An Acute Respiratory Distress Syndrome Drug Development Collaboration Stimulated by the Virginia Drug Discovery Consortium

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ABSTRACT

The genesis of most older medicinal agents has generally been empirical. During the past one and a half centuries, at least in the Western countries, discovering and developing drugs has been primarily the domain of pharmaceutical companies largely built upon concepts emerging from organic chemistry. Public sector funding for the discovery of new therapeutics has more recently stimulated local, national, and international groups to band together and focus on new human disease targets and novel treatment approaches. This Perspective describes one contemporary example of a newly formed collaboration that was simulated by a regional drug discovery consortium. University of Virginia, Old Dominion University, and a university spinout company, KeViRx, Inc., partnered under a NIH Small Business Innovation Research grant, to produce potential therapeutics for acute respiratory distress syndrome resulting from the ongoing COVID-19 pandemic.

Introduction

Ancient drugs were either natural organic products or inorganic materials. One can find the commonly used herbs and medicinal agents listed in the "Pharmacopeia" of China, India, Greece (De Materia Medica ~50 AD), and Arabia. These agents guided the treatment of diseases for centuries in many parts of the world. With the enormous advancements in our understanding of human physiology and disease pathology, contemporary drug discovery has become much less empirical and has focused on the specific biochemical and molecular causes or symptoms of diseases. One of the more interesting developments during the past two decades has been the emergence of academic centers formally focused on drug discovery. In the USA, this is due at least in part to the efforts of the National Institutes of Health, which hoped to leverage the products of the Human Genome Project and to focus on identifying new molecular targets for drugs with the launching of the Molecular Libraries Screening Centers in 2005 [1]. As members of one of the first pilot centers, some of the authors have watched how interdisciplinary academic groups have fostered innovative strategies to identify and refine potentially new therapeutics. This has been encouraged by international, national and local organizations, which have emerged and helped catalyze drug discovery communities. One example is the Academic Drug Discovery Consortium (ADDC) (https://www.addconsortium.org), which is a professional society established in 2012 and now has >2,200 individual registered members and includes 155 centers. The ADDC seeks to establish an interactive network of drug discovery scientists who can exchange technologies, encourage industrial partnerships, share educational materials, create formal meetings and symposia, and advocate for drug discovery funding from federal, state and non-profit agencies.

The Virginia Drug Discovery Consortium as a catalyst for regional collaboration

The Virginia Drug Discovery Consortium (VaDDC) (www.vaddc.org) is a regional organization located in the Commonwealth of Virginia that aims to promote collaborative work among the local universities, to assist in launching early stage pharmaceutical companies, and to improve the prestige of the region as a friendly environment for pharmaceutical and biotechnology companies. To date the VaDDC has hosted six annual conferences that have attracted on average approximately 150 attendees. The meetings have provided investigators and students with the possibility of connecting with peers and learning from national drug discovery leaders from academia, government, and industry. Representatives from the technology transfer offices of the local universities have

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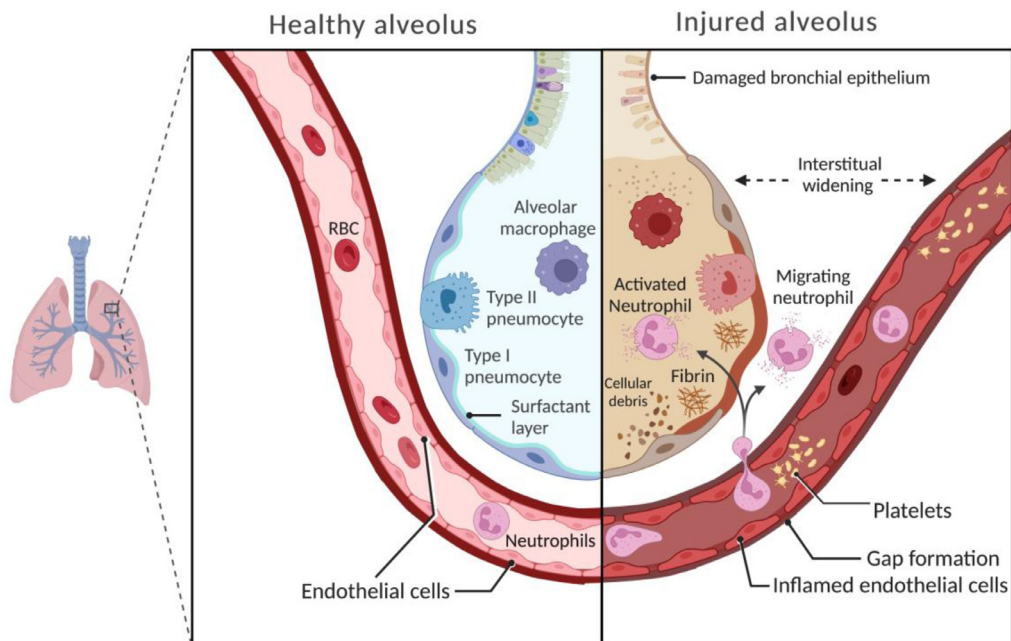*SLAS Discovery xxx (xxxx) xxx*

Fig. 1. Schematic representation of the acute injury caused to a healthy alveolus after exposure to SARS-CoV-2, other viruses and pulmonary toxins. Resident endothelial, epithelial, macrophages and fibroblasts become pro-inflammatory. Neutrophils migrate into the alveolus and are activated to a pro-inflammatory state. Created with the assistance of BioRender.com.

allowed the attendees to compare best practices and the leaders of local economic development organizations have discussed various funding vehicles. The meetings also provide a forum for vendors to display their products and scientific equipment.

This Perspective reviews how the VaDDC has helped create an ideal environment for independent laboratories with faculty and research staff from two Virginia universities, the University of Virginia and Old Dominion University, to rapidly collaborate on identifying new treatments for the unexpected Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic. SARS-CoV-2 like many other viral infections is lethal because of it causes acute lung injury (ALI) with the subsequent release of copious amounts of cytokines and chemokines often referred to as a "cytokine storm" (Fig. 1). A central feature of the Acute Respiratory Distress Syndrome (ARDS) is the loss of the pulmonary microvascular endothelial barrier, thus allowing an influx of serum and blood proteins and the formation of pulmonary edema, the most common cause of death from COVID-19. While ARDS and ALI terms define rapid-onset respiratory syndromes, their use has been somewhat controversial over time, and most often they only differ as ALI is mostly used in research settings, while ARDS is a clinical term [2]. The development of ARDS is a dramatic complication that can follow a direct initial respiratory injury (e.g., pneumonia, flu, inhalation injuries, trauma) or an indirect systemic insult (pancreatitis, reaction to blood transfusion or sepsis). Thus, ARDS is an extremely heterogeneous syndrome, associated with up to 40% mortality [3]. Besides the advances in airway management and mechanical ventilation, and the improvements in the short term management, and the consequent improvements in the short term treatment, the 1-year mortality for these patients is still high [4,5]. In the US, ARDS incidence has been reported to be as high as 78 cases per 100,000, with a prevalence of 10% of critical patients admitted to Intensive Care Units [6,7]. Due to the outbreak of the SARS-CoV-2 pandemic, however, its incidence is believed to have increased up to 10-fold, highlighting the importance for additional investigation into new therapeutic interventions.

Two of the co-authors of this Perspective, J.S. Lazo and J.D. Catravas, first collaborated on pulmonary endothelial dysfunction at the begin-

ning of their careers at Yale University four decades ago [8,9]. Using rabbits as a preclinical model, they found that acute treatment with the anticancer drug bleomycin, which continues to be clinically used, decreased pulmonary 5-hydroxytrypamine and norepinephrine lung clearance and they proposed that the clearance changes might reflect pulmonary endothelial injury, a hypothesis supported by light and electron microscopic studies showing microvascular endothelial blebbing [8]. Subsequent studies suggested the morphological microvascular damage caused by bleomycin was a transient effect [9]. After leaving Yale University, Lazo and Catravas enjoyed productive independent careers at other academic institutions while remaining in contact usually seeing each other at annual society meetings. Remarkably both investigators relocated to academic institutions in the Commonwealth of Virginia at a time when the VaDDC was being launched.

Research in the Catravas laboratory at Old Dominion University continued to focus on pulmonary microvascular injury, while Lazo, who was joined at the University of Virginia by Elizabeth R. Sharlow, focused on identifying new anticancer drugs that targeted the oncogenic protein tyrosine phosphatase PTP4A3. Not only is PTP4A3 widely expressed in tumor cells, it is also highly expressed in the endothelium of tumors [10] as well as in cultured endothelial cells [11], and developing vascular beds [12]. In the endothelium, PTP4A3 controls several important intracellular signaling pathways, including RhoA, Src, STAT3, p-38, and NF-kB (Fig. 2), which influence cellular migration, tight junctions and capillary permeability. Because of the potential role of PTP4A3 in vascular function during angiogenesis and metastases, we tested the hypothesis that PTP4A3 was a mediator of the angiogenic phenotype of vascular cells in tumor and nontumor microenvironments. Blood vessel development was contrasted in experimental colon tumors from wild type and Ptp4a3-null mice using CD31 immunocytochemistry [13]. The deletion of the Ptp4a3 gene blunted VEGF-mediated disruption of vascular permeability (Fig. 3) and its downstream signaling components, notably phosphorylation of Src. We confirmed disruption of human microvascular endothelium wound repair with a small molecule PTP4A3 inhibitor BR-1. Finally, we documented diminution of *in vivo* VEGF-mediated vascular permeability in mice lacking PTP4A3 [13].
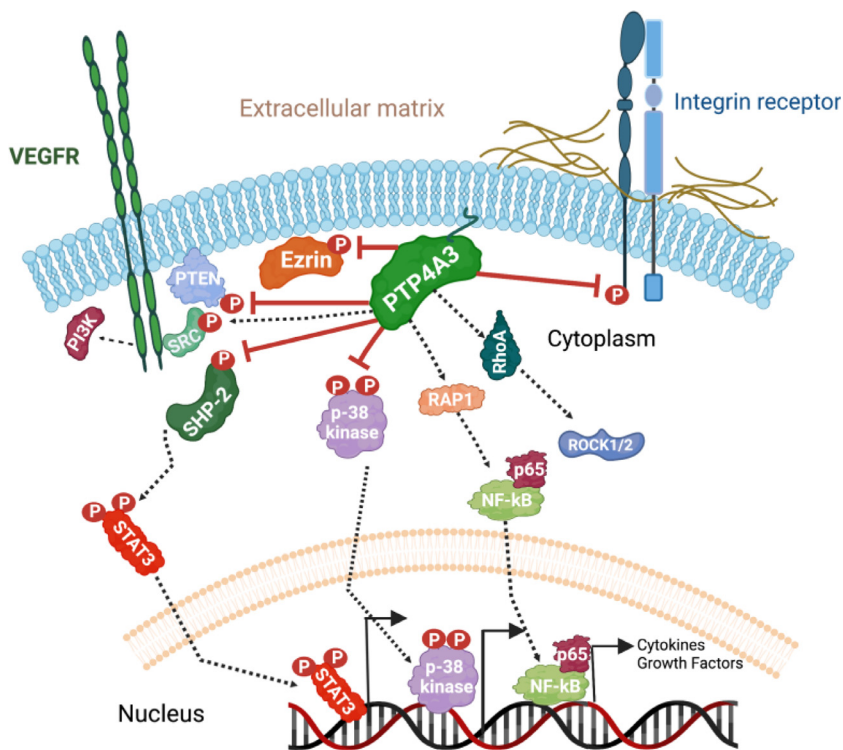
Fig. 2. PTP4A3 involvement in endothelial cell signaling. Red bars indicate inhibition and dotted black lines indicate activation by upstream pathways. The transcriptional activation by STAT3, p-38 and NF-kB cause increase production and extracellular release of cytokines and growth factors. The figure was designed with the assistance of BioRender.com.
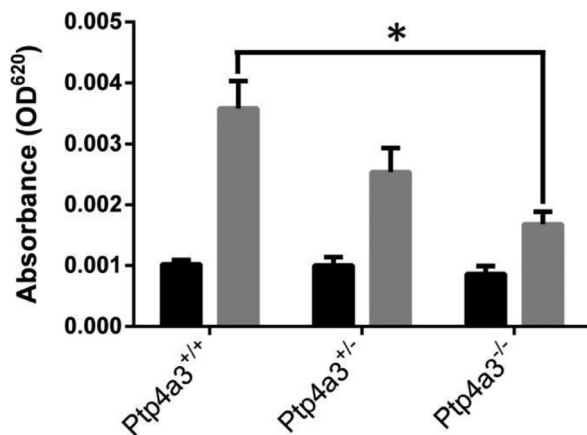

Fig. 3. Loss of PTP4A3 reduced VEGF-mediated vascular permeability. Mice were injected intravenously with Evans blue dye and 20 min later injected intradermally with 400 ng of recombinant murine VEGF (gray bars) or PBS (black bars) in the flank. Total dye absorbance in a 5 mm biopsy punch was determined with a spectrophotometer. Ptp4a3-null mice exhibited significantly reduced vascular permeability in response to VEGF relative to wild type mice (*, p < 0.01). Error bars, S.E. Further information can be found in Ref. [13].

We next assessed the ability of a more potent and selective small molecule PTP4A3 inhibitor, JMS-053, to normalize the barrier dysfunction of human lung microvascular endothelial cells (HLMVEC) caused by VEGF and LPS (Fig. 4) [14], which further implicated PTP4A3 in HLMVEC function.

Development of a murine model of COVID-19-like ALI

The ongoing pandemic has caused over 670 million infections and more than 6.8 million documented deaths worldwide (https://coronavirus.jhu.edu/map.html). However, it is believed that more than a third of the world's population has contracted the virus over the last two years. Since the first documented outbreak in December 2019 [15], a global effort has been undertaken to molecularly characterize COVID-19 and develop new therapeutic approaches.

A great challenge for the investigation of SARS-CoV-2 pathogenicity has been the establishment of a proper animal model. Indeed, while the most commonly used models for ARDS, especially sepsis-induced ARDS, rely on the intratracheal instillation of lipopolysaccharide (LPS), or the cecal ligation and puncture, investigators have been questioning how to develop a proper model of Covid-19-induced ARDS. Even though Coronaviruses are zoonotic diseases, able to jump from one species to another, the first variants of SARS-CoV-2 replicated minimally in common wild type mice strains (Mus musculus). It was soon discovered that SARS-CoV-2 enters host cells *via* the binding of its surface element, the spike protein, to the host Angiotensin Converting Enzyme 2 (ACE2) [16]. However, murine ACE2 cannot provide effective binding for the Spike protein (SP) and thus multiple transgenic models have been developed by engineering mice with the human ACE2 receptor [17].

One of the most common models utilized over the last two years has been the K18-hACE2 transgenic mouse, which uses the keratin 18 promoter to direct the expression of the human ACE2 receptor in the airway epithelium. K18-hACE mice develop a discrete pathology after being infected with SARS-CoV-2 [18], and if animals are exposed to a high concentration of viral particles, lethality is observed by seven days post-infection [19]. However, several issues have continued to affect the investigation of SARS-CoV-2 in laboratory settings. SARS-CoV-2 requires Biosafety Level-3 (BSL-3) facilities, making its investigation cumbersome, limiting its use to a small number of investigative groups, and thus reducing the testing of potential new drugs able to modulates its pathogenicity. In addition, after the initial evidence of lung injury, transgenic mice infected with SARS-CoV-2 display minimal residual pathology and only few models have been used for the investigation of persistent infections or long COVID [20]. Hamsters and ferrets are interesting models of COVID-induced ARDS, as their host ACE2 binds with high affinity to the SP, but they are more cumbersome models to use compared to mice and may have more limited translatability to the human condition [21].
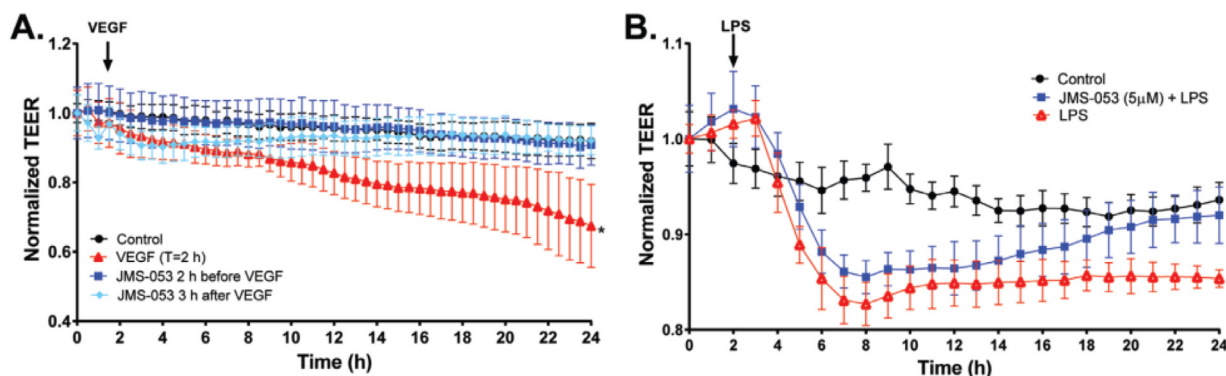
Fig. 4. Pharmacological inhibition of PTP4A3 normalizes VEGF- and LPS-induced decreases in transendothelial electric resistance (TEER). (A) Confluent HLMVEC were treated with VEGF alone or with JMS-053 (5 μM) 2 h before or 3 h after VEGF (100 ng/mL). (B) Confluent MVEC were pretreated with or without JMS-053 (5 μM) and 24 h later exposed to LPS (0.5 EU/mL). Further information can be found in Ref. [14].
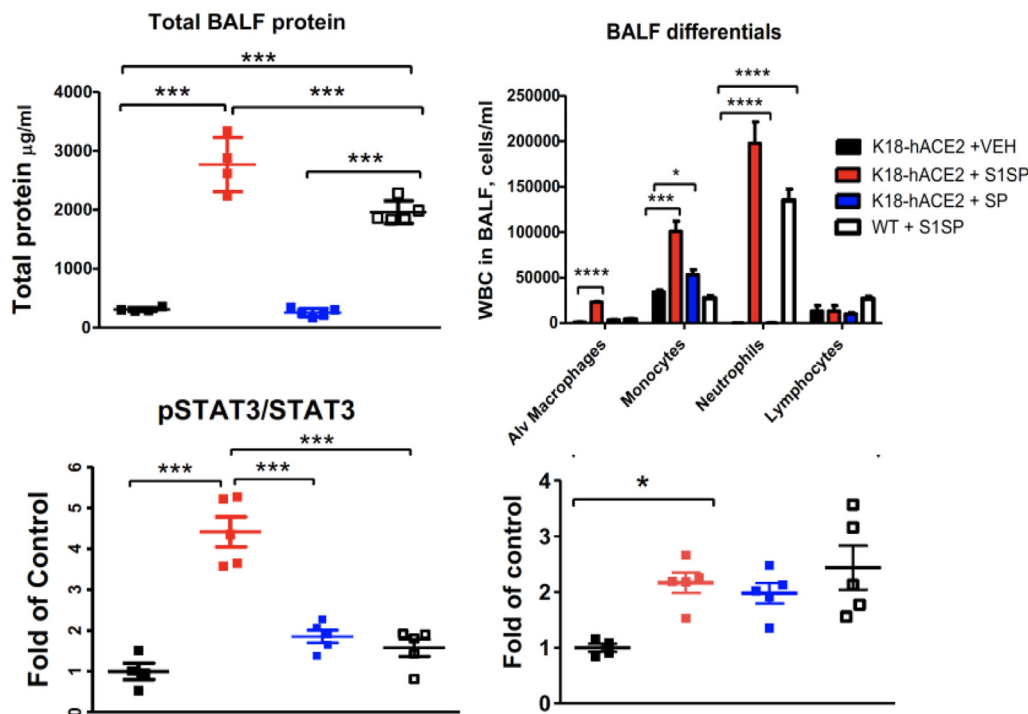

Fig. 5. Exposure of K18-hACE2 mice to SARS-CoV-2 subunit 1 of the spike protein (S1SP) induces alveolar inflammation. K18-hACE2 and wildtype C57Bl/6 (WT) mice were instilled intratracheally to Spike protein (SP), S1SP or vehicle and 72 h later the protein and cellular content in the bronchiolar alveolar lavage fluid (BALF) was determined. Top Left Panel. Total BALF protein. Top Right Panel. Differential number of leukocytes in the BALF. Bottom Left Panel. Phosphorylation of STAT3 T705 in lung homogenates. Bottom Right Panel. Phosphorylation of I-kappa B-alpha in lung homogenates. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$. More information can be found in Ref. [25].

Acute SARS-CoV-2 infection evokes a strong systemic inflammatory reaction with signs of vascular dysfunction that could evolve into multi-organ failure [22]. Significant contribution to the inflammatory reaction is believed to originate from the SP of SARS-CoV-2, which after binding the ACE2, is cleaved by proteases and released in the airway or vascular compartments [23,24]. Thus, we investigated the effects of the Spike Protein Subunit 1 (S1SP) in human lung microvascular endothelial cells and created a model of SARS-CoV-2-induced ALI, by intratracheally instilling the S1SP into K18-hACE2 transgenic animals (Fig. 5) [25]. This model not only can be investigated in BSL-2 facilities, but it is also associated with dose-dependent mortality and can also be used for the investigation of long COVID.

Discovering new therapies

A common strategy in the field of drug discovery is the repurposing of already approved drugs for new clinical applications. Many molecules

are active in multiple pathways and novel therapeutics, even when they inhibit a single target, may elicit wide biological effects. Examples of this can be seen in the work performed by two of the co-authors of this Perspective, Catravas and Lazo. Catravas' research has focused on the investigation of Heat Shock Protein networks and on Heat Shock Protein 90 (HSP90) inhibitors. This class of drugs was originally developed as new anti-cancer therapeutics, but due to the effects of HSP90 inhibitors on inflammation the drug class has gathered interest in the immunological and cardiovascular research fields.

HSP90 is a well-known chaperone that assists more than 400 "client" proteins during assembly, folding and stabilization, thus affecting their survival, activity and intended cellular signaling (Fig. 6) [26,27]. HSP90 resides in the cytoplasm, where depending on its activation state (phosphorylation and/or deacetylation), binds to its client proteins. Relevant to inflammation, HSP90 participates at multiple levels of STAT and NF-kB signaling. STAT3 directly colocalizes with HSP90, implying HSP90's role in inflammation [28]. HSP90 guards the Inflammasome NLRP3 in

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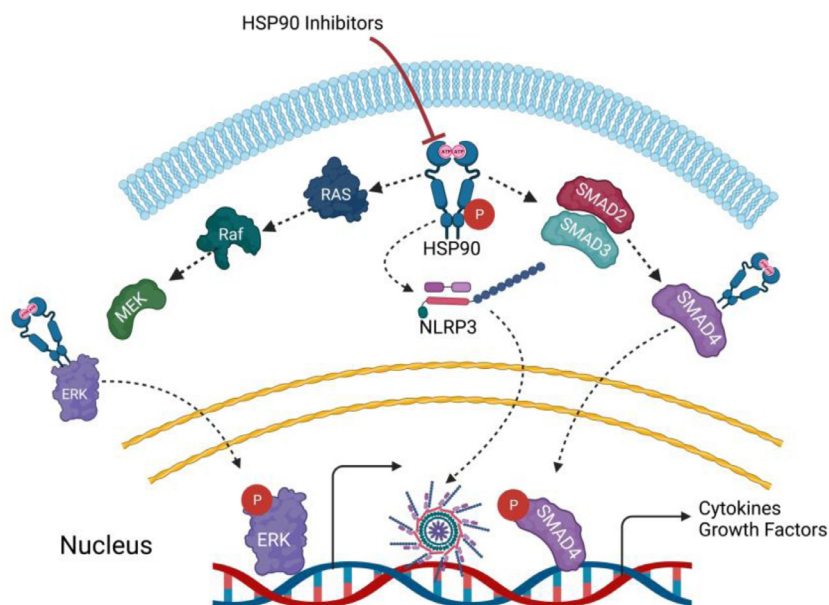
Fig. 6. HSP90 involvement in endothelial cell signaling. Red bars indicate inhibition and dotted black lines indicate activation by upstream pathways. The transcriptional activation by ERK, SMAD4 and NLRP3 cause increase production and extracellular release of cytokines and growth factors. The figure was designed with the assistance of BioRender.com.

the cytosol, and in response to stress is released, facilitating NLRP3 assembly and activation [29]. HSP90 interacts with NF-kB at multiple levels: it regulates NF-kB Inducing Kinase [30] and together with HSP70 modulates the IKK complex, thus altering NF-kB activation [31]. Additionally, two new mechanisms of HSP90/NF-kB interaction have been demonstrated: the first, mediated by HSP90 binding to type-2 Sirtuin histone deacetylase [32]; and the second mediated by HSP90 binding to the coactivator cAMP response element binding protein, required for RNA transcription [33]. Thus, HSP90 inhibitors exert anti-inflammatory effects and have shown encouraging results in preventing and repairing endothelial barrier dysfunction [34,35], lung fibrosis [36–41] and SARS-CoV-2 induced inflammation [41–43].

The second example, already mentioned in this Perspective, is the work of the Lazo group in developing novel phosphatase inhibitors. PTP4A3 is a phosphatase involved in cancer and specifically associated with tumor metastases (Fig. 2) [12,44,45] . PTP4A3 removes phosphorylated residues from target molecules and it is involved in the regulation of different cellular pathways. PTP4A3 promotes cell differentiation, epithelial-to-mesenchymal transformation, adhesion and vascular rearrangements [46]. However, PTP4A3 is also a crucial inflammatory mediator; it can be upregulated by STAT3, and reversely plays a role in STAT3 activation by promoting its re-phosphorylation [47]. Importantly, PTP4A3 modulates VEGF-induced vascular injury, and it is highly expressed in endothelial cells [10]. PTP4A3 dephosphorylates ERK1/2 and p-38 of the MAPK family, crucial mediators of lung dysfunction [48–50]. Similarly, PTP4A3 exerts a direct action on the PI3K/AKT/mTOR pathway [34,51], another well-known pathological pathway observed in ALI [52,53]. Cell lines in which the *Ptp4a3* gene has been deleted, display significant overexpression of crucial extracellular matrix proteins and adhesion molecules, such as Emilin-1 and ITGβ2 (Integrin Subunit Beta-2) [10].

Various PTP4A3 inhibitors have been identified (e.g., pentamidine, ginkgetin, and BR-1) but most have poor potency and specificity, structural liabilities and often irreversible binding [45]. JMS-053 is the most potent inhibitor of PTP4A3 with an *in vitro* 50% inhibitory concentration of <80 nM; it is a highly specific, reversible, allosteric and noncompetitive inhibitor [10]. JMS-053 was able to restore LPS-induced hyperpermeability [10], a mechanism likely related to PTP4A3's role in promoting GTPases of the Rho family [44], activated in endothelial dysfunction and hyperpermeability [54]. Currently, Catravas, Lazo and their colleagues are testing the hypothesis that PTP4A3 inhibitors pre-

vent and reverse S1SP-induced human lung microvascular endothelial and alveolar epithelial barrier dysfunction and S1SP-induced murine inflammation, ALI, ARDS, and lethality in mice.

Conclusions

Drug discovery and the investigation of new therapeutic targets are crucial steps in the advancement of modern medicine. Funding initiatives that encourage local collaborative work among research institutions can be enormously productive and can achieve high-quality investigation. They facilitate an ecosystem that encourages students, faculty, academic institutions, and commercial entities to engage in what is an inherently interdisciplinary undertaking. Other organizations in the Commonwealth of Virginia, such as the Virginia Innovation Partnership Corporation, CvilleBioHub, Virginia Beach Department of Economic Development, Virginia Venture Partners, and VABIO, also have also helped catalyze this dynamic ecosystem.

The repurposing of PTP4A3 and HSP90 inhibitors for the treatment of SARS-CoV-2, created under this initiative, is only one example of how the VaDDC has provided a significant platform that fosters collaboration among Institutions in the Commonwealth of Virginia. There have been many other unexpected productive interactions in the drug discovery space that have emerged in the past seven years among institutions, such as Virginia Tech, Virginia Commonwealth University, George Mason University, James Madison University, William and Mary University, Christopher Newport University, and Eastern Virginia Medical School. These interactions further validate the importance of encouraging local drug discovery consortia.

Declaration of Competing Interest

John S. Lazo is a co-inventor of intellectual property concerning phosphatase inhibitors that has been assigned to the University of Virginia and the University of Pittsburgh. John S. Lazo is co-founder of KeViRx, Inc., which has licensed the patented JMS-053 technology for further development. John S. Lazo is the Chief Scientific Officer of KeViRx, Inc. and has equity in the company.

CRediT authorship contribution statement

John S. Lazo: Conceptualization, Writing – review & editing. **Ruben M.L. Colunga-Biancatelli:** Conceptualization, Writing – review

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