

Vasoactive Intestinal Peptide treats Respiratory Failure in COVID-19 by rescuing the Alveolar Type II cell

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Abstract

Aviptadil, a synthetic form of human Vasoactive Intestinal Peptide (VIP) has been granted FDA Fast Track Designation for the treatment of Critical COVID-19 with respiratory failure and is now in phase 2/3 clinical trials, with initial determinations of safety and non-futility. Rapid recovery from Critical COVID-19 with respiratory failure as been seen in multiple patients treated with open label VIP under FDA Emergency Use IND. VIP binds uniquely to receptors on Alveolar Type II cells in the lung, the same cells that bind the SARS-CoV-2 virus via their ACE2 receptors. VIP protects those cells and the surrounding pulmonary epithelium by blocking cytokines, preventing apoptosis, and upregulating the production of surfactant, the loss of which is increasingly implicated in COVID-19 respiratory failure. Because of its lack of toxicity and low cost of manufacture compared to proprietary biologics, VIP may be uniquely attractive to those focused on global countermeasures against COVID-19.

Keywords

Vasoactive Intestinal Peptide, VIP, SARS-CoV-2, COVID-19, Acute Respiratory Distress Syndrome, ARDS, Acute Lung Injury, ALI, surfactant, Alveolar Type II

Vasoactive Intestinal Peptide (VIP) has been granted Fast Track Designation by FDA for the treatment of Critical COVID-19 with respiratory failure by FDA and has shown initial promise in FDA phase 2/3 clinical trials. Unfortunately, VIP has long been seen as an unattractive candidate for pharmaceutical development owing to its generic status as a natural peptide and its low cost of production, which limit its commercial potential and profitability. VIP may, however, be uniquely attractive from a global health perspective to combat the worst public health crisis since Spanish Flu killed more people than combat during World War I. VIP may also be the only candidate in human trials that could be produced at a cost structure that is compatible with the needs of the developing world.

Fifty years ago, Nature published a short report entitled “Potent peripheral and splanchnic vasodilator peptide from normal gut,” published by two young scientists working at the Karolinska Institute (Said, Mutt, 1970). Five decades of subsequent research documented VIP’s role as a potent natural anti-cytokine that has unique capability to block pathways of cell death in Alveolar Type II (ATII) cells – the cell targeted by the SARS-CoV-2 virus. Clinical reports are now emerging which document rapid recovery from Critical COVID-19 with respiratory failure within days of administering intravenous RLF-100 (Aviptadil), a formulation of VIP approved by the US FDA for human administration under Investigational New Drug (IND) 149,152, which has treated more than 60 patients (www.clinicaltrials.gov NCT 04311697). The Data Monitoring Committee of the clinical trial, in examining data on the first 30 patients has identified no safety concerns and determined that the trial has potential (i.e. non-futility) to detect a statistically significant difference in recovery from respiratory failure in Critical COVID-19 in those treated with Aviptadil vs. placebo (online only attachment 1).

Although the FDA phase 2/3 clinical trials remain blinded, some patients have been treated under open label Emergency Use IND on a named-patient basis. Rapid clearing of pneumonitis has been documented on radiography (Figure 1) with concurrent improvement in oxygenation and patient transition from intensive care with mechanical ventilation to home discharge on room air within days of IV infusion. It is hoped that this rapid recovery will be found to occur more frequently in patients treated with Aviptadil than in those treated with placebo. Recently, a German research group published similar findings from a patient with checkpoint inhibitor-induced pneumonitis who was treated with inhaled VIP (Frye 2020).

Acute respiratory failure is the primary cause of death in COVID-19. The injury is generally attributed to cytokine storm – i.e. a massive release of inflammatory cytokines as viral particles infect and then cause rupture of pulmonary epithelium cells. However, the cytokine storm is only produced after the SARS-CoV-2 virus enters the ATII cell through binding of its spike protein to Angiotensin Converting Enzyme 2 (ACE2) surface receptors (Mason 2020). ACE2 is not present on Type I alveolar cells, which comprise







	Before Infusion	24- hours post 3rd infusion
Portable Chest X-Ray		
Scout CT		
Chest CT		
PaO2:FiO2	146	285.19
SaO2	98	95
FiO2	HFLNC 30 L/min, FiO2 50%	2 L/min

Figure 1: Rapid recovery from Critical COVID-19 with respiratory failure in a patient with immunosuppressive agents for graft rejection following double lung transplantation. Treated under FDA emergency use IND. Reprinted under CC license from Youssef 2020.

95% of the pulmonary epithelium and those cells are not infected by the corona virus. Similarly, only the ATII cell expresses the VPAC₁ receptor to which VIP binds. VIP is shown to prevent their apoptosis in models of lung injury (Onoue, 2004). Hence, VIP represents a highly specific approach to rescuing the lung from the overwhelming failure of oxygenation seen in COVID-19 (figure 2).

Although VIP was once viewed as a promising drug and substantial resources were invested in elucidating its pharmacokinetics, toxicology, and safety pharmacology, its proposed uses in sarcoid and sepsis-related ARDS were not sufficiently compelling commercial targets to generate investment from major pharmaceutical companies. Aviptadil is seen as a “million dollar” drug in a world that invests in only “billion dollar” drugs.

Pulmonary drugs are notoriously difficult to develop, given regulatory requirements for long-term inhaled toxicology studies in multiple species, including primates (Tepper 2016). FDA has asserted that these preclinical toxicology requirements must be observed in the case of candidate drugs to treat COVID-19. VIP, on the other hand, completed four-species toxicology and safety pharmacology studies in both intravenous and inhaled dosage. Phase 2 trials in sarcoidosis (Prasse 2010), pulmonary hypertension (Leuchte 2008), pulmonary fibrosis, and allergy/asthma document that VIP has no major toxicities when inhaled at doses of 300µg/day. On July 14, 2020, FDA granted an IND for the study of inhaled VIP in the treatment of severe COVID-19 that has not yet progressed to respiratory failure in the hopes of preventing respiratory failure and ICU admission in such patients (NCT 04360096).

Vasoactive Intestinal Peptide (VIP) was first proposed as a modulator of lung inflammation by Said (Said 1988, 1991). VIP is known to have a beneficial effect in numerous models of lung injury (table 1) at the above doses. It has demonstrated clinical effects in clinical trials of sepsis-related Acute Respiratory Distress Syndrome (ARDS) and Sarcoid. Meaningful reduction in TNF-α and CD4/CD8 ratio was seen in sarcoid (Prasse 2010).

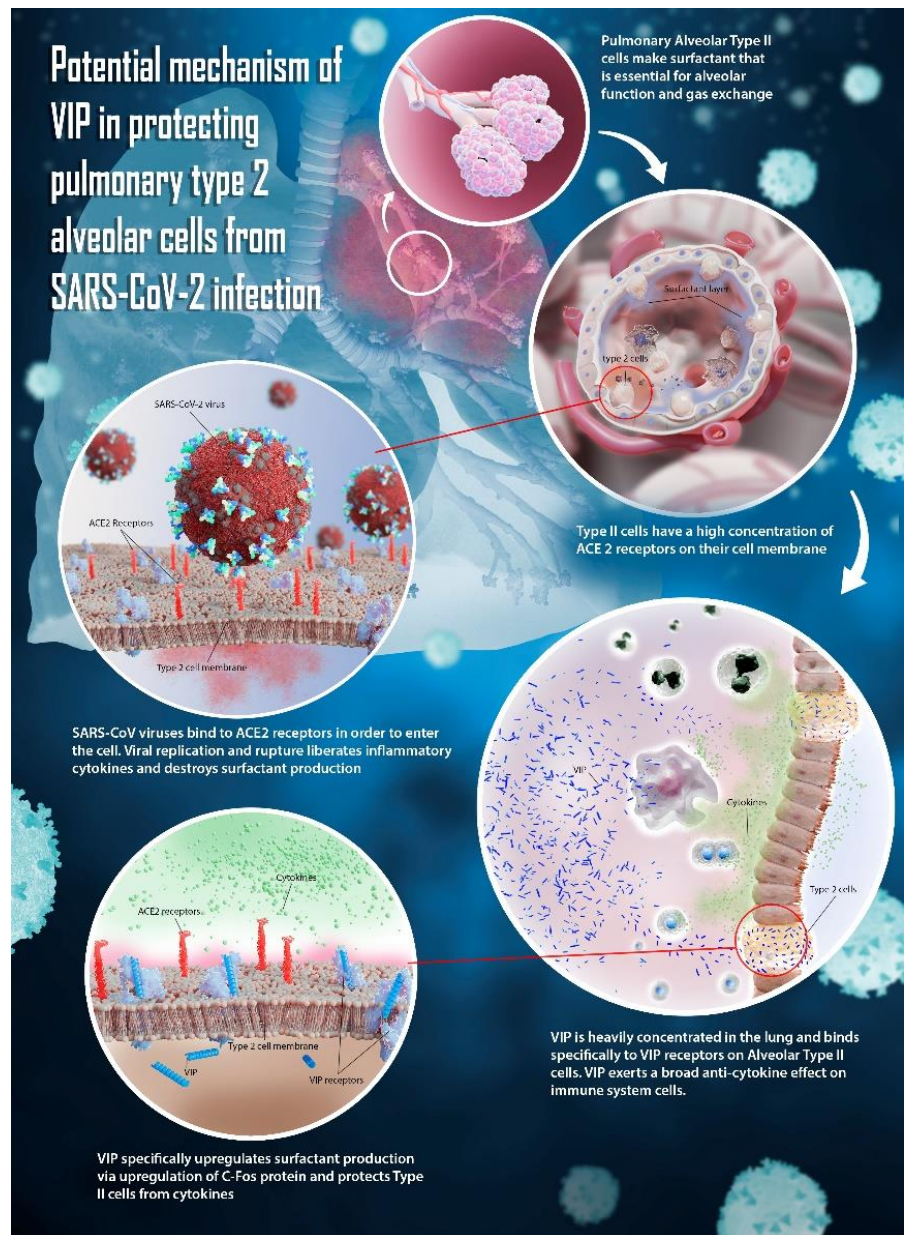


Figure 2: Potential mechanism of VIP in protecting the pulmonary Alveolar Type II cell.

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 & Dickman 2000
Rat	Xanthine/xanthine oxidase-induced lung injury in perfused lungs	Berisha 1990, Misra 1990
Guinea Pig	Paraquat (methyl viologen)	Pakbaz 1993, Said & 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

Although named (or mis-named) for the intestinal 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 (Figure 3: Virgolini 1995) but is a widely distributed immunomodulator with protective effects in 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 breathe, consistent with the theory that loss of surfactant and alveolar gas exchange is an early hallmark of COVID-19. 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, Li 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).

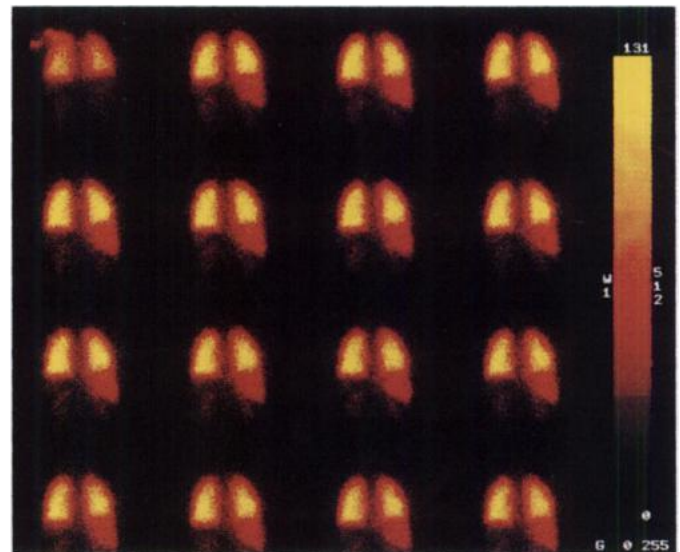


Figure 3. Concentration of VIP in the lung on scintigraphy: Virgolini 1995, reproduced with permission

Scientific Rationale for VIP effect in COVID-19

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 (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 and major increase in alveolar capillary permeability. 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 comprised 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 acts via degranulation of serine proteases (i.e. granzymes), together with the pore-forming protein perforin, to 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 & Dickman, 2000).

Decreased Pulmonary Inflammation: During the early phase of ALI, the lung is the site of an intense inflammatory process with sequential activation of cytokines, chemokines, and secretion of proteases, as well as concomitant collagen synthesis. Evidence of an acute inflammatory reaction in the alveolar-microvascular area also includes recruitment and activation of inflammatory cells, increased production of toxic oxygen metabolites, nitric oxide, and a protein rich exudate in the air spaces. A key regulator involved in a variety of these processes is the nuclear transcription factor NF- κ B. VIP has been shown to inhibit NF- κ B activation in numerous animal models of acute lung injury, which blocks the production of tumor necrosis factor alpha (TNF- α). (Delgado 1998).

Effect of VIP on Surfactant Production: A pathologic hallmark of the ALI/ARDS is the damage to the surfactant-producing alveolar type II cells (Mossel 2008). These cells are characterized by Angiotensin Converting Enzyme 2 (ACE2) receptors on the cell surface and, therefore, may be particularly susceptible to SARS-CoV-2 virus infection. Studies have demonstrated high density VIP binding sites on rat type II cells (Onoue, 2004). Li demonstrated 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, which is instrumental in promoting synthesis of pulmonary surfactant phospholipids (Li 2007) and induces surfactant protein A expression in ATII cells through activation of PKC/c-Fos pathway.

Conclusion:

VIP shows promise as a pulmonary therapeutic agent with potential both to rescue patients from respiratory failure in Critical COVID-19 and perhaps to prevent respiratory failure from developing in the first place. VIP is the only pulmonary therapeutic to have been granted Fast Track status by the US FDA and to be allowed into both phase 2/3 clinical trials, as well as an expanded use protocol for those who are unable to enter the clinical trial because of excluded comorbidity. Humanity faces a lethal viral threat that specifically attacks the ATII cell required for pulmonary oxygenation. The highly specific role of VIP in the lung may be key to combating the targeted lethal effect of SARS-CoV-2 infection. Unlike synthetic anti-cytokine and interleukin drugs, VIP has no known human toxicity. Because of its lack of toxicity and low cost of manufacture compared to proprietary biologics, VIP may be uniquely attractive to those focused on global countermeasures against COVID-19.

Illustration credit: Nicolás Fernández

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References

- 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, H.D., Said, S.I., Lodi, R., Pakbaz, H., and Schraufnagel, D.E. Vasoactive intestinal peptide enhances lung preservation. *Transplantation* 1993;56:964-973.
- Berisha, H., Foda, H., Sakakibara, H., Trotz, M., Pakbaz, H., and Said, S.I. 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, Brennehan 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.
- Foda, H.D., Iwanaga, T., Liu, L.-W., and Said, S.I. Vasoactive intestinal peptide protects against HCl-induced pulmonary edema in rats. *Ann NY Acad Sci* 1988;633-636.
- Frye, Bjoern & Meiss, Frank & Bubnoff, Dagmar & Zissel, Gernot & Müller-Quernheim, Joachim. (2020). Vasoactive Intestinal Peptide in Checkpoint Inhibitor–Induced Pneumonitis. *New England Journal of Medicine.* 382. 2573-2574. 10.1056/NEJMc2000343.
- 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, Z.Q., 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/PMC7144160>.
- 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, G.O., Smith, C.W., Anderson, D.C., Todd, R.F., III, and Ward, P.A. 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.
- 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, J.G.; Zahiruddin, F.; Al-Saadi, M.; Yau, S.; Goodarzi, A.; Huang, H.J.; Javitt, J.C. Brief Report: Rapid Clinical Recovery from Critical COVID-19 with Respiratory Failure in a Lung Transplant Patient Treated with Intravenous Vasoactive Intestinal Peptide . Preprints 2020, 2020070178 (doi: 10.20944/preprints202007.0178.v1).