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REQUEST FOR BREAKTHROUGH THERAPY DESIGNATION

Aviptadil [vasoactive intestinal polypeptide]
for the treatment of patients with Critical COVID-19 and respiratory failure

IND 149152

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Date: September 27, 2021

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1. PRODUCT NAMES:

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

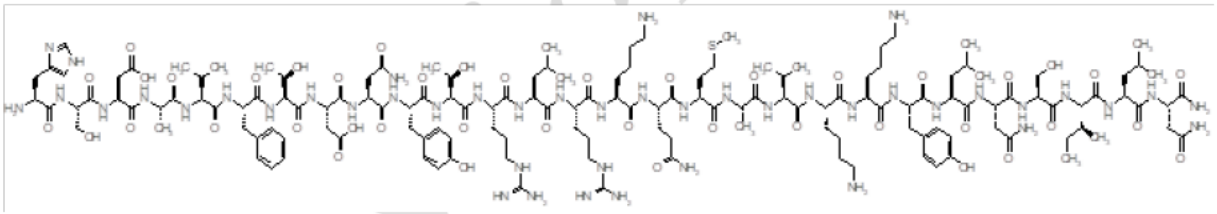
Application Numbers:

- 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
Molecular formula:	$C_{147}H_{238}N_{44}O_{42}S$ (net)
Molecular mass:	3325.8 g/mol (net)

3. PROPOSED INDICATION

Treatment of patients with Respiratory Failure in Critical COVID-19

4. BASIS FOR BREAKTHROUGH THERAPY DESIGNATION

NRx Pharmaceuticals, Inc. hereby requests that FDA grant Breakthrough Therapy Designation to aviptadil for the treatment of patients with Respiratory Failure in Critical COVID-19 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 an 80% 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: In this case, the clinically significant endpoints are survival at 60 days and being "alive and free of respiratory failure" at 60 days post treatment as measured in a 196-person phase 2b/3 randomized trial. These endpoints 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.

The clinical evidence demonstrated in the phase 2b/3 trial 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. 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: There are no approved therapeutic treatments for respiratory failure in Critical COVID-19, clearly an unmet medical need. The results of the Zyesami phase 2b/3 trial, combined with the open-label clinical study at Houston Methodist Hospital discussed in this document, provide preliminary clinical evidence that Zyesami may demonstrate a substantial improvement on a clinically significant endpoint in Critical COVID-19 patients with respiratory failure.

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 $VPAC_1$ 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. 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. No other COVID therapeutic has been reported to have an effect on surfactant production. Figure 1 illustrates the concepts described above.

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. 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.

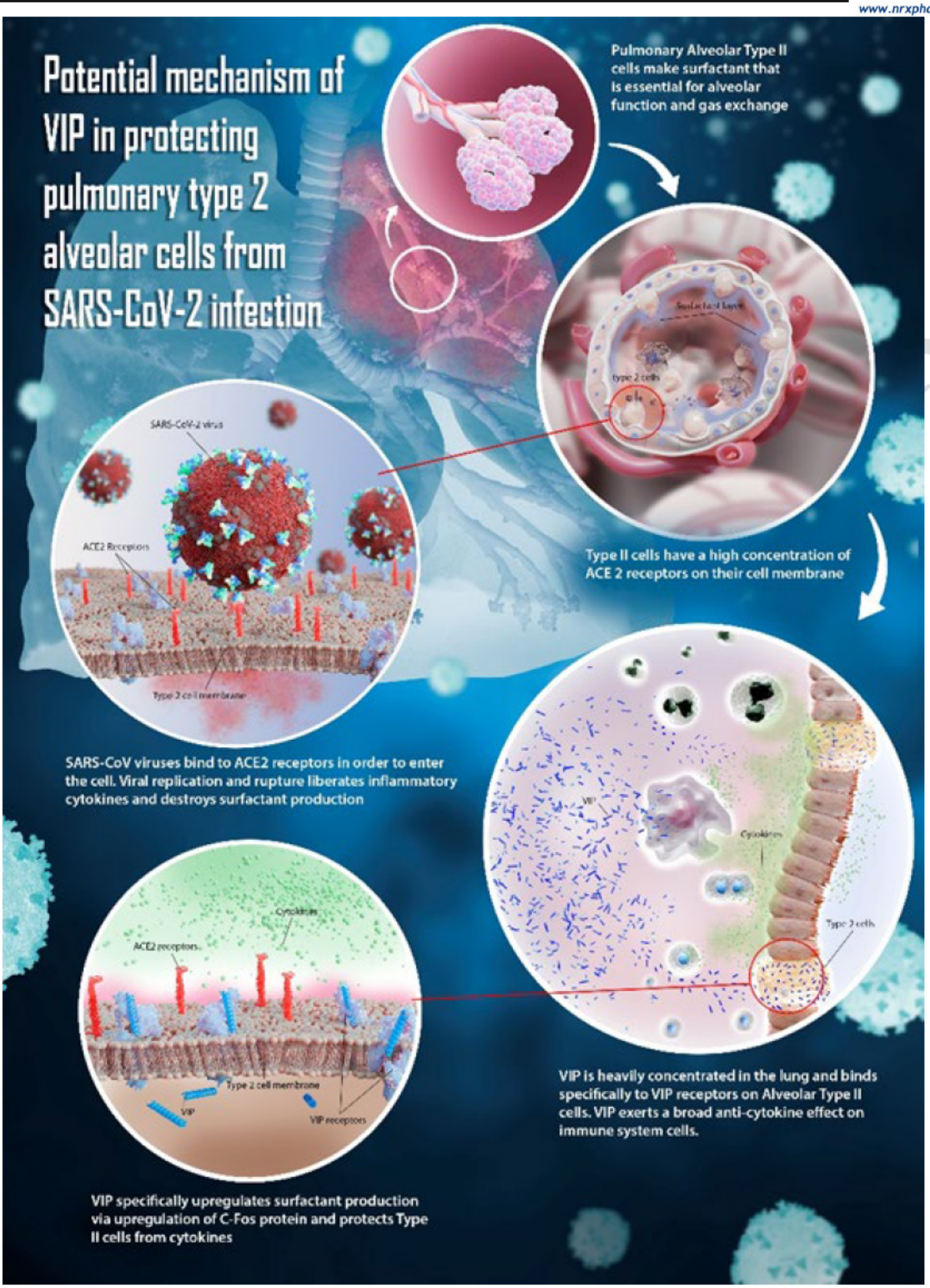


Figure 1: Potential mechanism of Vasoactive Intestinal Peptide in protecting pulmonary type 2 alveolar cells from SARS-CoV-2 infection

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-1β, and IL-8 (Channappanaver 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. While some have suggested that the blockade of IL-6 alone may block the cytokine storm in COVID-19, failures of IL-6–specific antagonists in recent clinical trials suggest that a broader anti-cytokine strategy may be needed. IL-6 is only one of numerous cytokines that are upregulated and attack the pulmonary epithelium.

If the mechanism of ALI in SARS-CoV-2 infection were driven by cytokine-induced inflammation alone, steroids and other anti-inflammatory drugs might be expected to have some salutary effect. 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 (Delagado 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-κB. This protein is normally located in cell cytoplasm and is bound to an inhibitory protein IκB in an inactive state. Inducers of NF-κB activation trigger a cascade of reactions, ending in liberation of NF-κB from IκB. NF-κB 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-κB 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 ($VPAC_2$) binding sites on rat AT2 cells (Groneberg 2001, Onoue, 2004). This binding site is distinct from the $VPAC_1$ 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 cytidylyltransferase (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-kB 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).

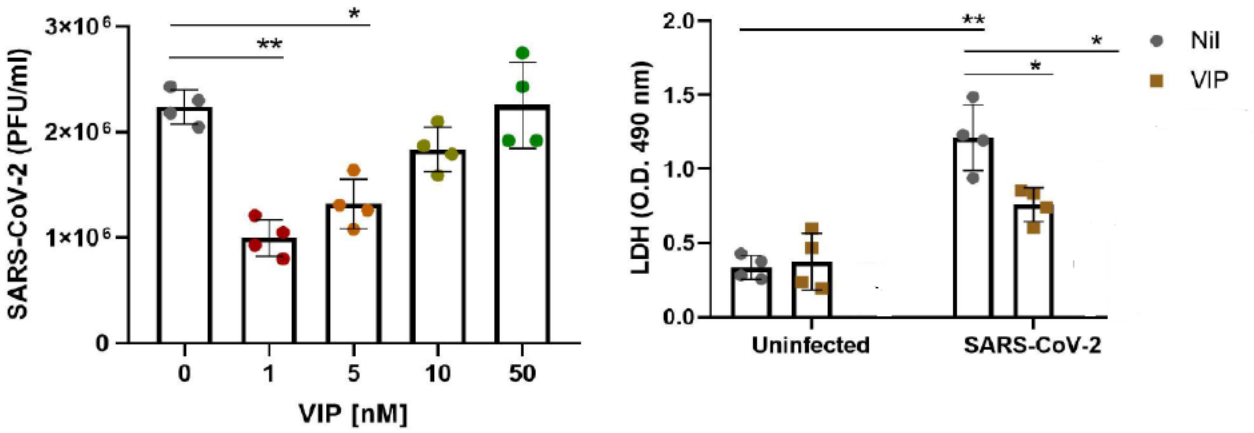


Figure 2: Effect of VIP on inhibition of SARS-CoV-2 replication in Calu-3 cells with statistically significant inhibition seen at 1 and 5 nM concentration (left). Effect of VIP in preventing cytopathy as measured by supernatant LDH levels in SARS-infected Calu-3 cells (right). Source: Temerozo (2020)

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).

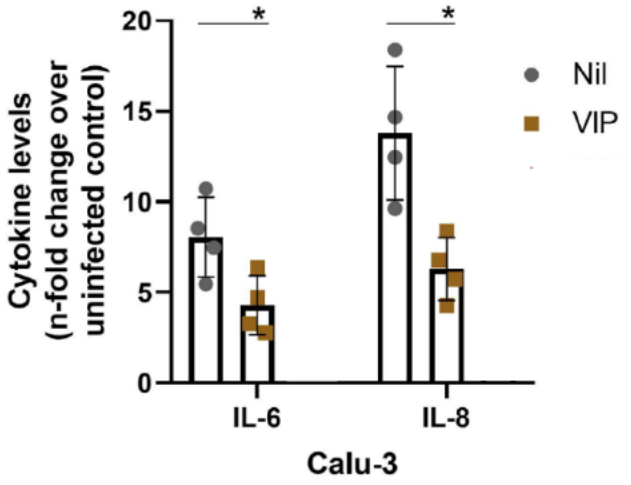


Figure 3: Effect of VIP in reducing IL-6 and IL-8 secretion from SARS-infected Calu-3 cells. Source: Temerozo (2020)

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 COVD-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 Endpoints: Recovery and Survival in Patients with Respiratory Failure

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).

Across all patients and all sites of care, the trial demonstrated 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, controlling for baseline severity. A trend towards significance (OR 1.63; P=.14) was seen for the endpoint of "alive and free from respiratory failure" at 60 days but was not significant without adjusting for hospital setting (i.e., tertiary vs. community).

Overall survival in both aviptadil- and placebo-treated patients was observed to be substantially lower in community hospitals than in tertiary care hospitals, as the study was monitored on a blinded basis prior to study completion and unblinding. Accordingly, the statistical analysis plan was modified to include a site of care variable to denote tertiary vs. community hospital. Tertiary care hospitals were defined as those with full-time board-certified critical care physicians, critical care nurses, critical care fellows, and respiratory therapists in the ICU around the clock. When adjusting for hospital setting as a baseline characteristic, 4-fold increased odds of survival and 2-fold increased odds of being "alive and free from respiratory failure" at 60 days are demonstrated when also controlling for baseline severity using the prespecified logistic regression.

The effect of site of care on 60-day clinical outcome, as distinct from a short-term biological effect of aviptadil, is not surprising. The ability of patients to survive respiratory failure on mechanical ventilation in the ICU is well known to be associated with multiple aspects of ICU care, including proning of the patient, minimizing the Positive End Expiratory Pressure (PEEP) on ventilation, controlling nosocomial infections, and many other factors.

Table 2: Logistic regression on primary and secondary endpoint, controlling for baseline severity of respiratory failure (NIAID 2 vs. NIAID 3) and site of care (tertiary vs. community hospital)

Endpoint	Percentages	Odds Ratio (95% CI) (controlling for baseline severity and site of care)
Alive and Free of Respiratory Failure at Day 60	ZYESAMI 62.8% Placebo 50.0%	2.621 (1.220, 5.628) P<.02
Survival to Day 60 (may still be in Hospital)	ZYESAMI 75.5% Placebo 54.0%	4.346 (1.909, 9.895) P<.001

Further confirmation of the statistical significance for primary and secondary endpoints was explored using the Wilks Global Hypothesis Test (Table 3) to adjust for treatment*site interactions identified in the prespecified logistic regression. As detailed below and described in the CSR, treatment with aviptadil was associated with a statistically significant benefit for all reported primary and secondary endpoints when controlling for baseline severity and site of care.

Table 3: Regression using Wilks' comprehensive global hypothesis test to confirm significance of all primary and secondary clinical endpoints, controlling for baseline severity and hospital setting

	Wilks' P-Value	
Endpoint	**Binary Endpoint**	**Time-to-Event**
Primary: Day 60 Alive and Free of Respiratory Failure	0.0226	0.0246
Key Secondary: Day 60 Alive	0.0009	0.0016
Day 60 Hospital Discharge	0.0258	0.0174
Day 60 NIAID Score Improvement ≥ 2	0.0167	0.0173
Day 60 ICU Discharge	0.0101	0.0068
Day 60 NIAID Score ≥ 6	0.0226	0.0242
Day 28 Alive and Free of Respiratory Failure	0.0588	0.0614

4.5.1.2 Secondary endpoint: Respiratory Distress Ratio—aviptadil vs. placebo

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 at the Horowitz index or PF ratio. This ratio depends upon measurement of arterial blood gas, which can either be drawn from an arterial line (in the case of an intubated patient) or by arterial puncture, which is painful to the patient and is generally performed only by a physician or respiratory therapist because of the potential for hematoma and injury to the patient. 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).

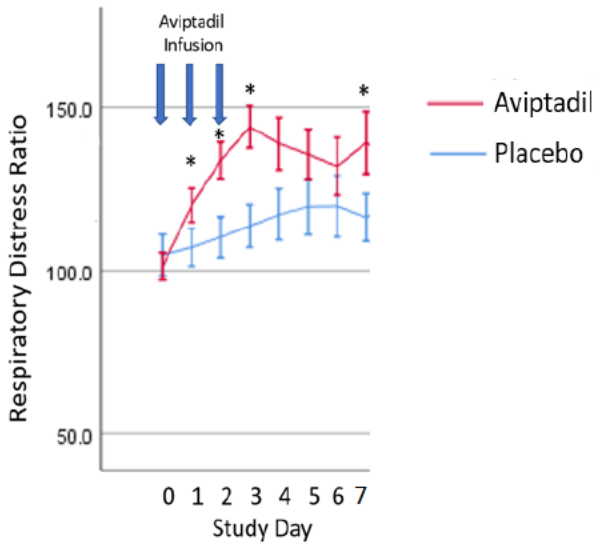


Figure 4 Respiratory Distress Ratio in patients treated with aviptadil and placebo. Significant differences in improvement are noted at days 1, 2, 3, and 7 (t-test P<.05) and across the 7-day interval on Mixed Measures Repeated Model (P<.02).

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).

The above data demonstrate a biologic and statistically significant response to treatment with aviptadil across all patients and all sites of care. The difference is larger in the HFNC subgroup and the tertiary care subgroup, where increased odds of 60-day survival and recovery from respiratory failure associated with aviptadil vs. placebo are also higher. 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).

4.5.1.3 Secondary Endpoint: NIAID Ordinal Scale

A subjective measure of patient improvement is the National Institute for Allergy and Infectious Diseases (NIAID) ordinal scale. 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.

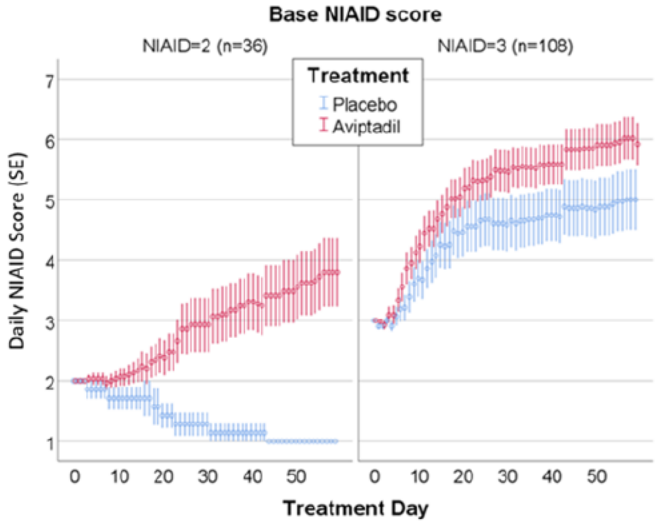


Figure 5 Among those treated in tertiary care centers, baseline NIAID score demonstrates an effect on 60 day outcome as measured by the NIAID scale. The difference is statistically significant for those with baseline NIAID =2 (P=.036) and at a trend level for baseline NIAID = 3 (P=.099)

4.5.1.4 Biomarker Endpoint (pre-defined): Cytokine IL-6

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<.02; Figure 6). This rise in IL-6 cytokine, colloquially known as a "cytokine storm," 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).

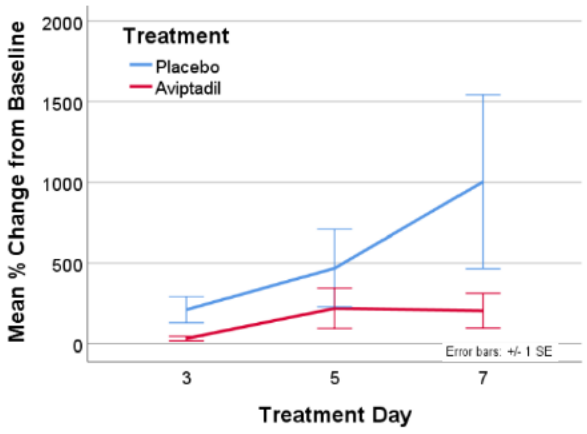


Figure 6: Percent change from pre-treatment (Day 0) baseline in IL-6. A significant difference is seen between day 3 and day 7 favoring aviptadil (P= .024) with independent significance on days 3 (P=.002) and 7 (p=.06).

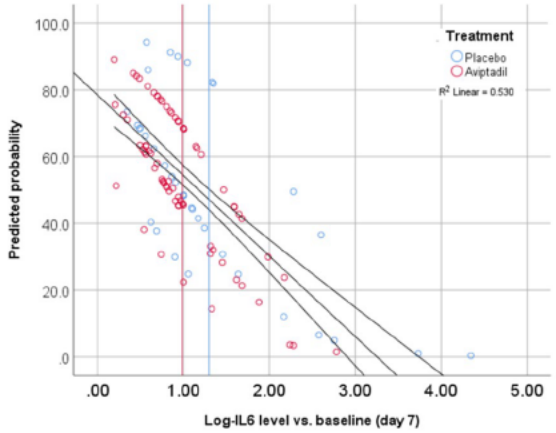


Figure 7: Predicted probability of primary outcome vs. change in Log IL-6 level from baseline to day 7. Increase in IL6 at day 7 predicted >50% of the variance in treatment outcome (R^2 - .53). Treatment with aviptadil is less likely to be associated with a day 7 increase in IL-6 and is associated with 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, χ^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 2b3 trial

Detailed information on safety is contained in the clinical study report (see Pre-EUA 103). Summary information (Table 4) shows that the primary drug-related Significant Adverse Events 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. No statistically significant difference in hypotension was seen between those treated with aviptadil and those treated with placebo.

Table 4: Incidence of Adverse Events

	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
HEPATOBILIARY 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 160 of the 660 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.

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).

Table 5: 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).

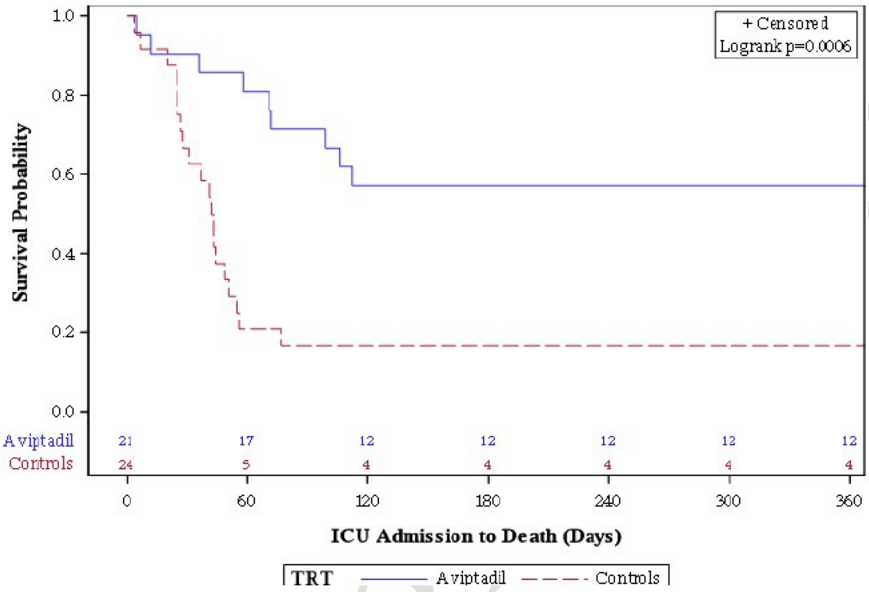


Figure 8: Survival in patients treated with aviptadil (n=21) vs SOC (n=24) from Time of ICU Admission (Hazard Ratio 0.26; 95% CL 0.12, 0.60)

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).

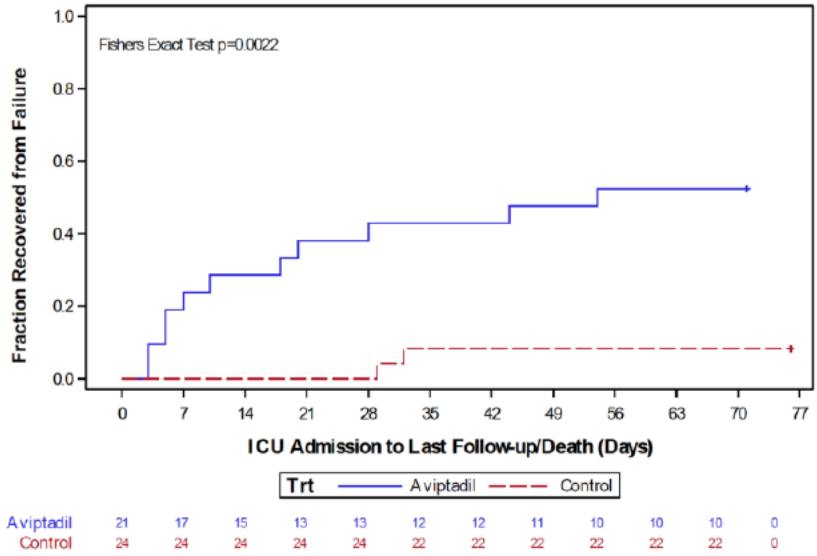


Figure 9: Time to recovery from Respiratory Failure. (Hazard Ratio 0.115 (95% CL: 0.0254, 0.5219)

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).

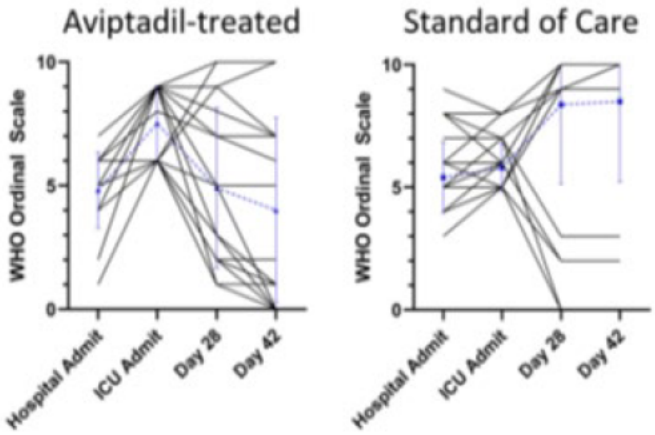


Figure 10: Change in 10-Point Ordinal Scale from Enrollment Through Day 24

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 (PAO_2:FiO2)

Aviptadil-treated patients demonstrated a significant, nearly 3-fold improvement in oxygenation as measured by the Respiratory Distress Ratio (RDR), also knowns as the $Pa0_2$: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 PaO_2: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).

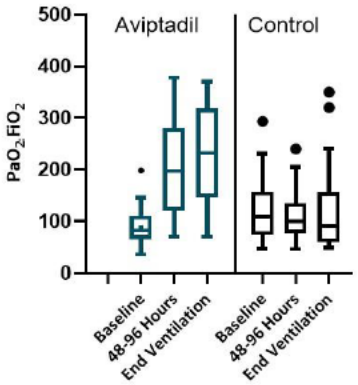


Figure 11: Respiratory Distress Ratio (PaO2:FiO2) in aviptadil-treated vs. control patients demonstrating statistically significant improvement in RDR among aviptadil-treated patients at 48-96 hours (P<.001).

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.

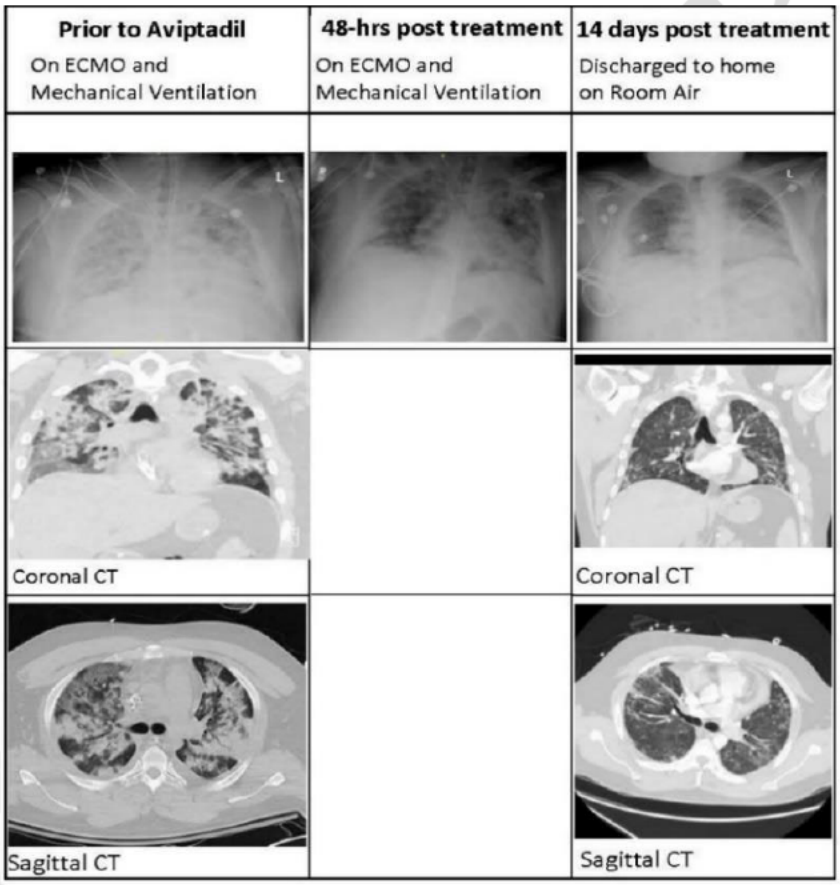


Figure 12: Chest x-ray and CT imaging of a patient initially treated while on mechanical ventilation and extracorporeal membrane oxygenation for Critical COVID-19 with respiratory failure

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% ±3%) and IL 6 (75% ±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.

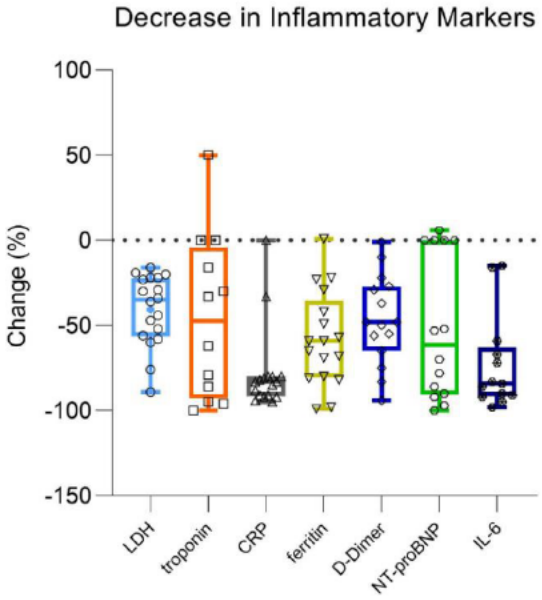


Figure 13: Decrease in inflammatory markers as a percent change from pretreatment value. The decrease is both clinically and statistically significant (P<.001

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 Clinical Evidence of Safety in the ACTIV3b TESICO Trial

The ACTIV3b TESICO trial (www.clinicaltrials.gov NCT04843761) is currently enrolling patients nationwide with funding from the National Institute for Allergy and Infectious Diseases (NIAID). The trial is approved by FDA as a phase 3 trial (IND 154701) and compares treatment of patients who have respiratory failure in Critical COVID-19 with aviptadil, remdesivir, aviptadil+remedesivir, and placebo in a full factorial design.

The NIAID DIADS Therapeutic and Prevention Data and Safety Monitoring Board met on August 16, 2021 and conducted a safety review following enrollment of 140 participants, 70 of whom are assumed to have been randomly allocated to either aviptadil alone or aviptadil+remdesivir. The DSMB identified no new safety concerns. The DSMB further acted to broaden recruitment to non-ICU (i.e. stepdown unit) patients in the vanguard hospitals and further reduced blood pressure monitoring requirements while on aviptadil.

4.5.7 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).

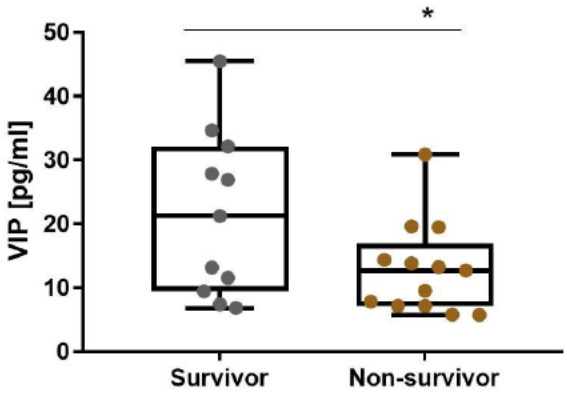


Figure 14: Case-control study (i.e. non-intervention study) of endogenous circulating VIP levels COVID-19 in survivors and non-survivors in a single ICU. Note the statistically significant higher level of circulating VIP among survivors (P<.05). Source: Temerozo (2020)

5. SAFETY OF AVIPTADIL AT CURRENT DOSING

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 231 patients as of October 1, 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.

6. SUMMARY

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 COVID-19.

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 survival benefit was seen across all patients when controlling for baseline severity (NIAID 2 vs. NIAID 3), a prespecified covariate ($p<.03$). Odds of Recovery from respiratory failure ("alive and free from respiratory failure" at 60 days) was 2-fold higher in favor of aviptadil among patients treated at tertiary care hospitals ($P < .02$), but this effect could not be demonstrated at community hospitals. 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.

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.

In both the phase 2b/3 and the open-label trial, Respiratory Distress Ratio ($Pa0_2$: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 that it addresses an unmet medical need in a serious medical condition (i.e. Respiratory Failure in patients with COVID-19) and demonstrates preliminary evidence of efficacy. 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.

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.

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, Brenneman 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 $VPAC_2$ 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 spetic 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/PMC7144II60/

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, Sacramenta Q, Fintelman-Rodriques 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