 patients; prophylactic or therapeutic loperamide was used in 86% of patients. The survival among the non-randomized concurrent controls was substantially lower, suggesting possible clinical efficacy. Approximately 200 patients have been studied under this EAP at multiple centers in the United States as of December 16, 2020. Reports from the full EAP cohort are pending.

In terms of observational data, Temerozo et al studied 24 patients with severe COVID-19 (i.e., requiring ICU admission), demonstrating significantly higher endogenous VIP levels among survivors than non-survivors.[5] In this observational cohort, no aviptadil was administered.

Randomized data in COVID-19. A randomized controlled trial (NCT04311697) has enrolled 196 patients (2:1 randomization) using the same intravenous dosing schedule as the Phase 1 trial in septic ARDS patients and the COVID-19 EAP experience. Final results from this trial are pending; preliminary results suggested survival of 71–72% at 28 days in both groups with exploratory signals suggesting possible benefit in time to recovery in the largest subgroup, those receiving high-flow nasal cannula at randomization. The DSMB did not identify any important safety concerns during interim monitoring; hypotension has been uncommon and has not generally resulted in changes to aviptadil infusion. Mild-moderate diarrhea occurred in approximately a third of patients.

1.1 Potential risk and benefits from aviptadil

Primary effects of VIP infusion, generally dose dependent, include facial flushing, changes in heart rate, decrease in blood pressure, and diarrhea. Effects on renal function and fluid status are transient and mild, with the possible exception of patients with advanced liver disease.

Facial flushing is common with VIP and is not dangerous. It is generally well tolerated and resolves when the VIP infusion is stopped. It is caused by dilation of cutaneous vasculature.

Increases in heart rate are common and rarely clinically significant. The increase in heart rate primarily reflects changes in cardiac preload and an adrenergic response to decreased afterload.

The primary known risk of intravenous VIP infusion is of decreased blood pressure. The clinician investigators with the most experience with the agent report (personal communication) approximately 25% incidence of hypotension during infusion in ICU patients with shock present before initiation of VIP. These rates are observed in treatment protocols that do not exceed 150 pmol/kg/hr. When present, the decrease in blood pressure appears to be approximately 10% of mean arterial pressure (e.g., a decrease from 80 mmHg to 72 mmHg). In other settings (generally healthy volunteers at

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

higher doses), a modest decrease in mean arterial pressure in most (but not all) studied populations has been observed. This is generally in the range of 10–15% decrease in MAP. The findings in normal volunteers are presented in Table 1.

Table 1. Hypotensive Effects Observed During VIP Infusion in Phase 1 or Similar Experience

Patient type	Patients infused with VIP	Rate in pmol/kg/hr	Blood pressure change	Study
Stable patients with stable cancer	79	300 pmol bolus (not adjusted for body mass)	7mm Hg nominal decrease (probably not significant)	Virgolini et al[2]
Healthy volunteers	6	400 pmol/kg/hr	MAP decrease by 12%	Frase et al[30]
Healthy volunteers	6	180 pmol/kg/hr	MAP decrease by 15%	Erikkson et al[31]
Healthy volunteers	8	360 pmol/kg/hr	MAP decrease by 5–10%	Unwin et al[32]
Healthy volunteers	4	198 pmol/kg/hr	DBP decrease by 15%/stable SBP	Domschke et al[33]
Healthy volunteers	6	360 pmol/kg/hr	MAP decrease by 7%	Calam et al[34]
Healthy volunteers	22	400 pmol/kg/hr	No change in blood pressure	Krejs et al[35]
Healthy volunteers	2	720 pmol/kg/hr	No change in blood pressure	Unwin et al[36]
Outpatient asthmatics	7	360 pmol/kg/hr	DBP decrease by 10%/stable SBP	Morice et al[37]
Cirrhotic patients	6	360 pmol/kg/hr	BP decrease by 10%	Calam et al[38]
BP: blood pressure; MAP: mean arterial pressure; DBP: diastolic blood pressure; VIP: vasoactive intestinal peptide				

The infusion rates used in this study are substantially lower than those used in healthy volunteers. Relevant to the proposed population for TESICO is the experience with aviptadil administered to patients with ARDS at infusion rates ranging from 50 to 150 pmol/kg/hr. In patients with septic ARDS, approximately 25% of patients encountered some decrease in blood pressure during infusion.[29] In the EAP experience with aviptadil for COVID-19 (unpublished data supplied to investigators by NeuroRX), which included patients on vasopressors, ECMO, CRRT, approximately 25% had hypotension during infusion, while the balance of patients either had stable or increased blood pressure, including several patients who weaned off vasopressors during aviptadil infusion. In the preliminary results of the randomized trial, hypotension was observed in 25.2% of aviptadil patients and 18.5% of placebo patients.

Diarrhea, which can lead to bicarbonate wasting and metabolic acidosis, was observed in 5 healthy volunteers receiving 400 pmol/kg/hr of VIP, reproducing the syndrome of “pancreatic cholera” associated with VIP-producing tumors.[39] Youssef and colleagues report (personal communication) that the diarrhea observed during infusion rates of 50–150 pmol/kg/hr are easily managed with enteral loperamide.

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

Hemoconcentration, presumably through diarrhea, has been observed with VIP infusion, primarily manifesting as a modest increase in hematocrit or serum albumin concentration. While urine output may decrease during aviptadil infusion, the glomerular filtration rate (GFR) does not.[34] The hemoconcentration does not persist after discontinuation of aviptadil infusion.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of aviptadil may be found in the Investigator's Brochure(s) (IB) and Participant Information Leaflet.

Given the high morbidity and mortality of COVID-19 ARDS, the short half-life of aviptadil, the close monitoring and early detection of abnormal vital signs present in the settings where the trial will be performed, and the ease of management of expected adverse events in care environments treating critically ill patients, the overall benefit-risk assessment of this study is considered favorable in the clinical settings where the trial will be performed.

1.2 Motivation for agent selection by the ACTIV Agent Selection Committee (ASC) and Trial Oversight Committee (TOC)

The ACTIV Agent Prioritization Committee (APC) Subteam reviewed the NeuroRX agent aviptadil (VIP) and voted in favor of the agent proceeding into ACTIV-3, and the TOC endorsed that recommendation. NeuroRX's aviptadil was supported because it binds to VPAC receptors on the pulmonary Alveolar Type II cell that is a selective target of SARS-CoV-2. The agent has suggested positive effects on lung function and clinical outcomes in small clinical studies of ARDS.

While the reviewers noted the mechanism of action in SARS-CoV-2 infection is not yet well elucidated, some published preclinical tests show a ~50% reduction in viral replication in infected Calu-3 cells, suggesting partial efficacy as an antiviral[5]; however, the agent has shown promising effects in clinical trials against SARS-CoV-2. In addition, the company provided a preprint of in vitro data, which suggests that this compound is efficacious as an antiviral. The Subteam also noted that its target within the host is a good candidate for preventing fluid accumulation and inflammation in the lung, which is a major factor in COVID-19, and the natural endogenous peptide is increased in survivors of severe COVID-19. Aviptadil is available in both IV and nebulized formulations, but the inhaled version may cause some nasal and respiratory epithelium degeneration; thus, the IV formulation is preferred for this trial. At the time of APC review, the Phase 2a trial of 50–150 pmol/kg/hr was close to completion—the company shared promising interim results from that trial.

Based on the positive response to the data presented for the agent, the Subteam discussed which ACTIV trial platform should test it. The agent already has safety data from indications other than COVID-19, which could allow it to proceed to a Phase III trial. The Subteam selected ACTIV-3 for effective testing of the agent, and the agent would fill a void in the more severely ill patients screened for that trial that are not eligible for the neutralizing antibodies currently being tested in the trial.

Finally, the APC Subteam found the manufacturing and scalability strategy for aviptadil sufficient for the full trial and beyond.

Statement regarding plans for licensure: NeuroRx, Inc., has filed IND 149,152 for Intravenous Use of Aviptadil with the FDA and been awarded Fast Track designation. FDA has indicated in writing that all preclinical data have been submitted that are required for NDA and that an NDA would be accepted based on efficacy as demonstrated in adequately controlled studies. EMA licensure will be sought by Relief Therapeutics AG (Geneva, Switzerland).

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

1.3 Justification for dose selected

Given temporal constraints imposed by the pandemic, selection of the dose and duration of therapy are based on preliminary observations from multiple sources, which together provide a reasonable basis for the dose and duration selected. Lines of evidence include pre-clinical observations, observations from cell models of SARS-CoV-2 infection, known serum pharmacokinetics, rapid trafficking to and accumulation in the target organ, lung, and an observational human cohort suggesting relevant differences in serum VIP concentrations between survivors and non-survivors.

Half life of VIP. The well-established serum half-life of VIP, due to degradation by serum peptidases, is 1 minute. In dogs, only repeat daily administration for 4 weeks was associated with effects that persisted for more than a few minutes after discontinuation of the infusion. The precise elimination dynamics from lung are not well established, but empirically, the accumulation of aviptadil in lung increases over time. In addition, concentrations in serum slowly increase over the course of a prolonged infusion.

Observations from cell models of SARS-CoV-2 infection. Temerozo and colleagues identified in multiple cell models of SARS-CoV-2 infection that a VIP concentration of approximately 10nM provided maximal anti-viral and cell-protective effects, especially in lung cells (Calu-3 cells) and monocytes. In some additional experiments, concentrations of 1nM demonstrated a relevant effect.[6]

An observational cohort of patients with COVID-19 ARDS. In a complementary observational cohort of 24 patients with severe COVID-19, Temerozo and colleagues demonstrated that VIP levels of 10–12 pg/ml were present among non-survivors (N=13), as opposed to 20 pg/ml among survivors (N=11). While these data are observational and do not provide causal evidence of the effect of ~10 pg/ml change in serum VIP levels, they nevertheless suggest the possibility that increases in VIP levels may be clinically relevant.

Expected blood and/or lung levels achieved with a given infusion. The infusion rates necessary to achieve serum levels have been demonstrated in pre-clinical experiment in dogs. Unverferth and colleagues infused 0.02 and 0.05 mcg/kg/min (360 and 900 pmol/kg/hr, respectively) in 12 dogs. The dogs had a baseline VIP blood level below the level of detection (<50 pg/ml), and the two infusion rates achieve blood levels of 540 pg/ml and 1200 pg/ml, respectively.[40] Extrapolating from these experiments (assuming a consistent relationship between infusion rate and resulting blood concentrations), 50 pmol/kg/hr would be expected to result in 71 pg/ml, and 100 pmol/kg/hr would result in 143 pg/ml in this model. These blood levels are substantially higher than those observed among survivors in the Temerozo cohort and also substantially higher than the difference between survivor and non-survivor VIP levels.

In a 10-hour infusion of 400 pmol/kg/hr of VIP among healthy volunteers, blood VIP levels rose over the course of infusion, achieving 782 pg/ml by the end of the 10-hour infusion.[39] Extrapolating this observed relationship between infusion rate and resulting blood concentrations to a 100 pmol/kg/hr infusion rate, we anticipate a blood level of 195 pg/ml by the 10-hour timepoint.

Following an intravenous dose, aviptadil rapidly distributes into tissue with approximately 45% of the dose distributing to the lungs within 30 minutes of administration.[2] The apparent volume of distribution following a 300 pmol dose is 135 mL/kg. Therefore, an initial aviptadil plasma concentration is estimated to be 0.03 nM for a 70 kg patient for which 45% of the plasma concentration is anticipated to be distributed into the lungs. Assuming dose-proportionality and drug-tissue accumulation, where dose escalation proportionally increases drug exposure, a 100 pmol/kg/hr aviptadil dose over 12 hours is estimated to achieve pulmonary concentrations within 10 nM for a 70 kg patient. A

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

150 pmol/kg/hr for 12 hours would with greater confidence achieve 10nM in lung. The 10nM concentration in lung is specific to a cell model of SARS-CoV-2 infection; lower concentrations may be protective. Extrapolation from serum concentrations suggest that rates as low as 50 pmol/kg/hr may have efficacy. The time course of subsequent decreases in lung concentrations is not well established, but the approach of interrupted infusion envisioned in this protocol is thought to represent the optimal balance of risk and benefit on the basis of current information.

Clinical experience. When Said and colleagues selected the range of doses/durations for the initial phase 1 trial in patients with septic ARDS,[29] they did so in the context of the infusion rates that were well tolerated in healthy volunteers (~300–400 pmol/kg/hr) and the awareness that even low infusion rates were associated with substantial increases in plasma VIP levels. That phase 1 trial envisioned dose escalation in small cohorts of patients, from 50 pmol/kg/hr for 6 hours up to 150 pmol/kg/hr for 12 hours. The investigators completed dosing through the 100 pmol/kg/hr for 12 hours (3 patients treated at that infusion rate). According to investigators (personal communication), VIP was infused daily for 3 days in the Phase 1 trial.

The COVID-19 experience to date (~200 patients in a 2:1 randomized trial and another ~200 treated open label under an expanded access program [EAP]) have employed a sequential dose escalation strategy, in which a 12-hour infusion is performed daily for 3 days. The initial dose is 50 pmol/kg/hr, followed on day 2 by 100 pmol/kg/hr and on day 3 by 150 pmol/kg/hr. Treatment is not continued after the patient leaves the ICU. If a patient develops intolerance at a given infusion rate, the infusion period is increased (commonly to 18 hours) without a change in the overall dose administered. These rates have been reasonably well tolerated (personal communication). The EAP experience (compared with non-randomized concurrent controls) suggested the possibility of clinical efficacy; the Phase 2a trial has not yet read out. Unpublished reports (personal communication from Dr. Youssef) from the EAP experience suggest that intolerance may be somewhat higher at the conclusion of the 100 pmol/kg/hr infusion and with the 150 pmol/kg/hr infusion among patients with ARDS and shock.

The maximum infusion rate used to date in COVID-19 (150 pmol/kg/hr) is substantially below the infusion rates used in healthy volunteers (300–400 pmol/kg/hr) which either elicited no hypotension or elicited an average of 10% decrease in mean arterial pressure. The approach taken in the present trial is thus designed to optimize tolerability while achieving adequate blood levels and lung tissue concentrations of aviptadil.

Given this context and background, the vanguard cohort of 40 participants (see below) is planned to evaluate and fine-tune the approach to managing aviptadil infusions and further assess anticipated feasibility/tolerance in the target population.

1.4 Vanguard cohort

In order to assure timely and sufficient evaluation of aviptadil using an optimal approach to managing aviptadil infusion in this target population, a vanguard cohort will be incorporated. It is recognized that prior experience with aviptadil in similar populations appears to be safe and well-tolerated, and that additional insights relevant to the conduct of the present trial can be gleaned from a vanguard cohort. The target population for the vanguard cohort will be identical to the overall trial, with the exception of the requirement that vanguard participants be admitted to an intensive care unit to facilitate more intensive monitoring. The vanguard cohort will be limited to approximately 10 sites and approximately 40 patients (randomized 1:1 to aviptadil vs. control). The focus in the vanguard cohort will be in understanding the usability and feasibility of infusion management guidelines and making minor adjustments to “fine-tune” the infusion guidelines. Investigators will received blinded adherence reports for receipt of study drug infusion as would be typical of a DSMB open report. Extensive unblinded data will also be provided on a regular basis to the DSMB, detailing blood pressure, heart

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

rate, vasopressors, fluid administration as well as data on the study drug infusions. These features will be monitored during the infusions and through 2 hours after the conclusion of the infusion.

In order to protect the overall blind and allow inclusion of vanguard participants in the final analytic cohort, investigators will only review (1) interviews with treating clinicians and site investigators regarding the utility and clarity of the infusion management guidelines, (2) blinded aggregate data on adherence with study drug infusion, and (3) recommendations from the DSMB. Standard firewalls between the DSMB and investigators will be maintained during the vanguard cohort.

The vanguard cohort is intended to assess and finetune guidelines for study drug infusion management. It is recognized that the small size of the vanguard cohort will not support conclusive inferences about safety or efficacy and is focused on feasibility and tolerance. If experience with the vanguard cohort reveals that the original infusion management guidelines are infeasible, the infusion management guidelines may undergo modification. If necessary, a second vanguard cohort may be enrolled to allow further assessment of feasibility/tolerance and further finetuning of the approach to management of aviptadil infusion. If a second vanguard cohort is required, the patients in the first vanguard cohort will not be included in the final trial analysis.

In general modifications to infusion management guidelines will not require an enrollment pause or protocol amendment, but will be managed through a protocol clarification memo and revision to the case report forms and PIM. The DSMB will also advise the study team and sponsor on the need for changes (or not) to the informed consent based on the experience in the vanguard cohort.

2. Agent-specific eligibility criteria

2.1 There is no change in inclusion criteria for this agent

2.2 Agent specific exclusion criteria

- *Refractory hypotension*, defined as infusion of vasopressors at or above norepinephrine equivalent of 0.1 mcg/kg/min (or infusion of more than one simultaneous vasopressor) in prior 4 hours to maintain MAP > 65 mmHg OR systolic blood pressure <90 mmHg or MAP < 65 mmHg at time of enrollment (or randomization, if the patient has already been enrolled) confirmed on two consecutive measurements at least 5 minutes apart (if a single measurement meets those criteria, a second measurement is required). Since aviptadil may induce hypotension, as noted above, patients with critical hypotension have a different risk:benefit profile that is less likely to favor aviptadil even where aviptadil is efficacious.
- *Severe diarrhea*, defined as 3 or more liquid bowel movements within the last 24 hours. Since diarrhea is a common side effect of aviptadil, if patients already have severe diarrhea, they may have a different risk:benefit profile that is less likely to favor aviptadil.
- *Current C. difficile infection (CDI)*. CDI generally causes diarrhea, its severity is often gauged in part by the volume of diarrhea, and anti-motility agents that may be used to manage aviptadil-associated diarrhea are contraindicated in CDI. These factors suggest that the risk:benefit ratio in patients with CDI may not be favorable.
- *Pregnancy or current breast-feeding*. Aviptadil was associated with involution of embryos in animal models and may be associated with changes in visceral and/or placental perfusion. It is thus felt not appropriate to infuse aviptadil in pregnant patients or in women who are breastfeeding.
- *End-stage liver disease (ESLD)*, defined as hepatic decompensation in a person with or without cirrhosis, usually associated with ascites (fluid in the peritoneal cavity), jaundice, variceal hemorrhage or hepatic encephalopathy (confusion, change in behavior, forgetfulness).

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

Liver function tests and/or coagulation profile are usually abnormal. An isolated elevation in serum bilirubin does not meet criteria for end-stage liver disease.

3. Description of investigational agent

3.1. Administration and duration
The approach to infusion is based on prior clinical experience with the use of aviptadil. Aviptadil is infused over 12 hours per day for three days. The day 1 infusion rate is 50 pmol/kg/hr, the day 2 infusion rate is 100 pmol/kg/hr, while the day 3 infusion rate is 150 pmol/kg/hr. The primary factors defining intolerance to aviptadil infusion are hypotension or diarrhea. The PIM will include infusion management guidelines to assist clinicians in responding to hypotension or diarrhea among patients receiving aviptadil. The total volume of the infusion (aviptadil vs. saline placebo) is generally less than 100 ml per day, although infusion volumes will vary by patient weight and dosing day.

3.2. Formulation and preparation
Aviptadil is a sterile drug product that must be formulated by a hospital pharmacist under sterile conditions according to the supplied pharmacy manual. Formulation is in 0.9% sodium chloride, with standard mixing procedures. Standard intravenous bags and tubing are used. Dosing is at 50/100/150 pmol/kg/hr.

3.3 Supply, distribution, and accountability
Procedures for ordering and accepting drug, for maintaining inventory of aviptadil, and for breaking the blind in the event of a medical emergency will be described in the Pharmacy Procedures.

3.4. Contraindicated medications
There are no known contraindicated medications. There is a theoretical consideration about use of nitric oxide or prostanoid therapy, but there is no compelling data to date to suggest that such medication should be restricted. Use of pulmonary vasodilators will thus be tracked with concomitant medications.

3.5. Precautionary medications
The clinical site should have necessary equipment and medications for the management of any infusion reaction. These include capacity to monitor vital signs, ability to infuse and monitor vasopressor agents if necessary, and capacity to manage diarrhea and electrolyte loss. Unrelated to aviptadil but centrally related to COVID-19, sites must be able to manage progression of respiratory failure.

4. Clinical and laboratory evaluations

Clinical and laboratory evaluations will follow the master protocol schedule of assessments.

4.1 Timing of Assessments
All assessments are outlined in the relevant section of the master protocol.

4.2. Pharmacokinetic Assessments
Pharmacokinetic assessments are being performed in a Phase 2 trial performed by NeuroRX.

5. Clinical management issues
All participants should be monitored closely for hypotension and diarrhea and any additional adverse events, with special attention to treatment-emergent adverse events.

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

5.1. Symptoms and Signs
Symptoms and signs that may occur as part of an infusion reaction, include, but are not limited to, decrease in mean arterial pressure, diarrhea, facial flushing. Infusion-related reactions' severity will be assessed and reported using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected version 2.1. Given the unique nature of the target population for this trial, hypotension will be graded according to the scale in Table 5 (Section 10) of the master protocol rather than the DAIDS AE Grading Table.

5.2. Site Needs
The clinical site should have necessary equipment, medications, adequately qualified and experienced staff with appropriate medical cover for the management of any infusion reaction, which may include, but is not limited to, hypotension and diarrhea.

5.3. Management of Infusion Reactions including Discontinuation
Infusion of aviptadil or its placebo will be guided by infusion management guidelines in the context of clinician judgment. If the complete infusion is not administered, all follow-up procedures and reporting outlined in the master protocol should be adhered to as indicated.

6. Agent-specific safety monitoring activities

Safety monitoring for aviptadil will be as specified in the master protocol. However, grade 3 or 4 diarrhea in the peri-infusion period will only be included in the composite safety endpoint if the diarrhea is a serious adverse event, or results in discontinuation of the study drug infusion. "Peri-infusion" refers to the time period during and up to 2 hours after an infusion.

The primary safety outcome was modified in order to avoid mistaken inferences regarding safety, because diarrhea is common with aviptadil and is generally well managed with loperamide in prior clinical experience. For example, diarrhea treated with loperamide would generally be classified as a grade 3 adverse event. All grade 3 or 4 diarrhea that occurs outside the peri-infusion time period will still be included in the primary safety outcome, as part of incident grade 3 or 4 AEs. Also, all peri-infusion diarrhea events will be reported to the DSMB as part of the infusion reaction summaries.

Specific to aviptadil study drug, there is one change to the safety monitoring schedule displayed in Table 3 of the master protocol: hypotension of any grade will be recorded daily through Day 28.

Note that as part of the oversight of this trial, the DSMB will review unblinded safety data regularly during the trial.

Hypotension is defined as a lower arterial blood pressure or low arterial blood pressure/perfusion leading to (1) initiation or clinically meaningful increase in vasopressor therapy, (2) administration of an intravenous fluid bolus (≥500 ml of crystalloid solution or equivalent volume of colloid), or (3) modification or discontinuation of study drug infusion. Specific grading of hypotension will be according to Section 10, Table 5 of the master protocol. Specific to the aviptadil vs. placebo comparison, in addition to standard data summaries for hypotension AEs, hypotension associated with organ dysfunction within the first 5 days after study entry will also be compared between aviptadil and its placebo. Hypotension associated with organ dysfunction is defined as hypotension plus concomitant or subsequent organ dysfunction. Organ dysfunction in this setting is a composite outcome consisting of items 5a-5f (excluding item 5b4) of the secondary outcome of clinical organ failure and serious infections (section 4.1.2 of the TESICO master protocol).

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022
For regulatory reporting purposes, including identification and potential expedited reporting of 'SUSAR' events, the following serious and/or non-serious Adverse Events/Reactions are considered expected for this study as applies to the aviptadil factor.

- Hypotension*
- Diarrhea

* For regulatory reporting purposes, hypotension up to Grade 3 is considered expected for aviptadil in this specific study, population, disease and setting. Hypotension occurring at Grade 4 or higher as described in Table 5 in the main protocol document is **not** considered expected.

In addition, the following adverse events/reactions are considered expected unless serious:

- Bradycardia
- Tachycardia
- Flushing

The DSMB reviews safety data on an ongoing and unblinded manner. If a pattern, frequency, or other characteristic of concern becomes evident to the DSMB with regard to the 'expected' events listed above, the study team and sponsor will be promptly notified and action will be taken as may be indicated for subject protection and/or reporting purposes.

6. References

1. Said SI, Mutt V. Potent peripheral and splanchnic vasodilator peptide from normal gut. Nature 1970;225:863-4.
2. Virgolini I, Raderer M, Kurtaran A, et al. Vasoactive intestinal peptide-receptor imaging for the localization of intestinal adenocarcinomas and endocrine tumors. N Engl J Med 1994;331:1116-21.
3. Onoue S, Ohmori Y, Endo K, Yamada S, Kimura R, Yajima T. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide attenuate the cigarette smoke extract-induced apoptotic death of rat alveolar L2 cells. Eur J Biochem 2004;271:1757-67.
4. Temerozo JR, Joaquim R, Regis EG, Savino W, Bou-Habib DC. Macrophage Resistance to HIV-1 Infection Is Enhanced by the Neuropeptides VIP and PACAP. PloS one 2013;8:e67701.
5. Temerozo JR, de Azevedo SSD, Insuela DBR, et al. The Neuropeptides Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase-Activating Polypeptide Control HIV-1 Infection in Macrophages Through Activation of Protein Kinases A and C. Frontiers in immunology 2018;9:1336.
6. Temerozo JR, Sacramento CQ, Fintelman-Rodrigues N, et al. The neuropeptides VIP and PACAP inhibit SARS-CoV-2 replication in monocytes and lung epithelial cells and decrease the production of proinflammatory cytokines in infected cells. BioRxiv 2020.
7. Ganea D, Hooper KM, Kong W. The neuropeptide vasoactive intestinal peptide: direct effects on immune cells and involvement in inflammatory and autoimmune diseases. Acta Physiol (Oxf) 2015;213:442-52.
8. Prasse A, Zissel G, Lützen N, et al. Inhaled vasoactive intestinal peptide exerts immunoregulatory effects in sarcoidosis. Am J Respir Crit Care Med 2010;182:540-8.
9. Dewit D, Gourlet P, Amraoui Z, et al. The vasoactive intestinal peptide analogue RO25-1553 inhibits the production of TNF and IL-12 by LPS-activated monocytes. Immunol Lett 1998;60:57-60.
10. Hernanz A, Tato E, De la Fuente M, de Miguel E, Arnalich F. Differential effects of gastrin-releasing peptide, neuropeptide Y, somatostatin and vasoactive intestinal peptide on interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha production by whole blood cells from healthy young and old subjects. J Neuroimmunol 1996;71:25-30.

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

11. Sun W, Tadmori I, Yang L, Delgado M, Ganea D. Vasoactive intestinal peptide (VIP) inhibits TGF-beta1 production in murine macrophages. J Neuroimmunol 2000;107:88-99.
12. Jobson TM, Billington CK, Hall IP. Regulation of proliferation of human colonic subepithelial myofibroblasts by mediators important in intestinal inflammation. The Journal of clinical investigation 1998;101:2650-7.
13. Li L, Luo ZQ, Zhou FW, et al. Effect of vasoactive intestinal peptide on pulmonary surfactants phospholipid synthesis in lung explants. Acta Pharmacol Sin 2004;25:1652-8.
14. Li L, She H, Yue SJ, et al. Role of c-fos gene in vasoactive intestinal peptide promoted synthesis of pulmonary surfactant phospholipids. Regul Pept 2007;140:117-24.
15. Li L, She H, Yue S, Feng D, Luo Z. Vasoactive intestinal peptide induces surfactant protein A expression in ATII cells through activation of PKC/c-Fos pathway. Peptides 2010;31:2046-51.
16. Sharma V, Delgado M, Ganea D. Granzyme B, a new player in activation-induced cell death, is down-regulated by vasoactive intestinal peptide in Th2 but not Th1 effectors. J Immunol 2006;176:97-110.
17. Said SI, Dickman KG. Pathways of inflammation and cell death in the lung: modulation by vasoactive intestinal peptide. Regul Pept 2000;93:21-9.
18. Foda HD, Iwanaga T, LIU LW, Said SI. Vasoactive Intestinal Peptide Protects Against HCl-Induced Pulmonary Edema in Rats. Annals of the New York Academy of Sciences 1988;527:633-6.
19. Said SI, Berisha HI, Pakbaz H. Excitotoxicity in the lung: N-methyl-D-aspartate-induced, nitric oxide-dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A 1996;93:4688-92.
20. Berisha H, Foda H, Sakakibara H, Trotz M, Pakbaz H, Said SI. Vasoactive intestinal peptide prevents lung injury due to xanthine/xanthine oxidase. Am J Physiol 1990;259:L151-5.
21. Pakbaz H, Foda HD, Berisha HI, Trotz M, Said SI. Paraquat-induced lung injury: prevention by vasoactive intestinal peptide and related peptide helodermin. Am J Physiol 1993;265:L369-73.
22. Saga T, Said SI. Vasoactive intestinal peptide relaxes isolated strips of human bronchus, pulmonary artery, and lung parenchyma. Trans Assoc Am Physicians 1984;97:304-10.
23. Mathioudakis A, Chatzimavridou-Grigoriadou V, Evangelopoulou E, Mathioudakis G. Vasoactive intestinal Peptide inhaled agonists: potential role in respiratory therapeutics. Hippokratia 2013;17:12-6.
24. Diamond L, Szarek JL, Gillespie MN, Altiere RJ. In Vivo Bronchodilator Activity of Vasoactive Intestinal Peptide in the Cat. American Review of Respiratory Disease 1983;128:827-32.
25. Cox CP, Linden J, Said SI. VIP elevates platelet cyclic AMP (cAMP) levels and inhibits in vitro platelet activation induced by platelet-activating factor (PAF). Peptides 1984;5:325-8.
26. Frye BC, Meiss F, von Bubnoff D, Zissel G, Müller-Quernheim J. Vasoactive Intestinal Peptide in Checkpoint Inhibitor-Induced Pneumonitis. N Engl J Med 2020;382:2573-4.
27. Leuchte HH, Baezner C, Baumgartner RA, et al. Inhalation of vasoactive intestinal peptide in pulmonary hypertension. The European respiratory journal 2008;32:1289-94.
28. Petkov V, Mosgoeller W, Ziesche R, et al. Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. The Journal of clinical investigation 2003;111:1339-46.
29. Youssef JG, Said S, Youssef G, Javitt MJ, Javitt JC. Treatment of Acute Respiratory Distress Syndrome with Vasoactive Intestinal Peptide. 2020.
30. Frase LL, Gaffney FA, Lane LD, et al. Cardiovascular effects of vasoactive intestinal peptide in healthy subjects. The American journal of cardiology 1987;60:1356-61.
31. Eriksson LS, Hagenfeldt L, Mutt V, Wahren J. Influence of vasoactive intestinal polypeptide (VIP) on splanchnic and central hemodynamics in healthy subjects. Peptides 1989;10:481-4.
32. Unwin R, Reed T, Thom S, Calam J, Peart W. Effects of indomethacin and (+/-)-propranolol on the cardiovascular and renin responses to vasoactive intestinal polypeptide (VIP) infusion in man. British journal of clinical pharmacology 1987;23:523-8.
33. Domschke S, Domschke W, Bloom SR, et al. Vasoactive intestinal peptide in man: pharmacokinetics, metabolic and circulatory effects. Gut 1978;19:1049-53.

TESICO Appendix H1: Aviptadil version 3.0, 08 March 2022

34. Calam J, Dimaline R, Peart WS, Singh J, Unwin RJ. Effects of vasoactive intestinal polypeptide on renal function in man. J Physiol 1983;345:469-75.
35. Krejs GJ, Fordtran JS, Fahrenkrug J, et al. Effect of VIP infusion in water and ion transport in the human jejunum. Gastroenterology 1980;78:722-7.
36. Unwin RJ, Calam J, Peart WS, Dimaline R, Lightman SL. Vipoma and watery diarrhea. N Engl J Med 1982;307:377-8.
37. Morice A, Unwin RJ, Sever PS. Vasoactive intestinal peptide causes bronchodilatation and protects against histamine-induced bronchoconstriction in asthmatic subjects. Lancet 1983;2:1225-7.
38. Calam J, Unwin RJ, Dorudi S, Taylor C, Springer CJ, Peart WS. Effects of vasoactive intestinal polypeptide infusions on renal function in patients with liver disease. Clin Exp Pharmacol Physiol 1988;15:491-5.
39. Kane MG, O'Dorisio TM, Krejs GJ. Production of secretory diarrhea by intravenous infusion of vasoactive intestinal polypeptide. N Engl J Med 1983;309:1482-5.
40. Unverferth DV, O'Dorisio TM, Muir WW, et al. Effect of vasoactive intestinal polypeptide on the canine cardiovascular system. Translational Research 1985;106:542-50.