Perspectives and potential approaches for targeting neuropilin 1 in SARS-CoV-2 infection

Svetlana P. Chapoval and Achsah D. Keegan

Abstract
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel type b coronavirus responsible for the COVID-19 pandemic. With over 224 million confirmed infections with this virus and more than 4.6 million people dead because of it, it is critically important to define the immunological processes occurring in the human response to this virus and pathogenetic mechanisms of its deadly manifestation. This perspective focuses on the contribution of the recently discovered interaction of SARS-CoV-2 Spike protein with neuropilin 1 (NRP1) receptor, NRP1 as a virus entry receptor for SARS-CoV-2, its role in different physiologic and pathologic conditions, and the potential to target the Spike–NRP1 interaction to combat virus infectivity and severe disease manifestations.

Keywords: ACE2, angiotensin converting enzyme 2, NRP-1, neuropilin-1, Molecular structures, SARS-CoV-2, severe acute respiratory syndrome coronavirus 2, Spike protein, COVID-19, coronavirus disease 2019, Host immune response, Immunotargets and strategies, Comorbidities, CendR C-end rule, RBD, receptor-biding domain

Background
SARS-CoV-2 infected over 224 million people worldwide leading to more than 4.6 million deaths. Currently, there is no FDA-approved treatment for the SARS-CoV-2 viral disease. Therefore, there is an urgent need for anti-viral therapeutics to treat this and future SARS infections. It is now well-established that SARS-CoV-2 entry into cells is initiated by its Spike protein priming by the transmembrane protease serine 2 (TMPRSS2) and binding to its main receptor in human tissues, the angiotensin-converting enzyme 2 (ACE-2) (Hoffmann et al. 2020b; Wang et al. 2020b; Yan et al. 2020). However, recent data suggest that there are additional mediators of viral entry and cell infectivity (Cantuti-Castelvetri et al. 2020; Daly et al. 2020; Radzikowska et al. 2020; Root-Bernstein 2021).

One such mediator is neuropilin 1 (NRP1) as reported in several recent preclinical and clinical studies (Cantuti-Castelvetri et al. 2020; Daly et al. 2020; Davies et al. 2020; McFarland et al. 2021; Moutal et al. 2021). In this review, we explored the details of NRP1 expression and function in health and diseases and perspectives of its targeting in order to prevent or abrogate a severe SARS-CoV-2 infection.

SARS-CoV-2 structure
Several recent publications provide detailed descriptions of SARS-CoV-2 structure and its unique features distinguishing it from SARS-CoV or MERS-CoV (Chen et al. 2020; Chuckran et al. 2020; Hoffmann et al. 2020a, b; Finkelstein et al. 2021; Kadam et al. 2021; Letko et al. 2020; Papageorgiou and Mohsin 2020; Sternberg and Naujokat 2020; Walls et al. 2020; Wang et al. 2020a; Witkowska 2020). SARS-CoV-2 is a single-stranded positive sense RNA virus. Its membrane is composed of several structural proteins such as membrane (M), spike (S),
and envelope (E) proteins. Viral RNA bound to helical nucleocapsid phosphoproteins (N) is positioned inside the virion. The spike protein of SARS-CoV-2 is an attractive antiviral target and main component (in form of mRNA or DNA) of all FDA- and EU CHFS-approved anti-COVID-19 vaccines (Chen et al. 2020; Letko et al. 2020; Wang et al. 2020a). Spike is a transmembrane homotrimeric glycoprotein of ~180 kDa that belongs to the class I of trimeric fusion proteins and consists of two subunits, S1 and S2 (Wang et al. 2020a). The S protein is cleaved by the transmembrane protease serine 2 (TMPRSS2) which is preferentially expressed on epithelial cells in the airways such as alveolar epithelial type II cells (ATII cells) (reviewed in Sternberg and Naujokat 2020; Hoffmann et al. 2020b). The TMPRSS2-mediated S protein cleavage and priming are required for its binding to ACE2 receptor, membrane fusion, and cell entry (Sternberg and Naujokat 2020). The S1 subunit of S protein contains a receptor-binding domain (RBD). The S2 subunit contains several domains including a fusion peptide for fusion of virus with a host cell membrane. The S1/S2 boundary includes the cleavage site for the subtilisin-like host cell protease furin which is expressed in all human tissues (Hoffmann et al. 2020a; Sternberg and Naujokat 2020; Walls et al. 2020; Wrapp et al. 2020). The furin cleavage site of S protein is believed to contribute to the high virulence and tissue tropism of SARS-CoV-2 in humans because of ubiquitous expression of furin and furin-like proteases (Hoffmann et al. 2020a; Walls et al. 2020; Wrapp et al. 2020). Moreover, the envelope proteins of several other viruses such as HIV, influenza, dengue fever, Ebola virus, and Marburg virus utilize furin or furin-like proteases for their cleavage and virus activation (Shiryaev et al. 2007).

ACE2 structure and function
Angiotensin-converting enzyme 2 (ACE2) is the main cell surface receptor for SARS-CoV-2 (Hoffmann et al. 2020b; Wang et al. 2020b; Yan et al. 2020). ACE2 functions as a physiological counter-balance molecule of ACE. It is a type I integral membrane carboxypeptidase which structure has been solved (Turner 2015; Wan et al. 2020). ACE cleaves vasodilating angiotensin 1 into angiotensin 2 which displays a vasoconstricting function (Hamming et al. 2004; Turner 2015). In contrast, ACE2 causes vasodilation by cleaving and hydrolyzing angiotensin 2 into angiotensin decapeptide 1–7 which signals through the G-protein coupled MasR to induce Akt phosphorylation and NOS production (Bader 2013). Structural biology techniques such as Cryo-Electron Microscopy (Cryo-EM) and X-ray crystallography techniques detail the Spike protein interactions with ACE2 guiding the ongoing therapeutic and vaccination efforts (reviewed in Papageorgiou and Mohsin 2020; Chekol Abebe et al. 2021). Rather low to absent ACE2 expression was detected in all compartments of human respiratory tract. The analysis of three distinct scRNAseq datasets demonstrated low ACE2 expression in ATII cells, bronchial, goblet, and ciliated cells within the airways (Hikmet et al. 2020). Other recent studies reported high ACE2 and transmembrane serine protease 2 (TMPRSS2) expression in nasal epithelium and in lung parenchyma (Sungnak et al. 2020; Yang et al. 2020). Interestingly, analysis of the ACE2 and TMPRSS2 expression pattern in individual cells in the lung and in subsegmental bronchial branches by scRNAseq demonstrated a strong TMPRSS2 expression in both tissues whereas ACE2 was predominantly detected in a transient secretory cell type (Lukassen et al. 2020). The transcriptome analysis also showed that ATII cells co-expressed ACE2 with TMPRSS2. No sex-, age-, or gender-related differences were observed in ACE2 expression in individual cell types in lung cells or in the subsegmental bronchial lung tissue. However, the cell surface receptor expression was not examined in this study. These single cell transcriptome data led to several explanations for the observed relatively high human-to-human transmission of SARS-CoV-2 when compared to SARS-CoV or MERS-CoV, namely: (a) the binding of SARS-CoV-2 to another, yet unknown receptor on the host cell surface, (b) enhanced cleavage of the SARS-CoV-2 S protein resulting in higher efficiency of the virus’ entry into the cell, and (c) additional host factors increasing the virus entry into the cell by facilitating membrane fusion. All these can explain why SARS-CoV-2 readily infected tissues with relatively low or absent ACE2 expression, such as the respiratory tract and nervous system, pointing to other potentially important factors/receptors for virus entry (reviewed in Chekol Abebe et al. 2021).

NRP1 structure and function
Two recent reports in Science demonstrated that the SARS-CoV-2 Spike protein can also bind to the b1b2 domain of NRP1 (Cantuti-Castelvetri et al. 2020; Daly et al. 2020). Unlike SARS-CoV, SARS-CoV-2 contains a polybasic amino acid sequence (682RRAR685) which serves as furin cleavage site which, when cleaved, directly binds NRP1 (Fig. 1) significantly potentiating viral entry and increasing the in vitro cell infectivity with WT virus by 40–70% depending on the cell type used in the experiments as shown by blocking of NRP1 with Ab or shRNA (Cantuti-Castelvetri et al. 2020; Daly et al. 2020). NRP1 is a single-pass transmembrane glycoprotein, its extracellular portion is involved in semaphorin 3A (Sema3A) and VEGF165 binding (Kolodkin et al. 1997; Chapoval et al. 2009; Wild et al. 2012) whereas the c-domain and
transmembrane portion are involved in receptor dimerization and heteromerization (Wild et al. 2012) (Fig. 1). NRPI was originally identified in the nervous system. Recent studies have shown its expression in dendritic cells, macrophages (alveolar, bronchial, and intravascular; tumor-associated macrophages), T cell subpopulations (CD8+ T cells, Treg cells, Th cells, and NKT cells), and mast cells (Bruder et al. 2004; Chekol Abebe et al. 2021; Marone et al. 2016; Roy et al. 2017; Tordjman et al. 2002) demonstrating the important role of NRPI in the regulation of immune response and in respiratory diseases. NRPI was also found to be expressed on osteoblasts, renal glomerular mesangial cells, glomerular epithelial cells, neuroendocrine cells of the gastrointestinal tract, adipocytes, kidney’s podocytes, olfactory epithelium, and olfactory neurons (reviewed in Chekol Abebe et al. 2021; Roy et al. 2017; Ellis 2006). Mouse NRPI is a critical receptor for Sema4A and for Treg cells to regulate their stability and function (Delgoffe et al. 2013). In humans, Plexin B1 plays this Sema4A-dependent potentiating function for Treg cells which lack NRPI expression (Chapoval et al. 2019). NRPI is also expressed on CD4low PBMC-derived human monocytes (Chapoval et al. 2019). Therefore, these monocytes might potentially get infected with SARS-CoV-2 virus although the ACE-2 expression on these cells has not been assessed. Interestingly, NRPI expression was previously detected on lung tissue macrophages by IHC (Aung et al. 2016). These macrophages are believed to be a major source of cytokine overexpression in the “cytokine storm” phenomenon observed in severe SARS-CoV-2 infection (Merad and Martin 2020).

Peptides with a C-terminal basic sequence motif (C-end rule or CendR motif) bind to NRPI and are taken into cells by endocytosis (Teesalu et al. 2009). The binding of the S1 CendR motif generated by the furin cleavage of Spike protein to NRPI did not affect cell surface attachment but promoted cell entry and infection by SARS-CoV-2 (Daly et al. 2020). As NRPIIs known to mediate the internalization of CendR ligands through an endocytic process resembling micropinocytosis (Teesalu et al. 2009), it is possible that S1 interaction with NRPI alone, even in the absence of ACE2, may induce a complex internalization presumed by the receptor-mediated CendR endocytosis. This pathway was previously demonstrated in vitro using the NRPI plasmid-transfected HeLa cells (Pang et al. 2014). However, the intracellular consequences of such internalization for human primary cells have not been explored.

Host immune response to SARS-CoV-2

Host immune response plays a critical role in protection and fight against SARS-CoV-2. SARS-CoV-2, primarily distributed by air droplets, infects the host’s respiratory system. Multiple recent studies have shown that patients infected with SARS-CoV-2 demonstrated pathologic changes in multiple tissues and organs including the gastrointestinal and pancreaticobiliary systems, kidney, heart, and central nervous system (Cheng et al. 2020; Inamdar et al. 2020; Mao et al. 2020; Huang et al. 2021; Karas et al. 2021; Raman et al. 2021; Song et al. 2021a, b). It has been reported that many patients with severe disease have an exaggerated immune response to virus with elevated levels of proinflammatory cytokines IL-1, IL-6, IL-12, and increased IFNy, IFNy-inducible protein 10, IL-8 (neutrophil chemoattractant), and MCP-1 (monocyte chemoattractant protein 1) (Crestani et al. 1994; Wong et al. 2004a; Zhang et al. 2004). Within the lung tissue, ATII cells could serve as a source of those cytokines and chemokines as they were reportedly to make IL-6 in vitro and in vivo and participate in intra-alveolar cytokine networks secreting IL-8, IFN, MCP-1, TGFβ, and GM-CSF (Fig. 2) (Crestani et al. 1994; Lin et al. 1998; Yan et al. 2018). Therefore, ATII cells may contribute to a
local inflammatory response in viral infection by producing chemokines which attract inflammatory cell sequestration into the sites of infection and cytokines which activate those immigrant and local cells (Fig. 2). One recent study in SARS-CoV-2 patients have shown that in addition to the cytokines and chemokines listed above, TNFα, IL-2, and IL-7 were significantly elevated in a subset of patients with a fulminant and fatal hypercytokinemia (Mehta et al. 2020).

**Neurologic consequences of SARS-CoV-2 infection and role of NRP1**

SARS-CoV-2 directly and indirectly interacts with the peripheral nervous system and causes pain (McFarland et al. 2021). SARS-CoV-2 induces a complex neuroviroence in humans, a consequence of virus interaction with dorsal root ganglia and trigeminal ganglia. There are distinct neurological effects of SARS-CoV-2 infection on the early and late stages of disease. The human body respond to SARS-CoV-2 by the production and release of type I interferons (McFarland et al. 2021). These cytokines directly interact with corresponding receptor complexes expressed on nociceptors and thus promote pain. This mechanism of SARS-CoV-2 action explains headaches and body aches associated with the disease. However, some patients with severe COVID-19 infection lack type I interferon involvement due to selected SARS-CoV-2 proteins with antagonistic action against IFN-signaling pathways. Another subset of patients which also lack type I IFN involvement because of internal secretion of auto-Abs against type I INFs was particularly susceptible to severe disease (Bastard et al. 2020; McFarland et al. 2021; Lopez et al. 2021; Zhang et al. 2020). Additional cytokine-related mechanism of body aches and pain in SARS-CoV-2 infection is directly linked to ACE2 expression on human skeletal muscles which leads to muscle tissue damage during infection, tissue inflammation, and elevated levels of IL-6 which is known for its nociceptive effects. The inflammatory neuronal demyelination resembling Guillain-Barre syndrome was observed in a subset of patients with severe infection could lead to chronic muscle weakness and fatigue (Finsterer et al. 2021).
Dorsal root ganglia are reported to express ACE2, furin, and NRP1, the complete receptor machinery for SARS-CoV-2 infection. Interestingly, SARS-CoV-2 interaction with NRP1 through its Spike protein binding on nociceptors causes the pain-lowering effects in contrast to its pain-promoting interaction with ACE-2 (Finsterer et al. 2021). This NRP1-linked phenomenon might explain the increased disease transmission in asymptomatic infected individuals (Finsterer et al. 2021; Karuppan et al. 2021). Studies in human brain organoids have clearly demonstrated the SARS-CoV-2 neurotropism but these studies focused on ACE-2 expression and function and omitted the significance of NRP1 (Jacob et al. 2020; Ramani et al. 2021).

Viral infections cause damage to the nervous system with clinical features varying from encephalitis to meningitis with various degrees of severity (Big et al. 2009; Karuppan et al. Karuppan et al., 2021; Singh et al. 2021). SARS-CoV-2 invasion of central nervous systems caused many pathologic effects ranging from reversible brain dysfunction syndrome with headache, dysphoria, mental disorders, and delirium (Mao et al. 2020) to more severe pathologies such as edema of the brain tissue and partial neuronal degeneration (Dixon et al. 2020). The contributions of ACE2 and co-receptor NRP1 to these processes were the subjects of several recent studies (Davies et al. 2020; Karuppan et al. 2021; Khan and Gomes 2020). Whereas ACE-2 expression is rather low in human lung tissue and olfactory epithelium, NRP1 was found to be highly expressed in the lungs and olfactory tubercles and paraolfactory gyri of the human brain (Davies et al. 2020; Karuppan et al. 2021). The detailed examination of a precise NRP1 expression in human brain pointed to a hippocampal formation as the highest NRP1 expressor whereas on the cellular level its expression was detected in endothelial cells, mural cells, neuron clusters, perivascular macrophages and microglia (Davies et al. 2020). The SARS-CoV-2 interaction with NRP1 could explain the observed virus entry into olfactory epithelial cells and loss of olfactory function, the ability to detect odorous molecules (sense of smell) in COVID-19 disease. The reduced pain perception in infected asymptomatic patients could be, in part, related to the effect of Spike protein blocking nociceptive VEGF-A/NRP1 interaction (Moutal et al. 2021). SARS-CoV-2 genome was detected in cerebrospinal fluids or infected people and selected patients showed the presence of viral encephalitis (Karuppan et al. 2021). The documented brain dysfunction problems in SARS-CoV-2 infection suggested that the virus can cause infectious toxic or even acute necrotizing encephalopathy. In some patients the virus can lead to the formation of newly detected demyelinating lesions in the brain. All these reports together with known critical function of NRP1 in brain development including the neuronal axon guidance, target recognition, and synaptogenesis (Telley et al. 2016) point to an important role of NRP1 as the Spike protein receptor in the development of serious and prolonged neurological aspects of SARS-CoV-2 infection.

**ACE2 and NRP1 targeting strategies to combat SARS-CoV-2 infection**

Several therapeutic strategies for SARS-CoV-2 infection have been proposed, most of them targeting the SARS-CoV-2 Spike–ACE2 interaction (Chan et al. 2020; Kruse 2020; Montail et al. 2020; Wong et al. 2004b). They include: (1) small SARS-CoV-2 Spike protein molecule of 193 aa long containing ACE2-binding domain (RBD) which effectively blocked the virus entry in cell cultures; (2) anti-AE2 blocking Ab; and (3) soluble ACE2-Fc fusion protein (Lei et al. 2020) to bind and neutralize S protein (Table 1). The recently published review by Xiaojie et al. (2020) details the Spike protein targeting drugs based on ACE2 sequences. These drugs include: (1) recombinant soluble ACE2 ectodomain, and (2) its selective mutants which either bind Spike with a higher affinity or express a low catalytic activity which preserve ACE2 function in physiological processes (Table 1). The article by Xiaojie et al. (2020) also summarized the recent development and major structural characteristics of SARS-CoV-2 neutralizing mAbs isolated from convalescent patients, immunized animals, and phage-displayed human antibody libraries. Of note, all these mAbs currently in several clinical studies at different biotech companies were found to target S1 protein and none of them target S2 protein.

Three NRP1 targeting strategies were used to define its critical role in SARS-CoV-2 infection in vitro, namely: (1) anti-NRP1 neutralizing Ab (Cantuti-Castelvetri et al. 2020; Daly et al. 2020); (2) the small molecule EG00229 acting as a selective NRP1 antagonist (Daly et al. 2020), and (3) soluble extracellular b1b2 domains of NRP1 (Cantuti-Castelvetri et al. 2020) (Table 1). Incubation of Caco-2 cells with anti-NRP1 mAbs raised against the b1b2 ectodomain of NRP1 significantly (by ~40%) reduced SARS-CoV-2 infection as compared to a control mAb targeting avian influenza A virus (H11N3) hemagglutinin (Cantuti-Castelvetri et al. 2020; Daly et al. 2020). Other targeting approach of SARS-CoV-2 entry into cells was based on b1/b2 module of NRP1 as a binding site for VEGF165 (Perez-Miller et al. 2020). A library of 5 × 10^5 compounds was screened for specifically targeting this site; nine chemical series were reported with lead- or drug-like physical and chemical properties, out of which six compounds effectively disrupted VEGF-A–NRP-1 interaction, and all nine inhibited VEGF-A
triggered VEGFR2 phosphorylation. These series of lead compounds represent a first step in development of small molecule inhibitors for the SARS-CoV-2 S–NRP1 interaction (Fig. 3) and for certain types of cancer where NRP1 plays a disease-promoting role.

NRP1 in immune response and its targeting in SARS-CoV-2 infection in adults without comorbidities

The important roles of NRP1 in the immune response have been discussed in several published reviews (Chuckran et al. 2020; Roy et al. 2017; Ellis 2006; Chaudhary et al. 2014; Li et al. 2016). All NRP1-expressing cells may be potential targets for SARS-CoV-2 because Spike protein through its CendR motif that is exposed following furin processing interacts with NRP1 that can lead to a complex internalization through previously described endocytosis (Daly et al. 2020; Teesalu et al. 2009; Jobe and Vijayan 2021). Of note, recent reports clearly showed that without ACE2, NRP1 alone is not able to support efficient virus infection, not even in the immunocompromised HEK 293T cell system (Cantuti-Castelvetri et al. 2020; Daly et al. 2020). Moreover, NRP1 depletion did not affect virus binding to the cell surface but specifically affected the virus uptake (Daly et al. 2020). It is still debated whether SARS-CoV-2 might infect NRP1-expressing DC. However,
several previous studies have shown that MERS-CoV and SARS-CoV use DC-SIGN as an alternative receptor for DC infection or as a receptor which facilitates virus infectivity through ACE2 (Yang et al. 2004; Marzì et al. 2004; Campana et al. 2020). DC are unmatched in their ability to prime naïve CD4+ T cells and initiate and propagate the immune response to given antigen. Among immune cells, NR1P1 expression was first identified on human plasmacytoid dendritic cells (pDCs) (Dzionek et al. 2002). Recent findings detect a significant drop in circulating pDC number in SARS-CoV-2 infected patients (Zhou et al. 2020). The mechanisms of such SARS-CoV-2 effect on pDC were not defined. pDCs are known for their type I IFN secretion in response to viral infection. Human pDCs showed potent IFNα release in response to Respiratory Syncytial Virus (RSV) which was inhibited by anti-NR1P1 Ab pretreatment of cells (Grage-Grihenow et al. 2007). In addition to its expression on pDCs, NR1P1 was also detected on conventional DCs isolated from human peripheral blood, where it potentially promotes T cell priming by mediating the formation of immunological synapse between cells (Tordjman et al. 2002). NR1P1 is expressed on immature DC (iDCs). iDCs do not express costimulatory molecules, are not activated, and can not stimulated T effector cells. They induce T cell anergy and T cell deletion instead. However, they can stimulate Treg cells to secrete immunosuppressive cytokines such as IL-10 and TGF-β (Mahnke et al. 2002). NR1P1 was defined as a ‘glue’ between Tregs and DCs (Mizui and Kikutani 2008; Song et al. 2020). Thus, NR1P1 is a critical molecule for immune response to foreign antigen. At the same time, SARS-CoV-2 virus could use NR1P1 for DC invasion. It is unclear if DC co-express ACE2 or upregulate ACE2 expression after Spike binding to NR1P1. One current study reported little to no expression of ACE2 on immune cells in human peripheral blood including B cells, NK cells, monocytes, dendritic cells, granulocytes, and all subtypes of T cells (Song et al. 2020). However, ACE2 expression was induced or upregulated on CD3+ T cells obtained from infected patients while CD20+ B cells and the CD16+/HLA-DR+ monocytic/dendritic cells did not change a relatively low ACE2 level as compared to same cell populations obtained from healthy volunteers (Osman et al. 2020).

One published study reported the absence of any significant effect of anti-NR1P1 Ab raised to VEGF-binding site of NR1P1 on HUVEC cell proliferation and permeability (Pan et al. 2007). This Ab also did not affect VEGF-induced phosphorylation of Erk1/2 or Akt in endothelial cells but significantly inhibited VEGF-induced cell migration. There is one published report on the phase I study of anti-NR1P1 mAb in patients with advanced solid tumors where this was a well-tolerated approach with promising results (Weekes et al. 2014). Jung et al. (2020) used the NR1P1 antagonist [Fc(AAG)-TPP11] generated by fusion of the NR1P1-specific binding peptide TPP11 with the C-terminus of an effector function-deficient immunoglobulin Fc(AAG) variant to inhibits intratumoral NR1P1+ Treg cell function and stability. The intraperitoneal injections of Fc(AAG)-TPP11 into mice with established tumors demonstrated a potent anti-tumoral effect and inhibited tumor growth by >70%. The NR1P1 antagonist effect was comparable to that observed with the use of Treg-depleting anti-CTLA-4 Ab. Other NR1P1-directed drugs or biologics have never been examined in vivo.

**Perspectives of targeting NR1P1 in SARS-CoV-2 infection in pregnant women and infants**
NR1P1 is required for normal embryonic vascular, cardiac, and limb development (Kitsukawa et al. 1995; Kawasaki et al. 1999). It was reported to be expressed in a temporary restricted manner in the mouse embryo suggesting that its ectopic or excess expression could cause
abnormalities (Kitsukawa et al. 1995). Indeed, chimeric embryos with NRPI overexpression showed abnormal body vascularization and severe pathologies to the cardiovascular and nervous tissues. Such embryos demonstrated lethality at maximum 17.5 pdc (post-conception days of embryonic age) as the result of profound abnormalities to both systems. Therefore, the exogenous NRPI overexpression causes embryonic death. Such effect was attributed to the unique NRPI structure which has three domains all potentially involved in different molecular interactions. The observation that different systems are affected by NRPI overexpression also suggests that NRPI is a multifunctional molecule. The authors concluded that the correct spatiotemporal patterns of NRPI expression were essential for the growth and fasciculation of nerve fibers, formation of the cardiovascular system, and for the limb’s formation. Interestingly, a targeting disruption of nrp1 gene was also reported to be embryonic lethal and resulted in multiple defects to the cardiovascular system (Kawasaki et al. 1999). Therefore, NRPI targeting with Abs or small molecule inhibitors may lead to adverse events in SARS-CoV-2 infection in pregnant women and infants (Fig. 3). However, the drugs based on NRPI sequence/structure which selectively bind and neutralize SARS-CoV-2 spike protein could be potentially very useful in these groups.

**Targeting strategies for NRPI: cancer models**

A number of NRPI-targeting strategies have been developed to treat cancer. The overexpression of NRPI is reported in several types of cancers such as colon (Parikh et al. 2002), stomach (Mei et al. 2020), lung (Kawakami et al. 2002; Lantuejoul et al. 2003), osteosarcoma (Handa et al. 2000), breast (Stephenson et al. 2002), astrocytoma (Broholm and Laursen 2004), glioma (Hu et al. 2007), melanoma (Graziani and Lacal 2015; Bao et al. 2021), and is considered to be a negative prognostic marker for the disease outcome (reviewed in Ellis 2006; Rizzolio and Tamagnone 2011; Wild et al. 2012). Elevated NRPI expression was detected in malignant cells and in tumor microenvironment (TME) on macrophages, Treg cells, CD8+ T cells, and dendritic cells (DCs) (reviewed in Chuckran et al. 2020; Moutal et al. 2021; De Vlaemnick et al. 2020; Liu et al. 2020). All these together established a strong association between NRPI and cancer. Several NRPI-directed therapies such as anti-NRPI mAb, anti-NRPI nanobodies, and NRPI antagonist Fc(AAG)-TPP11 demonstrated significant retardation of established tumors without noticeable toxicity (Wild et al. 2012; Weekes et al. 2014; De Vlaemnick et al. 2020; Jung et al. 2020). These studies demonstrate that NRPI targeting can be highly effective with minimal adverse effects. It could also be considered as an approach in managing and treating the severe SARS-CoV-2 infection in cancer patients (Fig. 3).

**Targeting NRPI in SARS-CoV-2 infection in patients with pulmonary diseases**

Two research groups have described abundant expression of NRPI in alveolar epithelium (Ito et al. 2000; Roche et al. 2002). However, the functional significance of such expression is unknown. NRPI expression on epithelial cells may modulate the balance between VEGF and Sema3 signaling in nonepithelial cells (Bagnard et al. 2001; Castro-Rivera et al. 2004). Local lung NRPI levels gradually increase in the process of lung organogenesis (Roche et al. 2002). NRPI and its ligands Sema3A and VEGF contribute to the process of alveolar septation aimed to increase blood-gas interchange to supply oxygen to developing organism (Ito et al. 2000; Gerber et al. 1999; Jakkula et al. 2000; McGrath-Morrow et al. 2005; Kunig et al. 2005). Sema3A inhibits branching morphogenesis in lung bud organ cultures acting via NRPI (Roche et al. 2002), while Sema3C and Sema3F promote lung branching morphogenesis using both NRPI and NRPI2 (Kagoshima and Ito 2001). The NRPI expression was downregulated in smokers with diagnosed cigarette smoking-induced COPD which displayed defective lung function when compared with smokers with normal lung function and nonsmoking control subjects (Marwick et al. 2006). Therefore, NRPI plays a protective role in COPD pathobiology and direct targeting of NRPI in patients with COPD may not be beneficial in case of SARS-CoV-2 infection. Studies in murine models have demonstrated a critical role of NRPI in proper structural maintenance of lung alveoli (Le et al. 2009). There were more disruptions to alveolar structure in chronic cigarette smoke exposure in mice with conditional epithelial Nrpi deletion compared to similarly treated WT mice. Interestingly, bronchoalveolar lavage cell composition did not differ significantly between two experimental mouse groups.

The expression of NRP and its ligands in lung cancer is widely reported. Overexpression of both, NRPI and NRPI2 was reported in non-small cell lung carcinoma and it significantly correlated with tumor progression (Kawakami et al. 2002). High levels of NRPI expression correlated with shorter disease-free and overall survival, and combined overexpression of NRPI and NRPI2 was associated with a worse prognosis than when either NRP was singly overexpressed (Kawakami et al. 2002). A progressive upregulation of NRP levels was observed in different types of lung cancers including squamous cell carcinoma, small cell lung carcinoma, adenocarcinoma, large cell neuroendocrine carcinoma, basaloïd carcinoma, and typical and
atypical carcinoids (Lantuejoul et al. 2003). Expression progresses starting from benign bronchial hyperplasia to dysplasia and then to invasive carcinoma and was correlated with increases in VEGF expression (Lantuejoul et al. 2003). VEGF$_{165}$, one of NRP1 ligands, plays a critical role in allergic asthma (Lee et al. 2004; Bhandari et al. 2006; Chapoval et al. 2009). Expression of both, NRP1 and its another ligand, Sema3A, was found to be upregulated in sputum of asthmatic patients and in BAL and lung homogenates of mice with OVA-induced experimental asthma (Shim et al. 2013) suggesting NRP1 and its ligands play a pathologic role in allergic asthma (Fig. 3). It is possible that targeting of NRP1 to combat SARS-CoV-2 infection in lung cancer and asthma patients will reduce or prevent the severe form of infection.

**Targeting NRP1 in SARS-CoV-2 infection in patients with diabetes**

The rate of SARS-CoV-2 infection in people with diabetes is similar to that of general population but post-infection complications are different and much more severe (Apicella et al. 2020; Feldman et al. 2020; Zhou et al. 2021). Moreover, the prevalence of diabetes was 34.6% in patients with severe COVID-19 (Apicella et al. 2020). One recent report shows that transmembrane protein endothelial and smooth muscle cell-derived neuropilin-like protein (ESDN) serves as an inhibitor of insulin receptor signal transduction in the liver, muscle, and adipose tissue (Li et al. 2016). However, the mechanisms of ESDN action in angiogenesis are distinct from those defined for NRP1 (Nie et al. 2013). A recent study by Wu et al. (2021) found that b-cells in human pancreas express ACE2, TMPRSS2, and selectively high NRP1. SARS-CoV-2 infection in patients affected pancreatic insulin levels, its secretion, and induces b-cell apoptosis. All these events were rescued by NRP1 inhibition. Therefore, b-cells display the necessary molecular machinery for efficient SARS-CoV-2 infection and high NRP1 expression may, in part, explain the SARS-CoV-2 tropism for b-cells. Indeed, a comprehensive analysis of human pancreatic tissue employing the immunofluorescence, immunohistochemistry, RNA scope and electron microscopy demonstrated that 70% of ACE2 expression was found on pancreas vasculature, whereas b-cell are ACE2+ only in 30% (Steenblock et al. 2021). The high NRP1 expression on b-cells suggested that the virus uptake could be facilitated by NRP1 when ACE2 expression is low. Therefore, it is tempting to speculate that specific targeting of NRP1 in severe SARS-CoV-2 infection may be a potentially useful strategy in patients with diabetes (Fig. 3).

**Other receptors involved in SARS-CoV-2 infection**

Other receptors can facilitate SARS-CoV-2 entry into cells. One such receptor is CD147 or basigin, a transmembrane glycoprotein of the immunoglobulin superfamily which has been shown to increase infectivity of both SARS-CoV and SARS-CoV-2 viruses (Chen et al. 2005; Wang et al. 2020c). The study by Wang et al. (2020c) demonstrated a co-localization of CD147, Spike protein, and Rab5 in BHK-21 cells induced to express CD147 and in lung tissues from patient with SARS-CoV-2 infection. This observation let the authors to conclude that Spike protein binding to CD147 induced a CD147-mediated endocytosis of virus. The results of this study also showed the infection of lung T cells obtained from COVID-19 patients which was mediated by CD147 but not by ACE2 as T cells lack ACE2 expression. The cell infectivity was inhibited by a humanized IgG2 antibody targeting CD147, meplazumab, in a dose-dependent manner. Moreover, the constructed hCD147 transgenic mice, unlike WT littermates, were susceptible to SARS-CoV-2 infection and developed virus-induced pneumonia what further establishes CD147 as an alternative receptor for SARS-CoV-2 entry in an ACE2-deficient environment. However, a recent report by Shiels et al. (2021) showed no evidence for a direct interaction of the SARS-CoV-2 spike protein with basigin. HEK293 cells transfected with basigin full-length cDNA overexpression plasmid were not able to bind a full-length Spike or its S1 domain. Moreover, CRISPR-Cas9-mediated CD147 knockdown in a lung cell line had no effect on cell’s susceptibility to SARS-CoV-2 infection whereas knockdown of ACE2 completely blocked viral infection in CaLu-3 cells. Based on these new data, the authors suggested to re-consider all hypotheses relying on Spike protein binding CD147 to explain viral tropism but rather focus on indirect CD147 involvement in SARS-CoV-2-induced disease.

Several recent studies established apilimod, a known pharmacologic blocker of PIKfyve (a 240 kDa class III lipid PI-3P-5-kinase present on the cytosolic face of endosomal membranes) as a potent anti-SARS-CoV-2 agent (Baranov et al. 2020; Kang et al. 2020; Kreutzberger et al. 2021; Riva et al. 2020). Apilimod inhibits the viral capsid fusion with the host cell membrane by inhibiting different lysosomal proteases such as TMPRSS2, furin, trypsin, matriptase, and cathepsin B/S/L (Baranov et al. 2020).

To study a potential interaction between Spike protein and distinct TLRs, the 3D structures of Spike and TLR1, TLR2, TLR4, and TLR5 were retrieved from the RCSB Protein Data Bank and used in the in silico molecular docking studies (Choudhury and Mukherjee 2020). Significant binding of SARS-CoV-2 Spike protein to TLR1, TLR4, and TLR6 was detected with...
a respective binding energy value of $-57.3$, $-120.2$, and $-68.4$. Interestingly, the strongest interaction of Spike protein was with TLR4. This finding was further supported by the in vitro studies where a direct Spike-TLR4 interaction was demonstrated employing TLR4-expressing THP1 cells. Spike protein induced IL-1b production in these cells in a dose-, NF-kB-, and MyD88-dependent manner which was inhibited by the TLR4-specific blocker Resatorvid. MD2 and CD14 were also involved in TLR4 activation by Spike as demonstrated by use of specific blockers such as T5342126 and anti-CD14 Ab, respectively.

Therefore, several immune and non-immune receptors can potentially be utilized by SARS-CoV-2 Spike protein to infect different cells. Such Spike-receptor association may play either a protective or a damaging role in virus-induced disease, an area of research that needs to be further investigated and clarified.

**Conclusions**

NRP1 has been shown to participate in the entry of SARS-CoV-2 into cells. Importantly, recent ‘omic’ analyses revealed a significant upregulation of NRP-1 in biological samples from COVID-19 patients compared to healthy controls (Xiong et al. 2020). Moreover, the increased levels of one NRP1 ligand, VEGF$_{165}$, in bronchial alveolar lavage fluid from COVID-19 patients were also reported (Xiong et al. 2020). VEGF$_{165}$ is a physiological ligand for the b1b2 pocket in NRP-1 (Moutal et al. 2021). Spike protein, the major surface antigen of SARS-CoV-2, could block VEGF$_{165}$/NRP-1 signaling. Noteworthy, Sema3A and Sema4A also bind NRP-1 but into a1a2 pocket (Fig. 1). Nevertheless, Sema3A or Sema4A binding may modify the ability of NRP-1 to interact with Spike. The multiple roles of these ligand-receptor interactions in SARS-CoV-2 infection of different cells, tissues, and organs need to be further evaluated. This is an emerging field in research as the roles of NRP1 in different diseases are not well established. Nevertheless, published studies point to NRP1 as potential player in SARS-CoV-2 infection and recently developed approaches for NRP1 targeting provide a foundation for designing novel anti-viral therapies.

**Acknowledgements**

We thank Dr. Matthew B. Frieman from the Department of Microbiology and Immunology, University of Maryland School of Medicine, for his expert review, comments, and suggestions for this article.

**Authors’ contributions**

SPC conceptualized the idea, wrote a first draft, and revised the manuscript according to ADK’s comments, modifications, and suggestions. Both authors read and approved the final manuscript.

**Funding**

S.P.C. is supported by NIH/NIAID RO1 AI076736 and RO1 AI143845 grants (both awarded to A.D.K.). A.D.K. is also supported by the Merit Review Award Number I01 BX001850 from the US Department of Veterans Affairs Biomedical R&D Service.

**Availability of data and materials**

The dataset supporting the conclusions of this article is included within the article.

**Declarations**

**Ethics approval and consent to participate**

Not applicable for this manuscript.

**Consent for publication**

Both authors understand that the text and figures published in the article will be freely available on the internet and may be seen by the general public. The figures and text may also appear on other websites or in print, may be translated into other languages or used for commercial purposes. Both authors contributed to the manuscript. The manuscript is not under consideration elsewhere. The content of the manuscript has not been published. Both authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria.

**Competing interests**

The authors declare no competing interests.

**Author details**

¹Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA. ²Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, 800 West Baltimore Street, Baltimore, MD 21201, USA. ³Program in Oncology at the Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA. ⁴SemaPlex LLC, Ellicott City, MD, USA. ⁵VA Maryland Health Care System, Baltimore VA Medical Center, Baltimore, MD, USA.

**Received:** 20 September 2021  **Accepted:** 13 December 2021

**Published online:** 27 December 2021

**References**

Aicellia M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes Endocrinol. 2020;8(9):782–92. https://doi.org/10.1016/S2213-8587(20)30238-2.

Aung NY, Ohe R, Meng H, Kabasawa T, Yang S, Kato T, Yamakawa M. Specific neuropilins expression in alveolar macrophages among tissue-specific macrophages. PLoS ONE. 2016;11(2): e0147358. https://doi.org/10.1371/journal.pone.0147358.

Bader M. ACE2, angiotensin-1(−7), and Mas: the other side of the coin. Pflugers Arch. 2013;465(1):79–85. https://doi.org/10.1007/s00424-012-1120-0.

Bagnard D, Vaillant C, Khuth ST, Dufay N, Lohrum M, Puschel AW, Belin MF, Bolz J, Thomasset N. Semaphorin 3A vascular endothelial growth factor-165 balance mediates migration and apoptosis of neural progenitor cells by the recruitment of shared receptor. J Neurosci. 2001;21(10):3332–41. https://doi.org/10.1523/JNEUROSCI.21-10-03332.2001.
Bao R, Surriga O, Olson DJ, Allred JB, Strand CA, Zha Y, Carl T, Labadie BW, Bastos BR, Butler M, Hogg D, Musi E, Ambrosini G, Munster P, Schwartz GK, Luke JJ. Transcriptional analysis of metastaticveal melanoma survival correlates NRP1 as a therapeutic target. Melanoma Res. 2022;31(1):27–37. https://doi.org/10.1097/CMR.0000000000000401.

Baranov MV, Bianchi F, van den Bogaart G. The PIKfyve inhibitor apilimod: a double-edged sword against COVID-19. Cells. 2020;10(2):130. https://doi.org/10.3390/cells10020130.

Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, Dorgham B, Baranov MV, Bianchi F, van den Bogaart G. The PIKfyve inhibitor apilimod: a therapy for SARS-CoV-2 infection and a potential therapeutic target. Biologics. 2021;15:143–52. https://doi.org/10.2147/BTT.S30735.

Chen Z, Mi L, Xu J, Wu D, Wang X, Jiang X, Jia J, Jiang Y, Zhang P, Qian A, Li Y, Shaw PX, Wang J, Duan S, Ding L, Fan C, Zhang H, Yang Y, Yu X, Feng Q, Li B, Yao X, Zhang Z, Li L, Xue X, Zhu P. Function of HAB18G/CD147 in infection of host cells by severe acute respiratory syndrome coronavirus. J Infect. 2005;195(1):75–80. https://doi.org/10.1016/j.jinf.2004.07.011.

Chen WH, Struyf H, Hotje P, Bottazzi ME. The SARS-CoV-2 vaccine pipeline: an overview. Curr Trop Med Rep. 2020. https://doi.org/10.1007/s40475-020-0201-6.

Cheng Y, Luo R, Wang X, Wang K, Zhang N, Zhang M, Wang Z, Dong L, Li J, Zeng R, Yao Y, Ge S, Xu G. The incidence, risk factors, and prognosis of acute kidney injury in adult patients with coronavirus disease 2019. Clin J Am Soc Nephrol. 2020;15(10):1394–402. https://doi.org/10.2215/CJN.04650420.

Choudhury A, Mukherjee S. In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. J Med Virol. 2020;92(10):2105–13. https://doi.org/10.1002/jmv.25987.

Chuckran CA, Liu C, Bruno TC, Workman CJ, Vignali DA. Neurtilin-1: a checkpoint target with unique implications for cancer immunology and immunotherapy. J Immunother Cancer. 2020;8(2):e000967. https://doi.org/10.1186/s41413-020-000967.

Crestani B, Cornillet P, Dehoux M, Randeva HS, Gueronou M, Aubier M. Alveolar type II epithelial cells produce interleukin-6 in vitro and in vivo. Regulation by alveolar macrophage secretory products. J Clin Invest. 1994;94(2):73–40. https://doi.org/10.1172/JCI17392.

Daly JL, Simonetti B, Klein K, Chen KE, Williamson MK, Anton-Piçãgor A, Shoomark D, Simon-Giacia L, Bauer M, Hollandier R, Greber UF, Horvath P, Sessions RB, Helenius A, Hiscox JA, Teesalu T, Matthews DA, Davidson AD, Collins BM, Cullen PJ, Yamauchi Y. Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science. 2020;370(6518):861–5.

Davies J, Randeva HS, Chatka H, Hall M, Spandidos DA, Karteris E, Kyrou I. Neuropilin-1 as a new potential SARS-CoV-2 infection mediator implicated in the neurologic features and central nervous system involvement of COVID-19. Mol Med Rep. 2020;22(5):4221–6. https://doi.org/10.3892/mmr.2020.11510.

De Vaemlink Y, Bonelli S, Awad RM, Devilde M, Rizzolio S, Lecocq Q, Bolli R, Teesalu T, Hepojoki J, Maun J, Adeler J, TIMem M, Nus, Goyvaert C, Soteras J, Rong P, Soteras J, Rong P. The role of neuropilins in cancer. Mol Cancer Ther. 2006;5(5):1099–107. https://doi.org/10.1158/1535-7109.MCT-05-0282.

De Vlaeminck Y, Bonelli S, Awad RM, Devilde M, Rizzolio S, Lecocq Q, Bolli R, Teesalu T, Hepojoki J, Maun J, Adeler J, TIMem M, Nus, Goyvaert C, Soteras J, Rong P, Soteras J, Rong P. The role of neuropilins in cancer. Mol Cancer Ther. 2006;5(5):1099–107. https://doi.org/10.1158/1535-7109.MCT-05-0282.
guide therapy and vaccine design strategies. Viruses. 2021;13(1):134. 
https://doi.org/10.3390/v13010134.

Finsterer J, Scorza FA, Fiorini AC. SARS-CoV-2-associated Guillain–Barre syn- 
drome in 62 patients. Eur J Neurol. 2021;28(1):e10–2. 
https://doi.org/10.1111/ene.14544.

Gerber HP, Killian KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, Wright BD, 
Radtké F, Aguet M, Ferrara N. VEGF is required for growth and survival in 
neonatal mice. Development. 1999;126(6):1149–59.

Grage-Griebenow E, Liske S, Kauth M, Gehlhar K, Zawatzky R, Bufe A. 
Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, Wright BD, 
Jung K, Kim JA, Kim YJ, Lee HW, Kim CH, Haam S, Kim YS. A neuropilin-1 antag-
onist exerts antitumor immunity by inhibiting the suppressive function of 
intratumoral regulatory T cells. Cancer Immunol Res. 2020;8(1):46–56. 
https://doi.org/10.1158/2326-6066.CIR-19-0143.

Kadarm SB, Suhramani GS, Bishnoi P, Pable AA, Barvkar VT. SARS-CoV-2, 
the pandemic coronavirus: molecular and structural insights. J Basic Micro-
bol. 2021;61(3):180–202. 
https://doi.org/10.1016/j.jbmb.202009537.

Kagoshima M, In T. Diverse gene expression and function of semaphorins in 
developing lung: positive and negative regulatory roles of semaphorins in 
lung branching morphogenesis. Genes Cells. 2001;6(6):559–71. 
https://doi.org/10.1046/j.1365-2443.2001.00441.x.

Kang YL, Chou YH, Rothlauf PW, Liu Z, Soh TK, Cureton D, Case JB, Chen RE, 
Diamond MS, Whelan SPI, Kirchhausen T. Inhibition of PIKfyve kinase 
preserves infection by Zaire ebolavirus and SARS-CoV-2. Proc Natl Acad 
Sci USA. 2020;117(34):20803–13. 
https://doi.org/10.1073/pnas.20078 
37117.

Karras A, Livrozset M, Lazareth H, Benichou N, Hulot JS, Fayol A, Chauvet S, 
Jannot AS, Penet MA, Diehl JG, Golider A, Sanchez O, Miraúlt T, Thervet 
Pallet N. Proteinuria and clinical outcomes in hospitalized COVID-19 
patients: a retrospective single-center study. Clin J Am Soc Nephrol. 
2021. https://doi.org/10.2215/CJN.09130620.

Kurappar MVK, Devadoss D, Nair M, Chand HS, Lakshmana MK. SARS-
CoV-2 infection in the central and peripheral nervous system-associated 
neurological morbidities and their potential mechanism. Mol Neurobiol. 
2021;58(6):2465–80. 
https://doi.org/10.1007/s12035-020-2245-1.

Kawakami T, Tokunaga T, Hatanaka H, Inoue H, Nishioka K, Yagi T, Ohara 
M, Yagi T, Fujisawa H. A neuropilin-1 antag-
onist exerts antitumor immunity by inhibiting the suppressive function of 
intratumoral regulatory T cells. Cancer Immunol Res. 2020;8(1):46–56. 
https://doi.org/10.1158/2326-6066.CIR-19-0143.

Lancet. 2021;397(10270):220–32. https:// 
doi.org/10.1016/s0140-6736(20)32656-8.

Kondoh H, Fujisawa H. Overexpression of a membrane protein, neuropilin, in 
chimeric mice causes anomalies in the cardiovascular system, nervous system 
and limbs. Development. 1995;121(12):4309–18.

Kolodkin AL, LeVengood DV, Rowe EG, Tai YT, Giger JR, Ginty DD. Neuropilin 
as a semaphorin III receptor. Cell. 1997;90(4):753–62. 
https://doi.org/10.1016/s0092-8674(00)80535-8.

Kreutzberger AJB, Sanjal A, Ojha P, Pyle JD, Vapalathi O, Balistreri G, Kirch-
hauser T. Synergistic block of SARS-CoV-2 infection by combined 
drug inhibition of the host entry factors PIKfyve kinase and TMPRSS2 
protease. J Virol. 2021;95(21):e0097521. 
https://doi.org/10.1128/JVI. e00975-21.

Kruke RL. Therapeutic strategies in an outbreak scenario to treat the novel 
coronavirus originating in Wuhan, China. F1000Res. 2020;9:72. 
https://doi.org/10.12688/f1000research.22211.2.

Kurig AM, Balasaumbrahmanian V, Markham NE, Morgan D, Montgomery G, 
Grover TR, Abman SH. Recombinant human VEGF treatment enhances 
alveolarization after hyperoxic lung injury in neonatal rats. Am J Physiol 
Lung Cell Mol Physiol. 2005;289(4):L529–35. 
https://doi.org/10.1152/ajplung.00336.2004.

Lantuejoul S, Constantin B, Drabink H, Brambilla C, Roche J, Brambilla E. 
Expression of VEGF, semaphorin SEMA3F, and their common receptors 
neuropilins NP1 and NP2 in preinvasive bronchial lesions, lung tumours, 
and cell lines. J Pathol. 2003;200(3):336–47. 
https://doi.org/10.1002/path.1367.

Le A, Zielenki R, He C, Crow MT, Biswal S, Tuder RM, Becker PM. Pulmo-
nary epithelial neuropilin-1 deletion enhances development of 
cigarette smoke-induced emphysema. Am J Respir Crit Care Med. 
2009;180(5):596–406. 
https://doi.org/10.1164/rccm.200809-1483OC.

Lee CG, Link H, Baluk P, Homer RJ, Chapoval S, Bhandari V, Kang MJ, Cohn L, 
Kim YK, McDonald DM, Elias JA. Vascular endothelial growth factor 
(VEGF) induces remodeling and enhances TH2-mediated sensitization 
and inflammation in the lung. Nat Med. 2004;10(10):985–103. 
https://doi.org/10.1038/nm1105.

Lei C, Qian K, Li T, Zhang S, Fu W, Ding M, Hu S. Neutralization of SARS-CoV-2 
spike pseudotyped virus by recombinant ACE2-Ig. Nat Commun. 
2020;11(1):2070. 
https://doi.org/10.1038/s41467-020-16048-4.

https://doi.org/10.1038/s41467-020-16048-4.
Mizui M, Kikutani H. Neuropilin-1: the glue between regulatory T cells and Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol. 2019;20(6):355–62. https://doi.org/10.1038/s41577-020-0331-4.

Parikh AA, Fan F, Liu WB, Ahmad SA, Stoeltzing O, Reinmuth N, Bielenberg D, Papageorgiou AC, Mohsin I. The SARS-CoV-2 spike glycoprotein as a drug and vaccine target: structural insights into its complexes with ACE2 and antibodies. Cells. 2020;9(11):2343. https://doi.org/10.3390/cells9112343.

Pan Q, Chantrney Y, Liang WC, Stawicki S, Mak J, Rathore N, Tong RK, Kowalski J, Yee SF, Pacheco G, Ross S, Cheng Z, Le Couter J, Plowman G, Peale F, Koch AW, Wu Y, Bagri A, Tisser-Lavigne M, Watts RJ. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. Cancer Cell. 2007;11(1):53–67. https://doi.org/10.1016/j.ccr.2006.10.018.

Parish ML, Routledge PE, Ruidizad ME, Sugahara KN, Teasal MD, Ruoslahti E. An endocytosis pathway initiated through neuropilin-1 and regulated by nutrient availability. Nat Commun. 2014;5:4904. https://doi.org/10.1038/ncomms5904.

Papaioannou AC, Mohsin I. The SARS-CoV-2 spike glycoprotein as a drug and vaccine target: structural insights into its complexes with ACE2 and antibodies. Cells. 2020;9(11):2343. https://doi.org/10.3390/cells9112343.

Pardoll DM, Pardoll DM. Immune checkpoint inhibitors: impact and future potential. Nat Rev Cancer. 2021;21(3):162–72. https://doi.org/10.1038/s41564-020-0688-y.

Pan Q, Chantrney Y, Liang WC, Stawicki S, Mak J, Rathore N, Tong RK, Kowalski J, Yee SF, Pacheco G, Ross S, Cheng Z, Le Couter J, Plowman G, Peale F, Koch AW, Wu Y, Bagri A, Tisser-Lavigne M, Watts RJ. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. Cancer Cell. 2007;11(1):53–67. https://doi.org/10.1016/j.ccr.2006.10.018.

Parish ML, Routledge PE, Ruidizad ME, Sugahara KN, Teasal MD, Ruoslahti E. An endocytosis pathway initiated through neuropilin-1 and regulated by nutrient availability. Nat Commun. 2014;5:4904. https://doi.org/10.1038/ncomms5904.

Papaioannou AC, Mohsin I. The SARS-CoV-2 spike glycoprotein as a drug and vaccine target: structural insights into its complexes with ACE2 and antibodies. Cells. 2020;9(11):2343. https://doi.org/10.3390/cells9112343.

Pardoll DM, Pardoll DM. Immune checkpoint inhibitors: impact and future potential. Nat Rev Cancer. 2021;21(3):162–72. https://doi.org/10.1038/s41564-020-0688-y.

Pan Q, Chantrney Y, Liang WC, Stawicki S, Mak J, Rathore N, Tong RK, Kowalski J, Yee SF, Pacheco G, Ross S, Cheng Z, Le Couter J, Plowman G, Peale F, Koch AW, Wu Y, Bagri A, Tisser-Lavigne M, Watts RJ. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. Cancer Cell. 2007;11(1):53–67. https://doi.org/10.1016/j.ccr.2006.10.018.

Parish ML, Routledge PE, Ruidizad ME, Sugahara KN, Teasal MD, Ruoslahti E. An endocytosis pathway initiated through neuropilin-1 and regulated by nutrient availability. Nat Commun. 2014;5:4904. https://doi.org/10.1038/ncomms5904.
R, Schulz PG, Su AI, García-Sastre A, Chatterjee AK, Yuen KY, Chanda SK. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. Nature. 2020;586(7827):113–9. https://doi.org/10.1038/s41586-020-2377-z.

Rizzolo S, Tamagnone L. Multifaceted role of neuropilins in cancer. Curr Med Chem. 2011;18(23):3563–75. https://doi.org/10.2174/092986711796624544.

Roche J, Drabkin H, Brambilla E. Neuropilin and its ligands in normal lung and cancer. Adv Exp Med Biol. 2002;515:103–14. https://doi.org/10.1007/978-1-4615-0119-0_9.

Root-Bernstein R. Innate receptor activation patterns involving TLR and NLR synergisms in COVID-19. ALI/ARDS and sepsis cytokine storms: a review and modeling novel predictions and therapeutic suggestions. Int J Mol Sci. 2021;22(4):2108. https://doi.org/10.3390/ijms22042108.

Roy S, Bag AK, Singh RK, Talmadge JE, Batra SK, Datta K. Multifaceted role of neuropilins in the immune system: potential targets for immunotherapy. Front Immunol. 2017;8:1228. https://doi.org/10.3389/fimmu.2017.01228.

Shitals J, Czrozer TWM, Greenwood EJD, Lehrner PJ, Wright GJ. No evidence for basigin/CD147 as a direct SARS-CoV-2 spike binding receptor. Sci Rep. 2021;11(1):413. https://doi.org/10.1038/s41598-020-80464-1.

Shim EJ, Chun E, Kang HR, Cho SH, Min KJ, Park HW. Expression of semaphorin 3A and neuropilin 1 in asthma. J Korean Med Sci. 2013;28(10):1435–42. https://doi.org/10.3346/jkms.2013.28.10.1435.

Shiryaev SA, Remacle AG, Ratnikov BI, Nelson NA, Savinov AY, Wei G, Bottini M, Root-Bernstein RR, Baretton G, Lindemann D, Solimena M, Ludwig B, Bornstein SR. Viral neuropilin-1-dependent cell, vascular, and tissue penetration. Proc Natl Acad Sci USA. 2009;106(38):16157–62. https://doi.org/10.1073/pnas.0908201106.

Talley L, Cadilla C, Cioni JM, Saywell V, Janhannaut-Talignani C, Huettet RE, Sarnali-Favre C, Dayer A, Huber AB, Ango F. Dual function of NRP1 in axon guidance and subcellular target recognition in cerebellum. Neuron. 2016;91(6):1276–91. https://doi.org/10.1016/j.neuron.2016.08.015.

Tordjman R, Lepelletier Y, Lemarchand-Vel, Cambot M, Gauliard P, Hermine O, Rompolo P. A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. Nat Immunol. 2002;3(5):477–82. https://doi.org/10.1038/nri789.

Turner AJ. Chapter 25. ACE2 cell biology, regulation, and physiological functions. In: The protective arm of the renin-angiotensin system (RAS). Amsterdam: Elsevier; 2015. p. 185–9. https://doi.org/10.1016/B978-0-12-803146-9.00025-0.

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281–92.e6. https://doi.org/10.1016/j.cell.2020.02.058.

Wan Y, Zhang J, Graham R, Banc RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of coronavirus SARS. Virol J. 2020;17(4):1–20. https://doi.org/10.1186/s13027-019-01227-0.

Wang MY, Zhao R, Gao Lj, Gao XF, Wang DP, Cao JM. SARS-CoV-2: structure, biology, and structure-based therapeutics development. Front Cell Infect Microbiol. 2020a;10(5):587–269. https://doi.org/10.3389/fcimb.2020.587269.

Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuan K, Wang Q, Zhou H, Yan J, Qi J. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell. 2020b;181(4):894–904.e9. https://doi.org/10.1016/j.cell.2020.03.045.

Wang K, Chen W, Zhang Z, et al. CD147-splice protein is a novel route for SARS-CoV-2 infection to host cells. Sig Transduct Target Ther. 2020c;5:283. https://doi.org/10.1038/s41392-020-00426-x.

Weekes CD, Beeram M, Tolver AW, Papadopoulos KP, Gore L, Hegde P, Xin Y, Yu R, Shih LM, Xiang H, Brachmann RK, Patnaik A. A phase I study of the human monoclonal anti-NRP1 antibody MNRP1665A in patients with advanced solid tumors. Invest New Drugs. 2014;32(4):653–60. https://doi.org/10.1007/s10637-014-0071-z.

Wild JR, Staton CA, Chapple K, Corfe BM. Neuropilins: expression and roles in the epithelium. J Int Exp Pathol. 2012;93(2):81–103. https://doi.org/10.1111/j.1365-2613.2012.00810.x.

Witkoswska D. Mass spectrometry and structural biology techniques in the studies on the coronavirus–receptor interaction. Molecules. 2020;25(10):2248. https://doi.org/10.3390/molecules25102248.

Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan H, Lit LC, Hui DS, Chan MH, Chung SS, Sung JJ. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol. 2004;141(1):95–103. https://doi.org/10.1111/j.1365-2249.2004.02415.x.

Wong SK, Li W, Moore MJ, Choe H, Farzan M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotsin-converting enzyme 2. J Biol Chem. 2004b;279(5):3197–201. https://doi.org/10.1074/jbc.C400520200.

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh Y, Li F, Weng D, Gao XF, Wang DP, Cao JM. SARS-CoV-2: structure, genome and implications for a novel coronavirus emerging from the蝙蝠. Science. 2020;367(6483):1260–3. https://doi.org/10.1126/science.abb2507.

Xiaojie S, Yu L, Lei Y, Guang Y, Min Q. Neutralizing antibodies targeting SARS-CoV-2 spike protein and its receptor in COVID-19 patients. Emerg Microbes Infect. 2020;9(1):1–12. https://doi.org/10.1080/22221751.2020.1747363.

Xin Y, Deng C, Liu X, Chen Y, Ye J, Cai R, Shen Y, Tang H. TNF-α induction of IL-6 in alveolar type II epithelial cells: contributions of TNF-α/APP-1
element, C/EBPδ/C/EBP binding site and IKK/NF-κB p65/κB site. Mol Immunol. 2018;101:585–96. https://doi.org/10.1016/j.molimm.2018.05.004.

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020;367(6485):1444–8. https://doi.org/10.1126/science.abb2762.

Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, Subbarao K, Nabel GJ. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol. 2004;78(11):5642–50. https://doi.org/10.1128/JVI.78.11.5642-5650.2004.

Yang C, Li Y, Xiao SY. Differential expression of ACE2 in the respiratory tracts and its relationship to COVID-19 pathogenesis. EBioMedicine. 2020;60:103004.

Zhang Y, Li J, Zhan Y, Wu L, Yu X, Zhang W, Ye L, Xu S, Sun R, Wang Y, Lou J. Analysis of serum cytokines in patients with severe acute respiratory syndrome. Infect Immun. 2004;72(8):4410–5. https://doi.org/10.1128/IAI.72.8.4410-4415.2004.

Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, Ogishi M, Sabli IKD, Hodeib S, Korol C, Rosain J, Bilgicar K, Ye J, Bolze A, Bigio B, Yang R, Arias AA, Zhou Q, Zhang Y, Onodi F, Korniotis S, Karfp L, Philippot Q, Chbibi M, Bonnet-Madin L, Dorpham K, Smith N, Schneider WM, Razooky BS, Hoffmann HH, Michailidis E, Moens L, Han JE, Lorenzo L, Bizien L, Meade P, Neehus AL, Dittrich S, Liu A, Xia J, Zhang P, Rapaport F, Seeleuthner Y, Manry J, Masson C, Schmitt Y, Schlüter A, Le Voyer T, Khan T, Li J, Fellay J, Roussel L, Shahrooei M, Alosaimi MF, Mansouri D, Al-Saud H, Al-Mulla F, Almourfati F, Al-Muhesen SZ, Alshohime F, Al Turki S, Hasanato R, van de Beek D, Biondi A, Bettini LR, D’Angiol M, Bonfanti P, Imberti L, Sotinna A, Paghre S, Quirres-Roldan R, Rossi C, Oler AJ, Tompkins MF, Alba C, Vandernoot I, Goffard JC, Smits G, Migieotte I, Palear-Palacin P, Martin-Nafula A, Colobran R, Morange PE, Keles S, Çolkesen F, Ozcelik T, Yasar KK, Senoglu S, Karabela SN, Rodriguez-Gallego C, Novelli G, Hraiech S, Tandjaoui-Lambiotte Y, Duval X, Laouenian C, Snow AL, Dalgaard CL, Milner JD, Vinh DC, Mogensen TH, Mar N, Spaan AN, Boisson B, Boisson-Dupuis S, Bustamante J, Puel A, Ciancanelli ML, Meyts I, Manisaitis T, Soumelis V, Amara A, Nussenzweig M, Garcia-Sastre A, Krammer F, Pujol A, Duffy D, Lifton RP, Zhang SY, Gorochov G, Bézat V, Jouanguy E, Sancho-Shimizu V, Rice CM, Abel L, Notarangelo LD, Cobat A, Su HC, Casanovas JL, COVID-Storm Clinicians, COVID Clinicians, Imagine COVID Group, French COVID Cohort Study Group, Cov-Contact Cohort, Amsterdam UMC, Biobank, COVID Human Genetic Effort, NIAID-USUHS/TAGC COVID Immunity Group. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020;370(6515): eabd4570. https://doi.org/10.1126/science.abd4570.

Zhou R, To KK, Wong YC, Liu L, Zhou B, Li X, Huang H, Mo Y, Luk TY, Lau TT, Yeung P, Chan WM, Wu AK, Lung KC, Tsang OT, Leung WS, Hung IF, Yuen KY, Chan Z. Acute SARS-CoV-2 infection impairs dendritic cell and T cell responses. Immunity. 2020;53(4):864–77.e5. https://doi.org/10.1016/j.immuni.2020.07.026.

Zhou Y, Chi J, Lv W, Wang Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19). Diabetes Metab Res Rev. 2021;37(2): e3377. https://doi.org/10.1002/dmrr.3377.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.