



REQUEST FOR BREAKTHROUGH THERAPY DESIGNATION

Treatment of patients with Critical COVID-19 who are at immediate risk
of death from Respiratory Failure despite treatment with approved
therapy including remdesivir
IND 149152

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1. PRODUCT NAMES:

- Zyesami
- Aviptadil (USAN and INN names)
- Vasoactive Intestinal Polypeptide (synthetic)

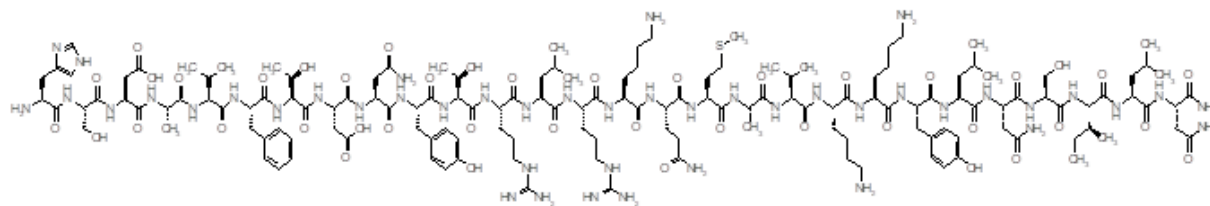
Application Numbers:

- IND 149152: Intravenous aviptadil
- IND 151070: Inhaled aviptadil

A note on nomenclature: When discussing physiologic effects of Vasoactive Intestinal Peptide (VIP), many of which were reported based on VIP purified from biologic tissues, the term “VIP” is used. When discussing the effects of pharmaceutical (synthetic) VIP, the term “aviptadil” is used.

2. CHEMICAL NAME AND STRUCTURE

Aviptadil is composed of synthetic vasoactive intestinal polypeptide whose final commercial presentation will be in the form of lyophilized VIP in glass syringes for reconstitution with saline for injection.



Abbreviated chemical name:	H-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-NH ₂
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Molecular formula:	C ₁₄₇ H ₂₃₈ N ₄₄ O ₄₂ S (net)
Molecular mass:	3325.8 g/mol (net)

3. PROPOSED BREAKTHROUGH THERAPY INDICATION

Treatment of patients with Critical COVID-19 who are at immediate risk of death from Respiratory Failure despite treatment with approved therapy including remdesivir.

4. BASIS FOR BREAKTHROUGH THERAPY DESIGNATION

NRx Pharmaceuticals, Inc. requests that FDA grant Breakthrough Therapy Designation (BTD) to aviptadil for the treatment of patients with Critical COVID-19 who have progressed to respiratory failure despite treatment with remdesivir on the following grounds:

Aviptadil is intended to treat a Serious Condition: Critical COVID-19 with respiratory failure is a serious and life-threatening medical condition. The mortality rate associated with Acute Lung Injury/Acute Respiratory Distress Syndrome has historically been reported at 35–50%. ARDS related to COVID-19 is reported to have a 50% or higher mortality rate. As such Critical COVID-19 with respiratory failure meets the requirements for a Serious Condition under 21 CFR 312.300(b)(1).

There is clinical evidence that aviptadil may demonstrate substantial improvement on a clinically significant endpoint over available therapies:

Trial RLF-100-001 was a randomized, double-blind, placebo-controlled trial of aviptadil for the treatment of critical COVID-19 patients with respiratory failure. Seventy percent of the randomized subjects had failed all approved therapies for critical COVID-19, including remdesivir. The analysis presented below uses Statistical Analysis Plan (SAP) Version 1.4, which FDA considers to be the pre-specified analysis plan.

The primary endpoint considered in this request for BTD is cumulative distribution of time to respiratory failure resolution with concurrent survival through the period of observation **in patients who have progressed to respiratory failure despite treatment with remdesivir and other approved therapies**. Sponsor believes that this endpoint is scientifically and linguistically equivalent to FDA's formulation of "alive and free of respiratory failure" and will therefore use the FDA-supported language. Although day 28 was the originally-anticipated period of observation, sponsor recognized the need to add a day 60 endpoint in December 2020, prior to unblinding because it was observed that 1/3 of the cohort remained in the ICU at day 28. FDA similarly recognized the need for follow-up through day 60 in critically-ill patients (FDA 2021).

In the subgroup of patients treated with remdesivir, those treated with remdesivir + aviptadil were significantly more likely to be alive and free of respiratory failure at Day 28 (OR 2.8; P=.03) and at Day 60 (OR 2.5; P=.03). A trend level of significance in survival favoring aviptadil vs. placebo emerges at day 28 (OR 2.3; P=.09) and demonstrates a high degree of statistical significance at Day 60 (OR 4.0; P=.006) (Appendix 1 page 7, 12, and referenced tables).

This subgroup of remdesivir-treated patients comprises approximately 70% of both the drug and the placebo randomization cohorts. Among those who were most acutely ill, the subgroup of NIAID 2 score at baseline (i.e. those on mechanical ventilation or ECMO) odds of death on Placebo were 10 fold higher compared to those treated with aviptadil (P= .02) (Appendix 1, p5 Table 2). In the overall trial – both those treated with remdesivir and those not treated with remdesivir, across all patients enrolled aviptadil demonstrated a statistically-significant (P<.02) 2-fold improvement in odds of survival (the secondary endpoint), but achieved the primary endpoint at 60 days only at a trend level (P=.08).

Prior treatment with remdesivir was a prespecified covariate for analysis of primary and secondary endpoint in the Statistical Analysis Plan (v 1.4). Analysis of the remdesivir-treated subgroup was performed in consideration of FDA's comments in FDA's response to sponsor's initial request for Breakthrough Therapy Designation (Ref ID 38890281). There is substantial rationale for considering this subgroup analysis on the grounds that 1) use of remdesivir at baseline is a prespecified covariate, 2) survival was a statistically-significant endpoint across the entire enrollment of the clinical trial, 3) the subgroup analysis was not prompted by sponsor's data exploration. Rather, it is presented to address an FDA suggestion to compare aviptadil to remdesivir.

The endpoints of survival and recovery from respiratory failure are specified in the FDA Guidance to Industry (FDA 2021 COVID-19: Developing Drugs and Biological Products for Treatment or Prevention). The clinical and biological plausibility of these endpoints is supported by (1) statistically significant improvement in the NIAID ordinal scale in aviptadil-treated subgroups, (2) statistically significant improvement in oxygenation as measured by Respiratory Distress Ratio across all patients and all sites of care, and (3) a statistically significant rise in cytokine IL-6 levels among placebo-treated patients, compared to aviptadil-treated patients, across all patients and all sites of care.

Further support for Breakthrough Therapy Designation derives from the aviptadil benefit in the subgroup of patients who are NIAID 2 at baseline. The NIAID score at baseline is a predefined basis for subgroup analysis in the trial protocol. **The odds of death on Placebo in the NIAID 2 group at baseline were 10 fold higher than the death rate on Aviptadil or conversely the odds ratio for Aviptadil was 0.1; the two-sided p-value was 0.031 (Appendix 1).**

The clinical evidence demonstrated in the phase 2b/3 trial (section 4.5.1) is also supported by a single-center, open-label trial conducted at Houston Methodist Hospital among patients whose comorbidity levels precluded their enrollment in the phase 2b/3 trial (section 4.5.5). As shown below, highly significant differences in recovery and survival were demonstrated, together with statistically significant differences in ordinal scale, oxygenation, and cytokine levels.

Aviptadil addresses an unmet medical need in patients who have exhausted approved therapy: By definition, the BTD indication sought represents an unmet medical need because BTD is sought for patients who have exhausted approved therapy, particularly remdesivir. The remdesivir label indicates that remdesivir is indicated for the treatment of coronavirus disease 2019 (COVID-19) requiring hospitalization. However, the package insert documents efficacy data only in patients with mild, moderate, and severe COVID-19. No evidence of benefit is identified in patients with Critical COVID-19 (i.e. those in respiratory failure). The National Institutes of Health COVID treatment guidelines recommend the use of remdesivir in those patients maintained on nasal oxygen but do not recommend remdesivir in those patients treated with mechanical ventilation or ECMO. Moreover, the same guidelines only recommend tocilizumab for patients within the first 24 hours of admission to the ICU. Data cited in this BTD application suggest a statistically-significant improvement in survival among those treated with both mechanical ventilation and ECMO. The NIH guidelines specify that the recommendation for remdesivir plus dexamethasone in patients on high-flow oxygen is not based on clinical evidence.



Figure 1: NIH COVID-19 treatment guidelines August 2021

Remdesivir's lack of demonstrated efficacy in patients who have developed respiratory failure is readily apparent from the remdesivir phase 3 clinical trial publication (Figure 2). Although remdesivir clearly demonstrates efficacy in improving recovery from mild, moderate, and severe COVID-19, this benefit is not seen in patients who require High Flow Oxygen, non-invasive mechanical ventilation, invasive mechanical ventilation, or ECMO. Aviptadil, on the other hand, has demonstrated preliminary evidence of efficacy in those subgroups where remdesivir has not demonstrated effectiveness.

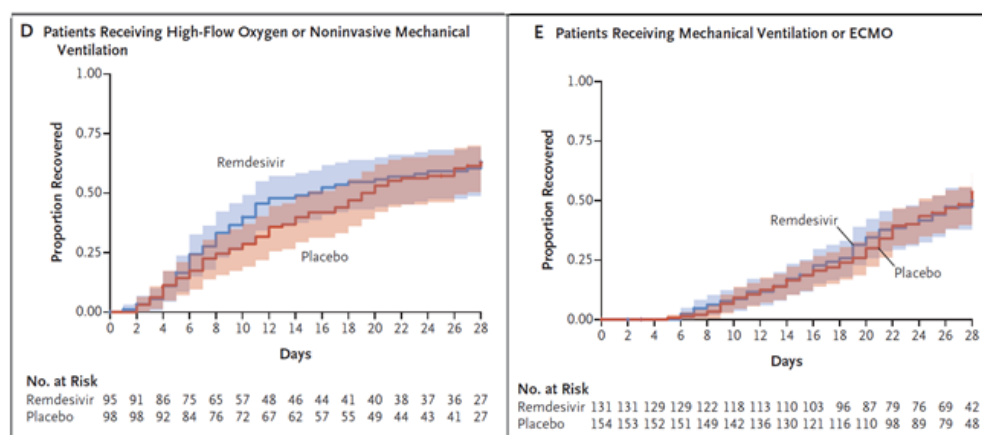


Figure 2: Effect of Remdesivir on recovery in patients with Critical COVID-19 and respiratory failure in the ACTT-1 trial (adapted from Beigel 2020)

The results of the aviptadil phase 2b/3 trial, combined with the open-label clinical study at Houston Methodist Hospital (HMH) discussed in this document, provide preliminary clinical evidence that aviptadil may demonstrate a substantial improvement on a clinically significant endpoint in patients who have progressed to Critical COVID-19 and respiratory failure despite treatment with remdesivir. In the case of the HMH trial, all those eligible to be treated with remdesivir were treated. Moreover, patients in the HMH trial had significant comorbidities that made them ineligible for the randomized phase 2b/3 trial. Such patients included lung transplant patients, who are particularly vulnerable to COVID-19 despite vaccination because of their immunosuppressive medication.

4.1 INTRODUCTION

Since its discovery in 1970 by Said and Mutt (Said and Mutt, 1970), VIP has been shown to protect the lung against a broad array of caustic, immune, and infectious injuries (Said 1988, Said 1991, Said 2000) through its binding to the VPAC1 receptor of the Alveolar Type II cell. This is the same cell to which the SARS-CoV-2 virus binds via the ACE2 receptor (Mason 2020). Intravenous VIP has previously demonstrated effectiveness in the treatment of sarcoid (Prasse 2010), pulmonary hypertension (Petkov 2003, Leuchte 2008), and ARDS related to sepsis (Said, unpublished data). More recently, VIP is the first COVID-19 therapeutic agent shown to block replication of the SARS-CoV-2 virus in human pulmonary epithelial cells and monocytes (Temerozo 2020). In numerous laboratory studies, VIP has been shown to block secretion of various inflammatory cytokines, most notably IL-6 (Delgado 1999).

4.2 ZYESAMI (AVIPTADIL ACETATE) IS INTENDED TO TREAT A SERIOUS CONDITION

ZYESAMI is intended to treat patients with Critical COVID-19 with respiratory failure. Critical COVID-19 is defined by the FDA (FDA 2020) by:

- Positive testing by standard RT-PCR assay or equivalent test
- Evidence of critical illness, defined by at least one of the following:
 - Respiratory failure defined as 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 by reinforced nasal cannula at flow rates > 20 L/min with fraction of delivered oxygen ≥ 0.5),
 - Non-invasive 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, diastolic blood pressure < 60 mmHg, or requiring vasopressors)
 - Multi-organ dysfunction/failure

Based on the above criteria, aviptadil is intended to treat a serious condition.

4.3 MECHANISM OF ACTION FOR ZYESAMI IN TREATMENT OF COVID-19

In order to understand why ZYESAMI is potentially effective in increasing survival and recovery from COVID-19 Respiratory Failure, it is first important to understand why SARS-CoV-2 infects all mammals but only causes COVID-19 and death in humans (except for genetically modified mice). Although SARS-CoV-2 causes general viremia in many mammals, in humans it binds to the human Angiotensin Converting Enzyme 2 (ACE2) receptor, which provides a route of entry to the alveolar type II (AT2) cell in the lining of the lung.

As described by Mason (Mason 2020) once SARS-CoV-2 has entered the AT2 cell, it shuts down surfactant production, replicates within the AT2 cell, and causes a release of numerous cytokines, ultimately resulting in AT2 cell death. Frequently, the first symptom of COVID-19 in the respiratory tract is loss of sense of smell because the cells of the olfactory nerve also express ACE2. Once the infection reaches the lower respiratory tract, the patient suffers a loss of surfactant production with alveolar collapse, which explains the onset of hypoxia prior to other signs of cytokine storm. The subsequent cytokine storm phase of COVID-19 is associated with shock, multisystem organ failure, and death.

AT2 cells contain VPAC₁ receptors that bind to VIP. When VIP enters the AT2 cell, it is believed to block SARS-CoV replication (Temerozo 2020), upregulate surfactant production (Mason, personal communication), inhibit cytopathy (Temerozo 2020), and inhibit cytokine release. All four of these effects have been demonstrated in vitro. The effect of blocking cytokine release has also been demonstrated in a randomized prospective clinical trial (see 4.5.2.4). The effect on cytokine production was also demonstrated in an open label clinical trial (see 4.5.6.4)

The mechanism of action for VIP differs from that of all known therapies aiming to treat COVID-19. The antiviral effect of VIP is uniquely targeted to the AT2 cell, as distinct from the generalized antiviral effect of remdesivir and other antiviral drugs. No other COVID therapeutic has been reported to have an effect on surfactant production. The anti-cytokine effect of VIP appears to prevent cytokine release, whereas the various monoclonal antibodies that target COVID-19 act downstream to block cytokine effects once cytokine release has occurred. Figure 3 illustrates the concepts described above.

For these reasons, the NIH-sponsored ACTIV3b program has included aviptadil in a head-to-head comparison with remdesivir alone and in combination. The BARDA-sponsored I-SPY trial has included aviptadil as one of the drugs being administered to patients in the ICU with Critical COVID-19, as defined in FDA Guidance (FDA 2021). NRx is an industry partner to both of these trials and manufactures the investigational product that is being tested.

In summary, aviptadil is believed to have a unique mechanism in preventing death and accelerating recovery from COVID-19—a mechanism that is distinct from all known COVID therapeutics and that is likely to be complementary to all therapeutics currently under consideration.

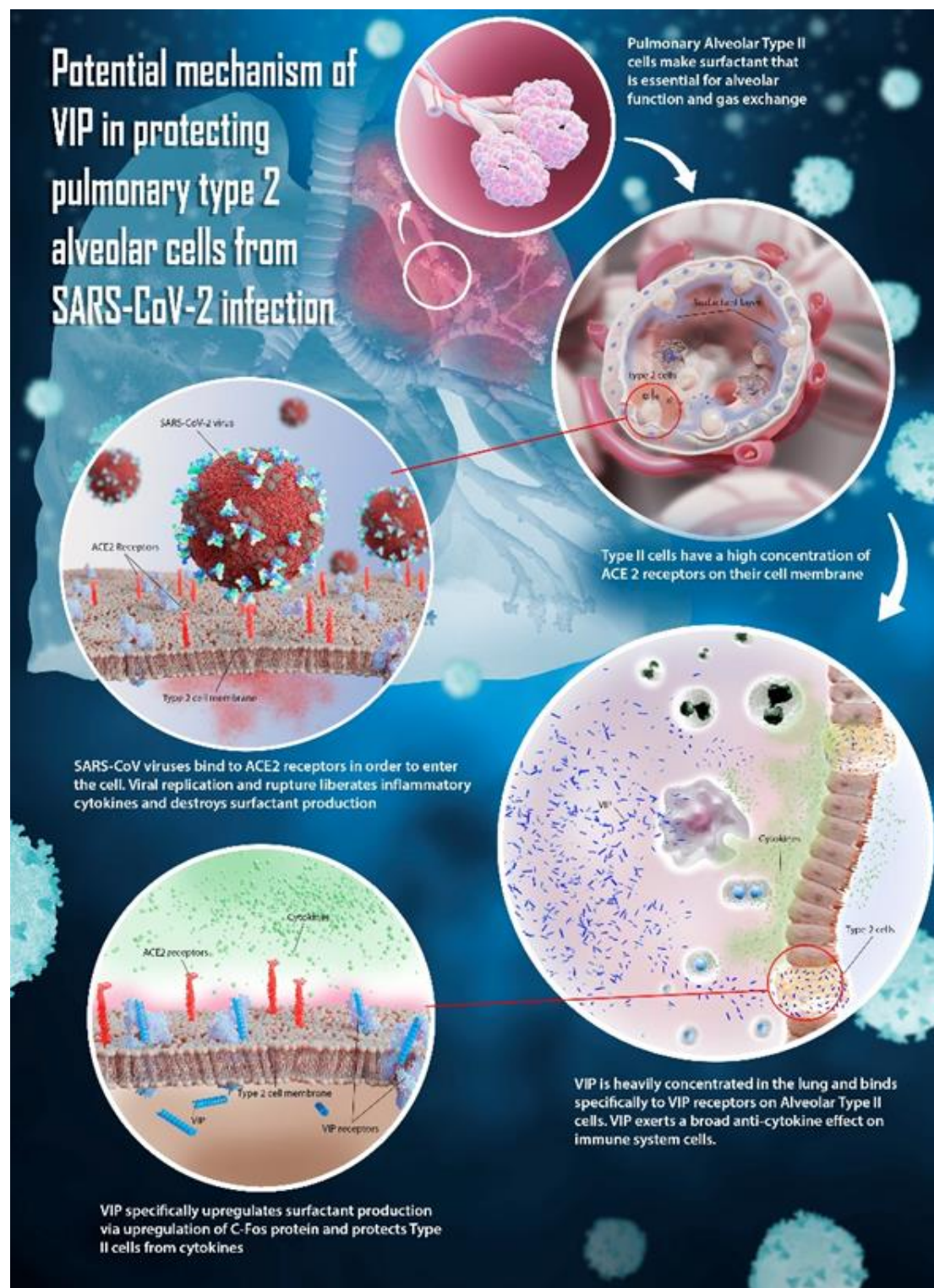


Figure 3: Potential mechanism of Vasoactive Intestinal Peptide in protecting pulmonary type 2 alveolar cells from SARS-CoV-2 infection

4.4 THERE IS EXTENSIVE PRECLINICAL EVIDENCE THAT AVIPTADIL PROTECTS HUMAN ALVEOLAR TYPE II CELLS BY UPREGULATING SURFACTANT PRODUCTION, INHIBITING CYTOKINE RELEASE, AND INHIBITING REPLICATION OF SARS-CoV-2

Vasoactive Intestinal Peptide (VIP) was first proposed as a modulator of lung inflammation by Said (1988, 1991). VIP is known to have a beneficial effect in numerous models of lung injury (Table 1) and has shown clinical effects in clinical trials of Acute Respiratory Distress Syndrome (ARDS) and sarcoid, with a meaningful reduction in TNF- α and CD4/CD8 ratio seen in sarcoid (Prasse 2010).

Table 1: Effect of VIP in experimental models of acute lung injury

Species	Etiology of lung injury	References
Rat	NDMD induced lung injury w/ arginine	Said (1996), Said and Dickman (2000)
Rat	Xanthine/xanthine oxidase-induced lung injury in perfused lungs	Berisha (1990), Misra (1990)
Guinea Pig	Paraquat (methyl viologen)	Pakbaz (1993), Said and Dickman (2000)
Rat	Hydrochloric acid induced pulmonary edema	Foda (1988)
Sheep	Intravenous infusion of platelet-activating factor	Pakbaz (1988)
Dog	Intravenous infusion of platelet-activating factor	Pakbaz (1988)
Guinea Pig	Phospholipase C	Pakbaz (1991)
Rat	Cobra venom factor model of septic shock	Mulligan (1992)

Multiple investigators have confirmed that the SARS-CoV family of viruses selectively attacks pulmonary Alveolar Type II (AT2) cells, as distinct from other pulmonary epithelial cells, because of the ACE2 receptors of the former. The AT2 cells manufacture surfactant, which is essential to gas exchange in the alveolus (Mason 2020).

VIP is a potent anti-cytokine in the lung that provides a key defense against numerous forms of acute lung injury. Although named (or mis-named) for the tissue in which it was first isolated, VIP is produced by neuroendocrine cells throughout the body and by T-lymphocytes, B-lymphocytes, and macrophages. VIP is highly localized in the lung (Virgolini 1995) but is a widely distributed immunomodulator with protective effects in the heart, thyroid gland, kidney, immune system, urinary tract, and genital organs. Early COVID-19 lung injury is characterized by a remarkable degree of hypoxia in the absence of overwhelming pneumonia, suggesting a primary injury to the pulmonary gas-exchange mechanism. Patients frequently report “crackling sounds” while attempting to breath, consistent with the theory that the loss of surfactant and alveolar gas exchange is an early hallmark of COVID-19. VIP is the body’s primary defense against cytokine injury in the lung and elsewhere. Unlike synthetic anti-cytokines, such as anti-IL6 drugs, VIP is shown to have a specific role in preserving surfactant production in the lung (Li 2004, 2010) and in protecting type 2 alveolar cells. Accordingly, VIP and longer-acting modifications of VIP have been proposed in the past as respiratory therapeutics (Mathioudakis 2013).

COVID-19 and Immune Response:

Coronavirus pathophysiology in humans has been studied since the 2004 SARS epidemic, in which pulmonary complications were traced to a release of proinflammatory cytokines, including IL-6, IL-12, and TNF- α . Patients with SARS-CoV-2 pneumonia admitted to an ICU had higher plasma levels of cytokines including IL-6, IL-2, IL-7, IL-10, granulocyte-colony stimulating factor (G-CSF), interferon- γ -inducible protein (IP10), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), and TNF- α (Wong 2004). Studies on MERS-CoV demonstrated marked upregulation of the cytokine genes of IL-6, IL-1 β , and IL-8 (Channappanavar 2017). This phenomenon is now widely called a “cytokine storm.” More recent data point to the specific attack of the virus on ACE2 receptors of the type 2 alveolar cells, resulting in the selective death of those cells.

In recent years, interleukin 6 (IL-6)–blocking drugs and biologics have been developed to treat arthritis, inflammatory bowel disease, and other autoimmune conditions. Blockade of IL-6 has been demonstrated to have a potent effect on outcome in nonclinical studies and aviptadil is now demonstrated to block IL-6 release in clinical trials (Figs 7 and 14). Blockade of IL-6 release is also highly correlated with survival in clinical trials (Fig 8).

Lung injuries seen in COVID-19 are increasingly recognized as similar to those in premature infants, in whom the loss of surfactant secreted by alveolar type II cells leads to demise despite mechanical ventilation. The SARS-CoV-2 virus is known to enter cells via binding to ACE2 receptors on the cell surface, and those receptors are predominantly found on the AT2 cell. Moreover, VIP receptors are preferentially expressed on AT2 cells, and VIP is shown to prevent their apoptosis in models of lung injury (Onoue, 2004).

4.4.1 SCIENTIFIC RATIONALE FOR VIP EFFECT IN COVID-19

4.4.1.1 Preservation of pulmonary tissue

Three studies validate the protective role of VIP on isolated and transplanted lung models. Isolated rat lungs stored in VIP-containing solutions had significantly more normal-shaped mitochondria, less mitochondrial edema, less distortion of mitochondrial cristae, thinner basal lamina, and less aggregation of nuclear chromatin than lungs stored in control solutions (Alessandrini 1993). VIP significantly delayed the onset of edematous lung injury in isolated perfused rat lungs (in control solution after 213 minutes, in VIP solution after 349 minutes) (Pakbaz 1994). VIP was effective in preventing ischemia-reperfusion injury in an *in vivo* rat lung transplantation model, as demonstrated by improved pulmonary function (Nagahiro 1998).

Inhibition of Apoptosis: One of the main features of coronavirus lung injury is destruction of the alveolar epithelium, with severe damage to the alveolar capillary barrier, as well as a major increase in alveolar capillary permeability. The alveolar epithelium of patients who die from lung injury is notable for evidence of DNA fragmentation. Extensive alveolar epithelial cell apoptosis is found in murine models of lipopolysaccharide-induced lung injury (Matute-Bello 2003). There are two independent cell death pathways involved in the destruction of lung cells in ARDS: the Fas/Fas ligand and the perforin/granzyme system (Hashimoto 2000). Several lines of evidence

point to the Fas/Fas ligand system as an underlying mechanism responsible for the epithelial cell apoptosis in acute lung injury and ARDS (Albertine 2002). The Fas/Fas ligand system is composed of the cell membrane surface receptor Fas (CD95) and its ligand. Alveolar and airway epithelial cells express Fas on their surface, and the expression of Fas in epithelial cells increases in response to inflammatory mediators. Soluble Fas ligand is present in bronchio-alveolar lavage fluids of patients with early ARDS and reaches higher concentrations in the lung fluids of patients who die. This detected Fas ligand is biologically active and causes apoptosis in normal human lung epithelial cells (Matute-Bello 1999). VIP is a potent inhibitor of Fas ligand expression and has been shown to inhibit Fas ligand-mediated cell death (Delgado 1998, 1999).

The second cell death pathway relevant to ARDS and COVID-19 lung injury occurs when the degranulation of serine proteases, granzymes, together with the pore-forming protein, perforin, induce rapid death of the target cells (Hashimoto 2000). VIP is a proven inhibitor of activation-induced perforin, as well as of granzyme B, and therefore actively contributes to the reduction of deleterious proinflammatory and cell death-inducing processes, particularly in the lungs (Sharma 2006). Caspase-3 has been identified as a key mediator of apoptosis in mammalian cells via its role in cleaving a variety of substrate proteins and inducing DNA fragmentation. In animal models of ALI, caspase activity is significantly increased compared to its activity in normal lungs, and VIP is shown to suppress caspase activation (Said and Dickman, 2000). Finally, NMDA-induced experimental lung injury is associated with the downregulation of the anti-apoptotic gene bcl-2, and this downregulation is reversed in lungs treated with VIP (Said and Dickman, 2000).

Inflammation: During the early phase of ALI, the lung is the site of an intense inflammatory process involving the sequential activation of cytokines and chemokines and the secretion of proteases, as well as concomitant collagen synthesis. Evidence of an acute inflammatory reaction in the alveolar-microvascular area also includes the recruitment and activation of inflammatory cells, the increased production of toxic oxygen metabolites and nitric oxide, and a protein-rich exudation in the air spaces. A key regulator involved in a variety of these processes is the nuclear transcription factor NF- κ B. This protein is normally located in cell cytoplasm and is bound to an inhibitory protein I κ B in an inactive state. Inducers of NF- κ B activation trigger a cascade of reactions, ending in liberation of NF- κ B from I κ B. NF- κ B trans-locates into the cell nucleus, where it binds to the promoter sequences of many defense and immune-response genes, thereby inducing their expression—for example, the expression of tumor necrosis factor alpha (TNF- α). VIP has been shown to inhibit NF- κ B activation in numerous animal models of acute lung injury, preventing or attenuating the injury (Delgado 1998).

Effect of VIP on Surfactant Production: A pathologic hallmark of the ALI/ARDS is the damage to the surfactant-producing AT2 cell (Mossel 2008). These cells, comprising 5% of the pulmonary epithelium express Angiotensin Converting Enzyme 2 (ACE2) surface receptors. ACE2 receptors are not expressed by the more prevalent Alveolar Type I cells. The spike protein complex of the SARS-CoV-2 virus, which binds specifically to ACE2 receptors, therefore binds to and infects AT2 cells.

Studies have demonstrated high-density VIP (VPAC₂) binding sites on rat AT2 cells (Groneberg 2001, Onoue, 2004). This binding site is distinct from the VPAC₁ binding site found on smooth

muscle that is associated with the vasodilatory effect of VIP (Groneberg 2001). Li demonstrated in rat lung explants that VIP increased the incorporation of methyl-choline into phosphatidylcholine—the major component of the pulmonary surfactants—by enhancing the activity of the enzyme choline-phosphate cytidyltransferase (Li 2004). VIP upregulates C-Fos protein expression in cultured type II alveolar cells. This upregulation is instrumental in promoting synthesis of pulmonary surfactant phospholipids (Li 2007), and it induces the expression of surfactant protein A in ATII cells through activation of the PKC/c-Fos pathway.

4.4.2 IN VITRO EVIDENCE IN HUMAN CELLS

Before a discussion of the preliminary clinical evidence, it should be noted that VIP has been shown to inhibit SARS-CoV-2 RNA synthesis/replication in human monocytes and viral production in lung epithelial cells (Temerozo 2020). VIP protected these cells from virus-induced cytopathy, reduced the production of pro-inflammatory mediators, and prevented the SARS-CoV-2-induced NF- κ B activation, which is critically involved in the production of inflammatory mediators.

The effect of VIP on human AT2 cells is illustrated in Temerozo's experimental findings with the Calu3 cell, a human-derived model of the AT2 cell (Fig 2). Temerozo documented the protective effects of VIP in inhibiting the replication of SARS-CoV-2 in Calu-3 cells (below left) and in inhibiting the production of lactose dehydrogenase, indicative of cell lysis (below right).

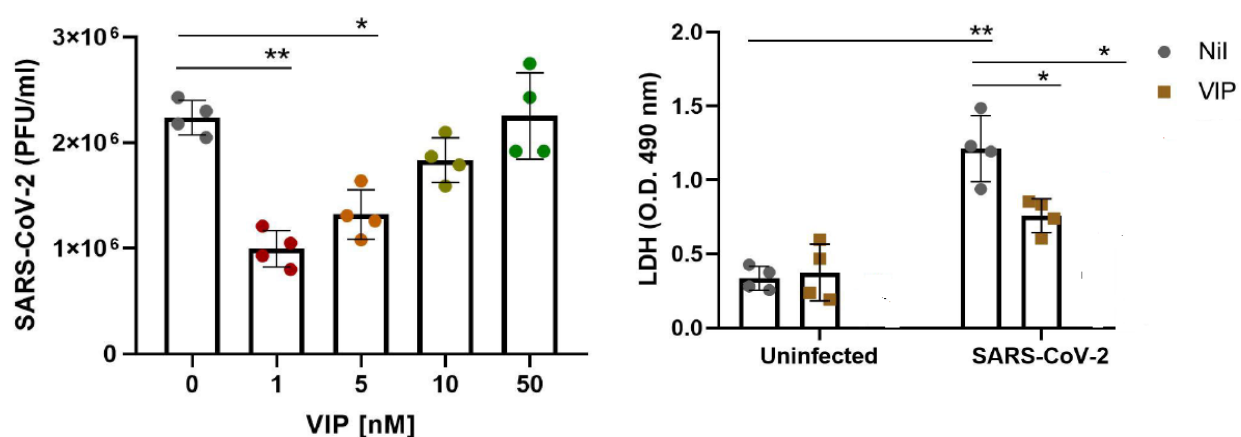


Figure 4: Effect of VIP on inhibition of SARS-CoV-2 replication in Calu-3 cells with statistically significant inhibition seen at 1 and 5 nM concentration (left). Effect of VIP in preventing cytopathy as measured by supernatant LDH levels in SARS-infected Calu-3 cells (right). Source: Temerozo (2020)

Temerozo further explored the effect of VIP in inhibiting the production of cytokines by SARS-CoV-2–infected Calu-3 cells and pulmonary monocytes. As summarized in the attached pre-print, a significant inhibitory effect of IL-6 and IL-8 is seen in Calu-3 cells in association with VIP administration (Figure 3).

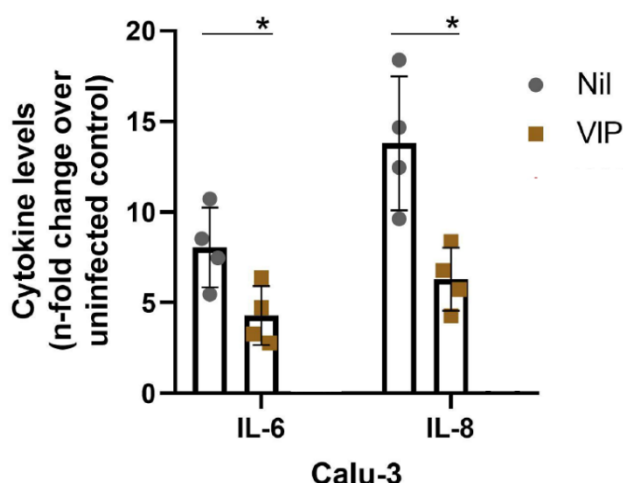


Figure 5: Effect of VIP in reducing IL-6 and IL-8 secretion from SARS-infected Calu-3 cells. Source: Temerozo (2020)

4.5 CLINICAL EVIDENCE THAT AVIPTADIL MAY DEMONSTRATE SUBSTANTIAL IMPROVEMENT ON A CLINICALLY SIGNIFICANT ENDPOINT OVER AVAILABLE THERAPIES, WITH FAVORABLE BENEFIT/RISK.

Clinical evidence of efficacy and safety is derived from a retrospective case/control study (section 4.5.1) and four prospective trials:

1. A phase 2b/3 randomized trial in 196 patients with Respiratory Failure in Critical COVID-19. (section 4.5.1)
2. An expanded access program that has enrolled more than 300 patients with Respiratory Failure in Critical COVID-19. (section 4.5.4)
3. An administratively controlled, open-label trial in 45 highly comorbid patients with Critical COVID-19 and respiratory failure. (section 4.5.5)
4. Safety information from an NIH-sponsored trial of aviptadil vs. remdesivir alone and in combination for patients with Respiratory Failure in Critical COVID-19. (section 4.5.6)

Higher serum levels of VIP are also reported to be associated with increased odds of survival among intubated patients with critical COVID-19 (section 4.5.7)

In addition to evidence of efficacy in prospective studies, both the phase 2b/3 trial and the open label trial demonstrate two objective biomarker measures upon which aviptadil demonstrates a clear advantage over placebo across all patients and all sites: RDR (4.5.1.2 and 4.5.5.2) and Cytokine IL-6 (4.5.1.4 and 4.5.5.4). Both are biologically plausible and highly predictive of long-term survival and recovery, as shown below. The primary and secondary outcomes of the clinical trial, 60-day recovery (alive and free of respiratory failure) and 60-day survival, are influenced both by the biologic effect of the investigational drug and by the plethora of clinical care factors that contribute to survival and recovery of critically ill patients. The immediate biologic response to the drug, therefore, should be considered as an early measure of whether aviptadil “may be effective” in the care of Critically Ill patients with COVID-19 Respiratory Failure.

4.5.1 CLINICAL EVIDENCE OF IMPROVED SURVIVAL AND RECOVERY FROM RESPIRATORY FAILURE IN PATIENTS WITH CRITICAL COVID-19 FROM A PHASE 2B/3 TRIAL

4.5.1.1 Primary and Secondary Endpoint: Recovery and Survival in Patients with Respiratory Failure: Overall Population

ZYESAMI has been studied in a randomized well-controlled study in patients with Critical COVID-19 with respiratory failure (Protocol RLF100-001, IND 149152; see attached preprint and CSR submitted in Pre-EUA 103). The clinical trial enrolled 196 participants with respiratory failure in Critical COVID-19, who were randomized 2:1 aviptadil:placebo (see [www.clinicaltrials.gov NCT 04311697](https://www.clinicaltrials.gov/NCT04311697)).

Across all patients and all sites of care, the trial demonstrated a 1.6 fold increase in the cumulative distribution of achieving the primary endpoint of being alive and free of respiratory failure at day 60 at borderline statistical significance (95% CI: .95 – 2.2; P=.08). A 2.0-fold increased odds of survival (95% CI 1.05-3.87; P<.035) among aviptadil-treated participants at 60 days post treatment based on NIAID score at baseline, simply controlling for baseline severity. (see appendix 1 pp 1-2)

4.5.1.2 Primary and Secondary Endpoint: Recovery and Survival in Patients with Respiratory Failure: Remdesivir Subgroup

At FDA’s direction, primary and secondary endpoint were analyzed for the subgroup of patients who were treated with remdesivir (approximately 70% of each randomization cohort. In this subgroup of patients, who form the basis of sponsor’s requested Breakthrough indication, **In the subgroup of patients treated with remdesivir, those treated with remdesivir + aviptadil were significantly more likely to be alive and free of respiratory failure at Day 28 (OR 2.8; P=.03) (appendix 1 page 12 table 2) and at Day 60 (OR 2.5; P=.03) (Appendix 1 page 7). A trend level of significance in survival favoring aviptadil vs. placebo emerges at day 28 (OR 2.3; P=.09) and demonstrates a high degree of statistical significance at Day 60 (OR 4.0; P=.006).** While the subgroup of remdesivir-treated patients was not prespecified, it was requested by FDA in the response to the initial BTB request. There is precedent at FDA for considering the results of a post-hoc subgroup analysis when the overall population shows a statistically-significant or near-significant effect and when the statistical significance seen in the subgroup is high.

Table 2 Primary and Secondary Endpoint analysis for Patients treated with Remdesivir

Endpoint	Odds Ratio (95% CI) controlling for baseline severity, age with clinical site as a random effect
Alive and Free of Respiratory Failure at Day 28	2.3 (.87 - 6.1) P=.09
Survival to Day 28 (may still be in hospital)	2.8 (1.1 – 7.1) P=.03
Alive and Free of Respiratory Failure at Day 60	3.1(1.13 – 8.34) P=.03 Appendix 1, p11, table 14
Survival to Day 60 (may still be in hospital)	4.1(1.5 11.5) P=.006 Appendix 1, p9, Table 10

4.5.1.3 NIAID 2 / Ventilator-treated Population

A further subgroup analysis, based on the pre-specified baseline NIAID covariate was conducted to assess the efficacy in the baseline NIAID 2 population where there is clearly an unmet need. We provide evidence that Aviptadil is effective in preventing death before 60 days in the NIAID 2 population. The site effect was modeled as a random effect. Among those who were most acutely ill, the subgroup of NIAID 2 score at baseline odds of death on Placebo were 10 fold higher compared to those treated with aviptadil (P= .02) (Appendix 1, p5 Table 2). The same results can be seen if treatment with ventilation at baseline is substituted for NIAID 2 classification at baseline (Appendix 1, p6 table 3).

4.5.1.4 Secondary endpoint: Respiratory Distress Ratio: Overall Population

Respiratory Distress Ratio (RDR) is measured as the ratio of arterial oxygen partial pressure (PaO₂) to fractional inspired oxygen partial pressure (FiO₂). This ratio (PaO₂/FiO₂) is also known as the Horowitz index or PF ratio. As patients recover and leave the ICU, PF ratio can no longer be measured because arterial blood gas is no longer obtained. Over the first three days of therapy, however, PF ratio is obtained on the entire study cohort and provides an early indication of biologic response to aviptadil vs. placebo.

Mean RDR was comparable at baseline (aviptadil=112.1, placebo=105.2), with differentiating improvement noted at Day 2 pre-dose (aviptadil=124.6, placebo=107.8; two-sided t-test=0.12) and at Day 3 pre-dose (aviptadil=140.1, placebo=107.7; two-sided t=0.01; Figure 4). A sustained mean numeric advantage at Day 7 pre-dose was seen (aviptadil=139.2, placebo=116.2; two-sided p=0.11). For patients treated at baseline with High Flow Nasal Cannula (HFNC), differentiating improvement was confirmed at Day 2 pre-dose (aviptadil=124.4, placebo=93.4; two-sided t-test=0.01), at Day 3 pre-dose (aviptadil=141.9, placebo=105.4; two-sided t=0.03), and at Day 7 (aviptadil=146.1, placebo=115.2; two-sided t=0.04). For patients treated in tertiary care medical centers, differentiating improvement was confirmed at Day 2 pre-dose (aviptadil=125.7, placebo=102.6; two-sided t-test=0.05), at Day 3 pre-dose (aviptadil=139.1, placebo=107.2; two-sided t=0.03), and at Day 7 (aviptadil=147.7, placebo=113.4; two-sided t=0.04).

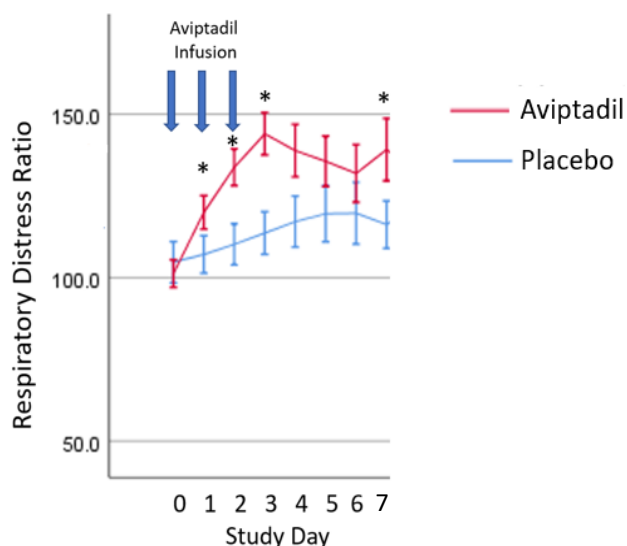


Figure 6 Respiratory Distress Ratio in patients treated with aviptadil and placebo. Significant differences in improvement are noted at days 1, 2, 3, and 7 (t-test $P<.05$) and across the 7-day interval on Mixed Measures Repeated Model ($P<.02$).

Prediction of Outcome: Mixed Model Repeated Measures (MMRM) regression was used to determine whether higher mean RDR was associated with a higher likelihood of achieving primary endpoint at 60 days. The higher mean RDR seen in aviptadil- vs. placebo-treated participants was predictive of achieving the primary endpoint on MMRM ($F 16.0$; $P<.001$).

This biologic effect of aviptadil on RDR is consistent with the improvement in RDR associated with aviptadil reported in the single-center, open-label trial conducted at Houston Methodist Hospital (Youssef 2021 and 4.5.6.2, below).

4.5.1.5 Secondary Endpoint: NIAID Ordinal Scale: Overall Population

A subjective measure of patient improvement is the National Institute for Allergy and Infectious Diseases (NIAID) ordinal scale. On this scale, 1 represents death, 2/3 represents respiratory failure, 4/5 represents continued hospitalization without respiratory failure, 6/7 represents ongoing need for oxygen, and 8 represents full recovery. This measure was applied to clinical trial patients in this study by study site investigators. No difference in daily NIAID scale was seen among the 25% of patients treated in community hospitals. However, substantial differences in daily NIAID score were seen among the 75% of patients who were treated in tertiary care medical centers. As shown in the below (Figure 5), a highly significant difference was seen among those who entered the trial at NIAID=2 ($P=.036$), and clear separation with a trend level of significance is seen among patients who entered the clinical trial at NIAID=3. The figure further illustrates that in this trial, there was minimal survival among patients who entered the trial at NIAID=2 and were randomly allocated to placebo.

4.5.1.6 Biomarker Endpoint (pre-defined): Cytokine IL-6: Overall Population

The effectiveness of aviptadil is further supported by biomarker evidence, in which patients treated with placebo demonstrated a five-fold higher mean plasma concentration of IL-6 than patients treated with aviptadil ($P<.001$; Figure 6). This rise in IL-6 cytokine, colloquially known as a “cytokine release syndrome,” was associated within this trial with increased likelihood of mortality, consistent with multiple reports of association between cytokine levels and mortality in COVID-19 (Figure 7).

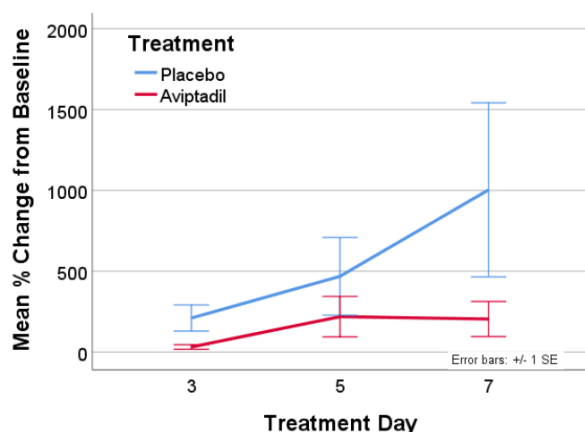


Figure 7: Percent change from pre-treatment (Day 0) baseline in IL-6. A significant difference is seen between day 3 and day 7 favoring aviptadil ($P=.024$) with independent significance on days 3 ($P=.002$) and 7 ($p=.06$).

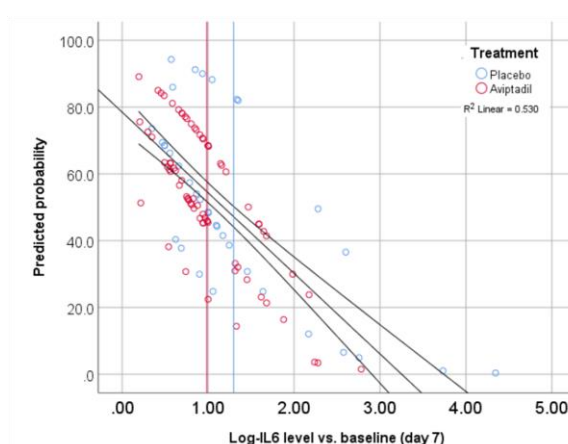


Figure 8: Predicted probability of primary outcome vs. change in Log IL-6 level from baseline to day 7. Increase in IL6 at day 7 predicted >50% of the variance in treatment outcome ($R^2 = .53$). Treatment with aviptadil is less likely to be associated with a day 7 increase in IL-6 and is associated with higher probability of primary endpoint success.

Among the subgroup of subjects for whom biomarker data were collected, treatment arm ($p=.034$) and baseline ventilation status ($p=.019$) were significant independent predictors of day 60 primary

endpoint success, independent of any interaction effects (fig 7). Type of hospital was not significantly associated with this biological effect. A lower level of IL-6 on Day 7 strongly predicted achieving the primary endpoint and survival at Day 60 (-2LL 136.3 vs. 261.3, $\chi^2=125$, $df=1$, $p<.0001$) and was collinear with treatment-type ($p=.95$). Baseline ventilation status ($p=.07$) demonstrated a trend level of significance as a covariate in this model.

All tables and listings supporting these and other endpoints may be found in the Clinical Study Report (see Pre-EUA 103) submitted May 31, 2021.

4.5.2 NO SIGNIFICANT SAFETY ISSUES HAVE BEEN IDENTIFIED IN THE PHASE 2B/3 TRIAL

Detailed information on safety is contained in the clinical study report (see Pre-EUA 103). Summary information (Table 4) shows that the most commonly reported adverse reactions seen in association with aviptadil treatment were hypotension and diarrhea. Mild to moderate diarrhea was seen in 30% of patients and is commonly seen among those treated in the Expanded Access Protocol (section 4.5.4). No statistically significant difference in hypotension was seen between those treated with aviptadil and those treated with placebo.

Table 3: Incidence of Adverse

	AVIPTADIL		PLACEBO	
	(N=131)	(N=820)	(N=65)	(N=360)
	# Patients	# Events	# Patients	# Events
ANY TEAE	102 (77.9%)	820	49 (75.4%)	360
BLOOD AND LYMPHATIC SYSTEM DISORDERS	18 (13.7%)	21	10 (15.4%)	13
CARDIAC DISORDERS	34 (26.0%)	75	15 (23.1%)	25
EYE DISORDERS	1 (0.8%)	2	1 (1.5%)	1
GASTROINTESTINAL DISORDERS	59 (45.0%)	88	10 (15.4%)	14
Diarrhea	43 (32.8%)	48	1 (1.5%)	1
GENERAL DISORDERS AND ADMIN SITE CONDITIONS	27 (20.6%)	37	13 (20.0%)	15
Multiple organ dysfunction syndrome	9 (6.9%)	9	9 (13.8%)	9
HEPATOBIILIARY DISORDERS	4 (3.1%)	4	2 (3.1%)	2
IMMUNE SYSTEM DISORDERS	1 (0.8%)	1	0	0
INFECTIONS AND INFESTATIONS	47 (35.9%)	61	19 (29.2%)	30
COVID-19	22 (16.8%)	22	10 (15.4%)	10
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	12 (9.2%)	15	5 (7.7%)	5
Infusion related reaction	7 (5.3%)	8	1 (1.5%)	1
INVESTIGATIONS	23 (17.6%)	140	8 (12.3%)	65
METABOLISM AND NUTRITION DISORDERS	28 (21.4%)	60	11 (16.9%)	28
Hyperkalemia	16 (12.2%)	16	5 (7.7%)	5
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	6 (4.6%)	10	3 (4.6%)	4
NERVOUS SYSTEM DISORDERS	13 (9.9%)	17	8 (12.3%)	10
PRODUCT ISSUES	2 (1.5%)	3	0	0
Device leakage	1 (0.8%)	1	0	0
Device malfunction	2 (1.5%)	2	0	0
PSYCHIATRIC DISORDERS	18 (13.7%)	25	7 (10.8%)	15
Anxiety	6 (4.6%)	6	4 (6.2%)	4
RENAL AND URINARY DISORDERS	36 (27.5%)	43	17 (26.2%)	23
Acute kidney injury	29 (22.1%)	30	14 (21.5%)	15
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	38 (29.0%)	102	25 (38.5%)	62
Acute respiratory distress syndrome	7 (5.3%)	7	2 (3.1%)	2
Respiratory failure	19 (14.5%)	21	11 (16.9%)	13
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	8 (6.1%)	12	3 (4.6%)	10
SURGICAL AND MEDICAL PROCEDURES	2 (1.5%)	2	2 (3.1%)	2
VASCULAR DISORDERS	50 (38.2%)	102	26 (40.0%)	36
Deep vein thrombosis	18 (13.7%)	21	9 (13.8%)	10
Flushing	13 (9.9%)	19	2 (3.1%)	2
Hypotension	34 (26.0%)	44	14 (21.5%)	19
Hypotensive crisis	1 (0.8%)	1	2 (3.1%)	2

4.5.3 CLINICAL EVIDENCE OF SAFETY FROM MIDPOINT ANALYSIS IN NIH-SPONSORED PHASE 3 TRIAL

The National Institute of Allergy and Infectious Diseases has sponsored the ACTIV3b Critical Care Trial (TESICO) that randomly allocates patients with Respiratory Failure in Critical COVID-19 to (1) aviptadil alone, (2) aviptadil + remdesivir, (3) remdesivir alone, or (4) placebo

(www.clinicaltrials.gov NCT04843761). The trial is currently being conducted at more than 20 sites in the US, with sites in Europe and Brazil anticipated to follow. Enrollment has surpassed 360 of the 640 patients targeted.

The independent Data Safety Monitoring Board (DSMB) of the TESICO trial met on August 9, 2021, to review safety data from the first 140 enrolled patients. No new or unexpected safety issues were identified by the DSMB. The DSMB advised the study investigators that the frequency of the blood pressure assessment could safely be reduced because hypotension-related adverse events were not been observed. The DSMB met most recently on December 13, 2021 and reviewed 348 randomized patients. No new safety concerns were identified and the study was allowed to continue. Because of continued demonstration of safety, the DSMB reduced the frequency of blood pressure monitoring and allowed treatment with aviptadil outside the ICU setting in various step down units.

4.5.4 CLINICAL EVIDENCE OF IMPROVED SURVIVAL AND RECOVERY FROM RESPIRATORY FAILURE IN AN EXPANDED ACCESS PROGRAM

In addition to the above clinical trial, aviptadil has been made available under both Emergency Use IND and the sponsor's Expanded Access Protocol to patients who do not qualify for the ongoing study due to serious exclusionary comorbidities (Table 5).

A total of 256 patients were enrolled and received at least one dose of aviptadil by March 19, 2021. 117 patients (46%) received the full regimen of three doses on consecutive days, and 8 received up to six doses in accordance with the protocol. Day 28 data were successfully collected on 240 patients, and 16 were lost to follow up. Of the 240 patients with day 28 data, 106 (44%) had a baseline WHO Ordinal score of 6 (HFNC or NIV), and 134 (56%) had a baseline score of 7 (IMV or ECMO). At 28 days post treatment with aviptadil, 44 (18%) patients were discharged to home and ambulatory, 24 (10%) patients were still hospitalized but not intubated, 26 (11%) patients were hospitalized and intubated (NIAID 7), 33 (14%) patients were known to be alive but with unknown ordinal scale status, and 113 (47%) patients were deceased.

196 received maximal SoC, and 54 patients received palliative care (withdrawal of life support). Overall survival for the 196 patients receiving maximal intensive care was 65%. In this subgroup, at 28 days after the initiation of treatment with aviptadil, 73 out of the 96 patients (76%) treated with high flow nasal cannula (HFNC) had been discharged from the hospital or were alive, compared to 54 out of 100 patients (54%) treated with mechanical ventilation (Fischer exact $P < .001$).

The best outcomes occurred for patients on baseline HFNC who received maximal intensive care, 76% of whom were discharged from the hospital or were alive, compared with 54% treated with mechanical ventilation (IMV) at baseline (Fisher's Exact $P < .001$). Overall survival for the 196 patients receiving maximal SoC was 65%.

All patients ($n=240$) were evaluated according to the 8-point WHO Ordinal Scale. Those enrolled at level 6 (treatment with non-invasive ventilation or high flow nasal cannula) were substantially more likely to survive to day 28 (68% vs 40%; Fisher Exact $P < .001$) than those enrolled at level 7 (treatment with mechanical ventilation or extracorporeal membrane oxygenation [ECMO]). In a subset of 192 patients in whom data could be obtained on time from

initial hospitalization to treatment with aviptadil, substantially higher survival was seen in those treated within the first five days of hospital admission than among those treated more than five days after admission, although the sample size is insufficient to reach statistical significance (59% survival vs. 48% survival).

These findings suggest that 28-day survival comparable to that seen in the randomized controlled trial can be obtained in regional hospitals when a commitment is made to maximal intensive care. Moreover, the achievement of these results in regional hospitals suggests that the disparity in outcome seen in tertiary vs. regional hospitals in the phase 2b/3 trial may have been dependent on specific characteristics and circumstances of two of the study sites included in the RCT. These findings also suggest that earlier treatment with aviptadil is associated with increased chance of survival from COVID-19 Respiratory Failure.

Table 4: Day 28 Data Summary: Aviptadil Expanded Access Protocol

	ZYESAMI (Aviptadil Acetate)	
	Discharged prior to or alive at day 28 (including withdrawal of life support) N = 240 n (%)	Discharged prior to or alive at day 28 (no withdrawal of life support) N = 196 n (%)
All Patients Evaluated	127 (53%)	127 (65%)
Mechanical Ventilation	54/134 (40%)	54/100 (54%)
Non-Invasive Ventilation	73/106 (69%)	73/96 (76%)

4.5.5 IMPROVED SURVIVAL AND RECOVERY FROM RESPIRATORY FAILURE IN AN ADMINISTRATIVELY CONTROLLED TRIAL OF HIGHLY COMORBID PATIENTS WITH COVID-19 RESPIRATORY FAILURE.

Youssef (2021) has reported on 21 consecutively admitted patients with Respiratory Failure in Critical COVID-19 and multiple co-morbidities, enrolled under Emergency Use INDs and an Expanded Access Protocol as detailed below. These patients were compared with 24 patients with comparable comorbidity from the same ICU, who were treated by the same clinical team during the same timeframe and who received maximal standard of care (SOC). All patients were treated with three successive 12-hour intravenous infusions of increasing doses of aviptadil (50/100/150 pmol/kg/hr). His initial 60 day trial has now been updated to one year for the purpose of assessing survival. All enrolled patients were followed to one year and survival status was assessed either at a clinic visit or by telephone call initiated by the principal investigator.

4.5.5.1 Survival

By Kaplan-Meier lifetable analysis (Figure 8), aviptadil-treated patients were 3-fold more likely to survive over one year than were those treated with Standard of Care (Hazard Ratio 0.26; 95% CL 0.12, 0.60). The difference is both dramatic and statistically significant (logrank test: $P < .0001$).

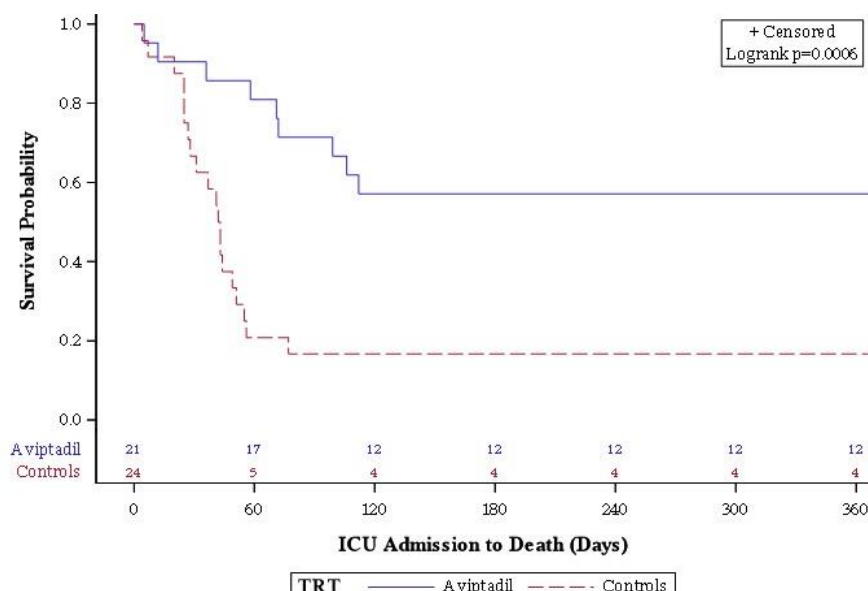


Figure 9: Survival in patients treated with aviptadil (n=21) vs SOC (n=24) from Time of ICU Admission (Hazard Ratio 0.26; 95% CL 0.12, 0.60)

4.5.5.2 Time to Recovery

Time to recovery from respiratory failure was similarly analyzed by lifetable analysis (Figure 9). Respiratory failure was defined by the FDA resource-based criterion (FDA 2021) of requirement for mechanical ventilation, noninvasive ventilation, or high flow nasal oxygen at 20L or greater. A similar 9-fold increase in likelihood of recovery from respiratory failure from time of ICU admission was seen (Fisher's Exact Test: $P < .001$). The hazard ratio is 0.115 (95% CL: 0.0254, 0.5219).

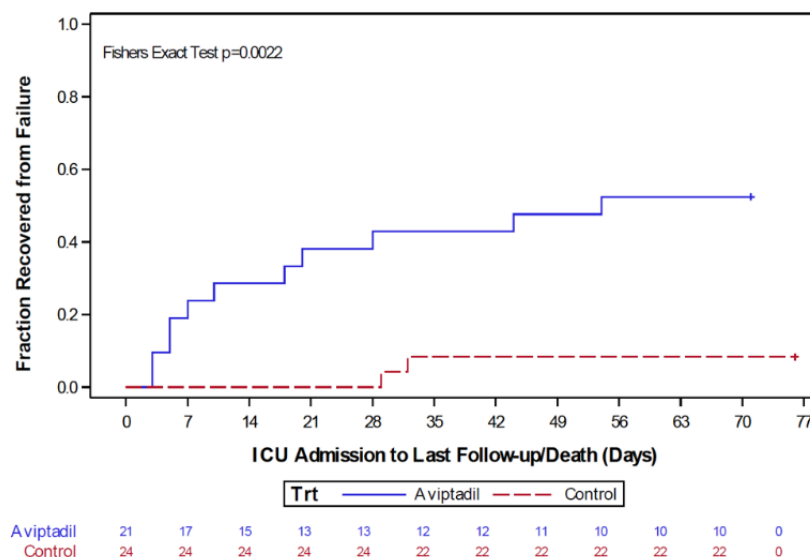


Figure 10: Time to recovery from Respiratory Failure. (Hazard Ratio 0.115 (95% CL: 0.0254, 0.5219))

Ten aviptadil-treated patients versus two SOC control patients have returned home with minimal oxygen requirement (48% vs. 8.3%; $P=0.007$).

All five of the aviptadil-treated patients who remain in respiratory failure are on minimal ventilator and oxygen support while undergoing rehabilitation for muscle weakness with tracheostomy in a long-term care facility. In contrast, the two SOC control patients who remain alive on ECMO show little sign of improvement with continued high ventilator support.

Four of the five aviptadil-treated patients on ECMO were successfully decannulated, compared to 3 of 13 control patients who developed the need for ECMO (80% vs. 23%; $P<.05$). The decannulation rate on ECMO seen among control patients in this study is consistent with survival rates for COVID-19 patients treated with ECMO across the country.

4.5.5.3 Improvement on WHO Ordinal Scale

A substantial and meaningful 6.1-point difference in the 10-point WHO Ordinal Scale for COVID-19 was seen between aviptadil-treated patients (Figure 10), who exhibited a 2.6-point median improvement from time of ICU admission, vs. those treated with standard of care, who exhibited a mean 3.5-point median decrement (Wilcoxon signed rank: $P<.001$).

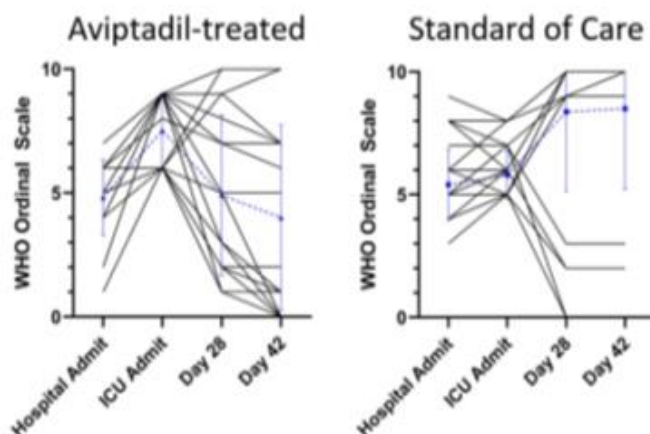


Figure 11: Change in 10-Point Ordinal Scale from Enrollment Through Day 24

As shown, aviptadil-treated patients demonstrated a median 2.6-point improvement compared with control patients, who demonstrated a 3.5-point median decrement at 42 days (Wilcoxon signed rank, $p < 0.001$). Note that the WHO scale is inverse in sign to the NIAID scale.

4.5.5.4 Improvement on Respiratory Distress Ratio ($\text{PaO}_2\text{:FiO}_2$)

Aviptadil-treated patients demonstrated a significant, nearly 3-fold improvement in oxygenation as measured by the Respiratory Distress Ratio (RDR), also known as the $\text{PaO}_2\text{:FiO}_2$ ratio. Control standard-of-care patients demonstrated no significant mean improvement (164 (SD 134) vs. 3 (SD 86): $P < .001$) (Fig 11). 15 of 21 aviptadil-treated patients demonstrated a 100-point or greater improvement in RDR, compared to 4 of 30 controls ($P < 0.001$). No aviptadil-treated patient demonstrated significant worsening in blood oxygenation, whereas 5 control patients demonstrated a decrement of 100 points or greater ($P < .05$). The improvement in patients on ECMO was similar to that seen in patients treated with conventional mechanical ventilation. Available data from blood gases showed clear increases in the $\text{PaO}_2\text{:FiO}_2$ ratio after the second dose (median increase = 92.5, IQR = 74) and at 24 hours after the third dose (median increase over baseline = 84.5, IQR = 110). A statistically significant difference in mean improvement is seen in aviptadil-treated patients vs. controls (164 vs. 3: $P < .001$).

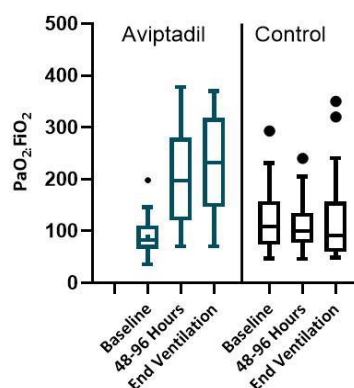


Figure 12: Respiratory Distress Ratio ($\text{PaO}_2\text{:FiO}_2$) in aviptadil-treated vs. control patients demonstrating statistically significant improvement in RDR among aviptadil-treated patients at 48-96 hours ($P < .001$).

Subsequent follow-up reveals that 7 additional aviptadil-treated patients returned either to home or long-term acute care for a total of 17 (81%), and 1 additional patient has died. In contrast, 2 additional control patients have died, and the remaining 2 were continuing on mechanical ventilation at 60 days. Thus, from a post-hoc perspective, aviptadil-treated patients with Critical COVID-19 were eight times more likely to return to home or long-term care than were patients given standard of care.

4.5.5.5 Changes on Radiographic Appearance

Radiographic evidence (Figure 12) on all patients is currently undergoing formal evaluation by a panel of blinded radiologists. Full or partial resolution of the “ground glass” parenchymal changes associated with COVID-19 pneumonitis occurred in 17 of 21 aviptadil-treated patients.

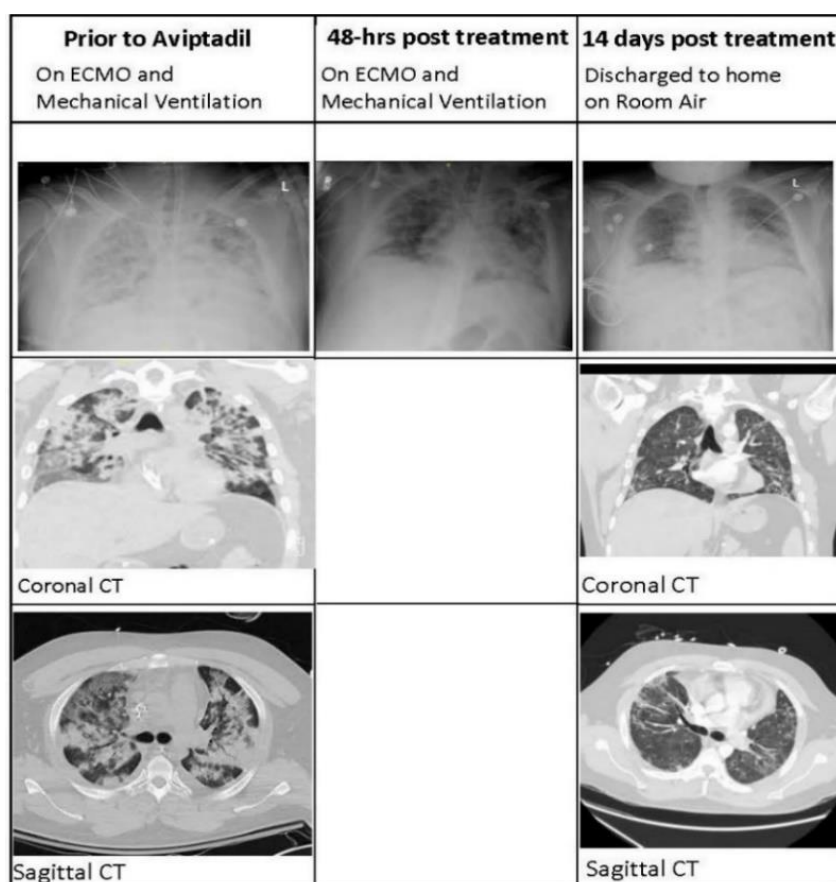


Figure 13: Chest x-ray and CT imaging of a patient initially treated while on mechanical ventilation and extracorporeal membrane oxygenation for Critical COVID-19 with respiratory failure

4.5.5.6 Changes in inflammatory markers

A laboratory panel of inflammatory markers, including LDH, troponin, C-reactive protein, ferritin, D-Dimer, and IL-6 was obtained prior to and post treatment with aviptadil (Figure 13). In all patients, improvement can be seen on each of the inflammatory markers. The largest average percent decrease was seen in C-reactive protein (76% \pm 3%) and IL 6 (75% \pm 3%). No patient demonstrated an increase in any of the inflammatory markers. Because of the high mortality rate in the control group, an accurate comparison in cytokine reduction between aviptadil-treated and standard-of-care patients is not feasible.

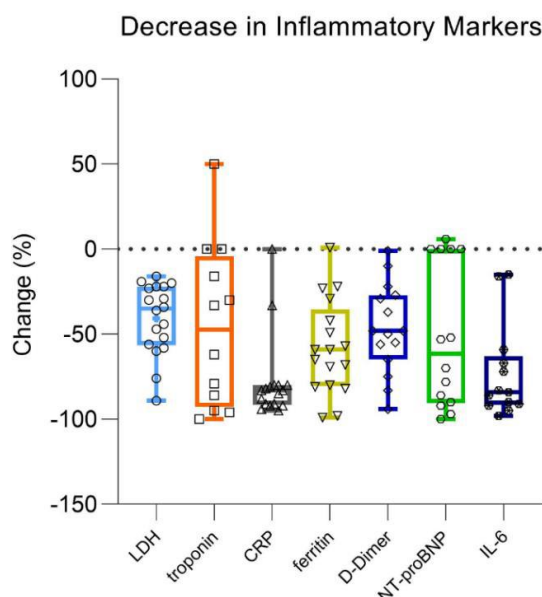


Figure 14: Decrease in inflammatory markers as a percent change from pretreatment value. The decrease is both clinically and statistically significant ($P < .001$)

4.5.5.7 Safety in the open-label trial

No drug-related Serious Adverse Events (SAEs), including mortality, were recorded. Only one patient developed a drug-related (non-serious) adverse event. Hypotension was seen in two patients and was successfully managed with pressors and did not require cessation of infusion.

Diarrhea was seen in four aviptadil-treated patients, consistent with the known metabolic effects of VIP, compared to three control patients (19% vs 10%; $p = .2$).

4.5.6 EVIDENCE OF IMPROVED SURVIVAL AT HIGHER LEVELS OF CIRCULATING VIP FROM A CASE-CONTROL STUDY

Temerozo documents a case-control study (Figure 14) in which VIP levels were measured in Critical COVID-19 survivors and non-survivors who received best-available intensive care. As documented in the figure below, those who survived Respiratory Failure in Critical COVID-19 were found to have approximately twice the circulating level of VIP as those who died in the ICU ($P < .05$).

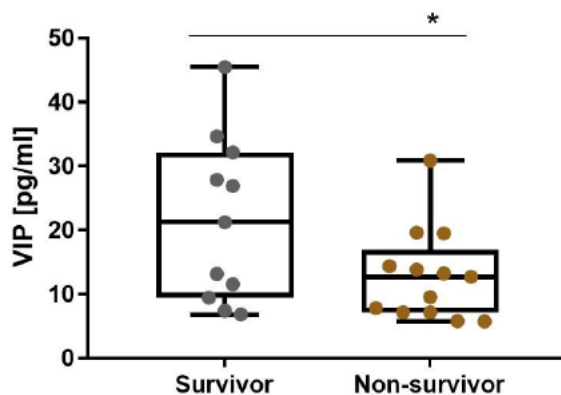


Figure 13: Case-control study (i.e. non-intervention study) of endogenous circulating VIP levels COVID-19 in survivors and non-survivors in a single ICU. Note the statistically significant higher level of circulating VIP among survivors ($P < .05$). Source: Temerozo (2020)

5. SAFETY OF AVIPTADIL AT CURRENT DOSING VS REMDESIVIR

In each of the studies (sections 4.5.2 and 4.5.5.7) described above, aviptadil was delivered intravenously in 3 daily infusions at escalating doses of 50/100/150 picomol/kg/hr for 12 hours. Table 4 (above) documents the absence of treatment emergent adverse events that are significantly more frequent in the aviptadil group vs. placebo or standard of care, other than mild-moderate diarrhea. As documented above (section 4.5.6), the NIAID-sponsored TESICO DSMB identified no new safety signals following enrollment of 348 patients as of December 13, 2021. Similarly, no new safety signals were identified in the expanded access program.

One IND safety report was filed from the expanded access program based on a patient who developed an anion gap acidosis (successfully treated with no long-term effects) that was deemed possibly associated with diarrhea caused by aviptadil.

Extensive nonclinical and human safety data are in IND 149,152 and have been reviewed by FDA. In pre-EUA meetings, FDA has noted that toxicology data on file are sufficient for drug approval (REF ID 4650082). In brief, no specific toxicities have been identified across 4 nonclinical species and no lethal dose of VIP is identified in large mammals.

Whereas aviptadil is generally well tolerated in clinical studies and there is no apparent lethal dose in large mammals, Remdesivir has a number of known toxicities and contraindications (see appendix 3). Remdesivir is known to cause a variety of allergic reactions and to have a propensity to cause transaminase elevations. It is contraindicated in patients with hepatic impairment and in those with renal impairment.

Table 5: Available safety data for intravenous aviptadil in treatment of COVID-19

Data Source	Total Patients in Safety Database
Patients treated in phase 2b/3 trial	131 on aviptadil (CSR attached)
Patients treated in Houston Methodist trial	21 on aviptadil (manuscript attached)
Patients treated in NIH ACTIV-3b trial	178+ on aviptadil (available from NIH)
Patients treated in Expanded Access	240 on aviptadil (CSR on File)
Total available safety database	570-patients

6. SUMMARY

Data reported to date show that aviptadil may be efficacious in patients with Critical COVID-19 with respiratory failure and is generally well tolerated in this same patient population. Aviptadil meets the criteria for FDA Breakthrough Therapy Designation in that it (1) treats a serious—in this case, lethal—medical condition and (2) offers clinical evidence that it may be effective in preventing mortality and improving survival in Critical COVID-19, especially among those previously treated with remdesivir.

The clinical evidence is supported by extensive preclinical evidence documenting a specific mechanism of action in protecting the Alveolar Type II (AT2) cell of the lung (Figure 1). SARS-CoV-2 binds to the AT2 cell, replicates within that cell, decreases surfactant production, stimulates the secretion of inflammatory cytokines, and causes cytopathy. Aviptadil similarly binds to the AT2 cell, decreases viral replication, increases surfactant production, blocks cytokine secretion, and reduces cytopathy.

Two prospective clinical trials, an open-label administratively-controlled trial, and a phase 2b/3 placebo-controlled trial, have demonstrated meaningful and statistically significant improvement in survival and recovery from Critical COVID-19 with Respiratory Failure. In the phase 2b/3 trial, the primary endpoint of “alive and free of respiratory failure at 28 and at 60 days was statistically significant for the subgroup of patients treated with remdesivir with a 3-fold increased odds of achieving the primary endpoint favoring aviptadil ($P=.03$). At 28 days, there was a 2.8-fold increased odds of survival at a trend level of significance ($P=.09$) which increased to a four-fold increased odds of survival favoring aviptadil at 60 days ($P=.006$). When considering all patients at all sites of care, the primary endpoint is demonstrated at near statistical significance ($P=.08$), with a 2-fold increased odds of survival at 60 days favoring aviptadil ($P=.03$). Odds of both survival and recovery were strongly in favor of aviptadil in the open-label study, which was conducted at a leading tertiary care hospital.

The expanded access protocol demonstrates that the benefits seen in sites selected for the phase 2b/3 trial can be seen when treatment is expanded to a broader array of less academic hospitals.

Daily change in ordinal scale (NIAID in the P2b/3 and WHO in the open-label study) was assessed as a measure of patient improvement. Clear and statistically significant benefits were seen in the open-label study and were seen among those treated in tertiary care hospitals in the P2b/3 trial and across all patients in the open-label trial.

In both the phase 2b/3 trial and the open-label trial, cytokine markers of inflammation were significantly reduced in aviptadil-treated patients. In the P2b/3 trial, there was a 5-fold increase in mean cytokine IL-6 level among placebo-treated patients compared to aviptadil-treated patients ($P<.02$). This difference was significantly associated with subsequent mortality. This finding suggests that the biological effect of aviptadil is seen regardless of the hospital setting, although the ultimate clinical outcome is dependent of many factors related to quality of intensive care and capacity of a site to provide critical care, family / patient decisions to pursue maximal intensive care, etc.

ICU capacity and % patient load is of particular relevance for survival. Utilization at levels above 50%-75% are associated with a HR of all-cause mortality of 1.19, and levels above 75% are

associated with a HR level of 1.96. During pandemic surges, particularly regional hospitals reached and are now again reaching levels well above 200%. (Bravata et. al, 2021) The aviptadil Expanded Access Program enrolled almost exclusively regional hospitals (30+), who were able to safely administer the drug..

In both the phase 2b/3 and the open-label trial, Respiratory Distress Ratio ($PaO_2:FiO_2$) was seen to improve in a clinically meaningful and statistically significant manner among those treated with aviptadil compared to those treated with placebo or standard of care. As is the case with cytokine levels, this finding suggests that the biological effect of aviptadil is immediate, significant, and not dependent upon the hospital setting.

Radiographic improvement in patients treated with aviptadil has been observed in a manner generally not seen in those treated with placebo or standard of care. Quantitative assessment of radiographic change remains in process.

In summary, NRx believes that aviptadil meets the statutory requirements for Breakthrough Therapy Designation in patients with COVID-19 whose respiratory failure continues to progress despite treatment with remdesivir. Aviptadil addresses an unmet medical need in a serious medical condition (i.e. Respiratory Failure in patients with COVID-19) given that the target indication is for patients who are at high risk of death despite all approved treatment. Therefore, sponsor believes that aviptadil has demonstrated preliminary evidence of efficacy in the subpopulation FDA identified in its response to the original Breakthrough Therapy request. Moreover, aviptadil demonstrates a biomarker effect as identified in the 21st Century Cures Act.

7. REFERENCES (available upon request)

- Albertine, KH, Soulier MF, Wang Z, et. al. Fas and Fas Ligand Are Up-Regulated in Pulmonary Edema Fluid and Lung Tissue of Patients With Acute Lung Injury and the Acute Respiratory Distress Syndrome. *Am J Pathol* 2002;161(5):1783-1796.
- Alessandrini F, Thakkar M, Foda HD, Said SI, Lodi R, Pakbaz H, and Schraufnagel DE. Vasoactive intestinal peptide enhances lung preservation. *Transplantation* 1993;56:964-973.
- Beigel JH, Tomashek KM, Dodd LE, et. al. ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med*. 2020 Nov 5;383(19):1813-1826. doi: 10.1056/NEJMoa2007764..
- Berisha H, Foda H, Sakakibara H, Trotz M, Pakbaz H, and Said SI. Vasoactive intestinal peptide prevents lung injury due to xanthine/xanthine oxidase. *Am. J. Physiol* 1990;259:L151-L155.
- Bravata, D, Perkins A, et.al. Associate of Intensive Care Unit Patient Load and Demand with Mortality Rates in US Department of Veteran Affairs Hospitals During the COVID-19 Pandemic. *JAMA Netw. Open*. 2021;4(1):e2034266
- Channapanaver R, Perlman S. Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol* 2017;39(5):529-539.
- Delgado, M, Martinez C, Pozo D, Calvo JR, Leceta J, Ganea D, Gomariz RP. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activation polypeptide (PACAP) protect mice from lethal endotoxemia through the inhibition of TNF-alpha and IL-6. *J Immunol*. 1999;162:1200-1205.
- Delgado M, Munoz-Elias EJ, Kan Y, Gozes I, Fridkin M, Brennenman DE, Gomariz RP, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit tumor necrosis factor alpha transcriptional activation by regulating nuclear factor-kB and cAMP response element-binding protein/c-Jun. *J. Biol. Chem*. 1998;273:31427-31436.
- FDA Guidance for Industry: COVID-19 Public Health Emergency: General considerations for Pre-IND meeting requests for COVID-19 related drugs and biological products. May 2020
- FDA Guidance for Industry: COVID-19: Developing Drugs and Biological Products for Treatment or Prevention Guidance for Industry. February 2021.
<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/covid-19-developing-drugs-and-biological-products-treatment-or-prevention>.
- Groneberg DA, Hartmann P, Dinh QT, Fischer A. Expression and Distribution of Vasoactive Intestinal Polypeptide Receptor VPAC₂ mRNA in Human Airways. *Lab Invest* 2001;81:749-755.
- Foda HD, Iwanaga T, Liu LW, and Said SI. Vasoactive intestinal peptide protects against HCl-induced pulmonary edema in rats. *Ann NY Acad Sci* 1988;633-636.

- Hashimoto S, Kobayashi A, Kooguchi K, et. al. Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2000; 161(1):237-243.
- Leuchte HH, Baezner C, Baumgartner RA, et. al. Inhalation of vasoactive intestinal peptide in pulmonary hypertension. *Eur Respir J* 2008;32:1289-1294.
- Li L, Luo ZQ, Zhou, et. al. Effect of vasoactive intestinal peptide on pulmonary surfactants phospholipid synthesis in lung explants. *Acta Pharmacol. Sin.* 2004;25:1652-1658.
- Li L, She H, Yue S, et. al. Role of C-Fos gene in vasoactive intestinal peptide promoted synthesis of pulmonary surfactant phospholipids. *Regul Pept* 2007;140(3):117-124.
- Li L, Hua S, Yue S, Luo Z, et. al., Vasoactive intestinal polypeptide induces surfactant protein A expression in ATII cells through activation of PKC/c-Fos pathway. *Peptides* 2010;31(11):2016-2051
- Mason R. Pathogenesis of COVID-19 from a cell biologic perspective. *Eur Respir J.* April 9 Epub ahead of print. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC71441160/>
- Mathioudakis AG, Chatzimavridou-Grigoriadou V, Evangelopoulou E, Mathioudakis GA. Vasoactive Intestinal Peptide Inhaled Agonists: Potential Role in Respiratory Therapeutics. *Hippokratia* 2013;17(1):12-16
- Matute-Bello G, Liles WC, Steinberg KP, Kiener PA, Mongovin S, Chi EY, Jonas M, Martin TR. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999;163(4):2217-2225.
- Matute-Bello G, Martin T. Science Review: Apoptosis in acute lung injury. *Crit Care* 2003;7(5):355-358
- Misra BR and Misra HP. Vasoactive intestinal peptide, a singlet oxygen quencher. *J. Biol. Chem* 1990;265:15371-15374.
- Mossel EC, Wang J, Jeffers S, et al. SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. *Virology* 2008;372:127-135.
- Mulligan MS, Varani J, Warren JS, Till GO, Smith CW, Anderson DC, Todd RF, and Ward PA. Roles of beta 2 integrins of rat neutrophils in complement- and oxygen radical-mediated acute inflammatory injury. *J. Immunol.* 1992;148:1847-1857.
- Nagahiro I, Yano M, Boasquevisque CH, Fujino, S, Cooper, JD, and Patterson, GA. Vasoactive intestinal peptide ameliorates reperfusion injury in rat lung transplantation. *J. Heart Lung Transplant.* 1998;17:617-621.
- 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: 1757-1767.
- Pakbaz H, Berisha H, Sharaf H, Foda HD, Said SI. VIP enhances and nitric oxide synthase inhibitor reduces survival of rat lungs perfused ex vivo. *Ann. N. Y. Acad. Sci.* 1994;723:426-428.
- 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-L373.
- Prasse A, Zissel G, Lützen N, Schupp J, Schmiedlin R, Gonzalez-Rey E, Rensing-Ehl A, Bacher G, Cavalli V, Bevec D, Delgado M, Müller-Quernheim J.. Inhaled vasoactive intestinal

- peptide exerts immunoregulatory effects in sarcoidosis. *Am J Respir Crit Care Med*. 2010;182:540-8.
- Petkov V, Mosgoeller W, Ziesche R, Raderer M, Stiebellehner L, Vonbank K, Funk GC, Hamilton G, Novotny C, Burian B, Block LH. Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J. Clin. Invest* 2003;111:1339-1346.
- Said SI, Mutt V. Potent peripheral and splanchnic vasodilator peptide from normal gut. *Nature*. 1970; 225: 863-864.
- Said SI. Vasoactive intestinal peptide in the lung. *Ann NY Acad Sci* 1988;527:450-464.
- Said SI. VIP as a modulatory of lung inflammation and airway constriction *Am Rev Respir Dis* 1991;143:S22-S24.
- Said SI, Dickman KG. Pathways of inflammation and cell death in the lung: modulation by vasoactive intestinal peptide. *Regul. Pept.* 2000;93:21-29.
- Sharma V, Delgado M, Ganea D. Granzyme B, a new player in activation-induced cell death is downregulated by vasoactive intestinal peptide in Th2 but not Th1 effectors. *J Immunol* 2006;176(1):97-110.
- Temerozo JR, Sacramento Q, Fintelman-Rodrigues N, et. al. The neuropeptides VIP and PACAP inhibit SARS-CoV-2 replication in monocytes and lung epithelial cells, decrease the production of proinflammatory cytokines, and VIP levels are associated with survival in severe Covid-19 patients doi: <https://doi.org/10.1101/2020.07.25.220806>
- Tepper JS. Symposium Summary: Breathe In, Breath Out, It's Easy. What you need to know about developing inhaled drugs. *Int J Toxicol*, 35(4):376-392
- Virgolini I, Kurtaran A, Raderer M, et. al. Vasoactive Intestinal Peptide Receptor Scintigraphy. *J Nucl Med* 1995;36(10):1732-1739
- Wong CK, Lam CWK, Wu AK, et al. Plasma inflammatory cytokines in severe acute respiratory syndrome. *Clin Exp Immunol* 2004;136(1):95-103.
- Youssef JG, Al-Saadi M, Zhiruddin F, et. Al. VIP in the treatment of Critical COVID-19 with Respiratory Failure in patients with severe comorbidity: A prospective externally-controlled trial. *J Infectious Dis and Treatment*. 2021 in press