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VIEWPOINT

Targeting neuropilins as a viable SARS-CoV-2 treatment

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The SARS-CoV-2 pandemic has significantly impacted global health. Research on viral mechanisms, highly effective vaccines, and other therapies is in progress. Neuropilins have recently been identified as host cell receptors enabling viral fusion. Here, we provide context to neuropilin's tissue-specific role in infection and the potential impact of NRP-based therapeutics. We conclude that the central roles of neuropilins in vascular, neural, and other pathways may render it a less suitable target for treating SARS-CoV-2 than agents that target its binding partner, the viral spike protein.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has significantly impacted global health and the daily functions of billions of people worldwide. Research relief efforts by laboratories across the globe is ongoing, examining patient symptoms, studying the immune response and elements of the virus such as cellular entry and engineering potential therapies. Efforts so far have identified SARS-CoV-2 S1 spike glycoprotein as a good vaccine antigen target. Characterizing the S1 protein, identifying the host cell receptors responsible for interactions with S1 and investigating the mechanisms of their interaction and its facilitated viral entry, could help develop approaches to block infection, enabling not just vaccines but also targeted therapies.

SARS-CoV-2 interactions with host cells

The host angiotensin-converting enzyme 2 (ACE2) is one docking site for the SARS-CoV-2 spike S1 protein. ACE2 appears to be an important and in some cases required factor for SARS-CoV-2 entry identified early in the pandemic as a potential key target [1,2]. Upon exposure to the SARS-CoV-2 virus, Furins expressed by host cells act to cleave and subsequently expose a polybasic Arg-Arg-Ala-Arg (RRAR) motif specific to SARS-CoV-2 on the viral S1 protein (which can bind directly to neuropilin1). This in turn enables S1 to interact with host cell surface proteins. Current knowledge on viral molecular interactions indicates that a number of cellular entry factors and facilitators

Abbreviations
ACE2, angiotensin-converting enzyme 2; ADAM17, ADAM metallopeptidase domain 17; AT1R, angiotensin II type 1 receptor; AVPR1B, vasopressin V1b receptor; CendR, C-end-Rule; DARPins, designed ankyrin repeat proteins; GAGs, glycosaminoglycans; HEK-293T, human embryonic kidney-293T; HSPGs, heparan sulfate proteoglycans; NRP, neuropilin; PLGF, placenta growth factor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SCARB1, Scavenger receptor class B type 1; SDC1, syndecan 1; TMEM106B, transmembrane protein 106B; TMPRSS2, transmembrane protease serine 2; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

are involved in SARS-CoV-2 fusion [3]. Among those are ACE2 (cleaved by TMPRSS2 and ADAM17) [1,4], HDL-scavenger receptor B type 1 [5], vasopressin V1b receptor (AVPR1B) [6], syndecans (SDC1–4) [7], angiotensin II type 1 receptor (AT1R) [8], transmembrane protein 106B (TMEM106B) [9], basigin (CD147) [10], and heparan sulfate proteoglycans (HSPGs) [11]. The expression of some of these factors such as CD147 is upregulated in patients with pre-existing morbidities such as obesity and diabetes. A number of recent studies have shown that neuropilin expression alone can promote viral entry and infection in the absence of ACE2 [12–14]. However, ACE2 expression confers the highest potential for entry [13,14]. Due to this and the large number of known SARS-CoV-2 attachment and/or co-factors on the host cells, ACE2 is currently widely regarded as an optimal target to inhibit infection.

However, neurological symptoms have been commonly reported in COVID-19 patients [15], and ACE2 is not present in most neurons [14,15]. Similarly, levels of ACE2 expression decline with age [15], while COVID-19 severity greatly increases with age [15]. These findings suggested that alternative viral entry routes may exist that ACE2 is not the sole receptor responsible for SARS-CoV-2 cell entry. Involvement of other transmembrane proteins (as independent docking sites or potentially as co-receptors of ACE2) has also come into focus to explain SARS-CoV-2 tissue tropism. The cellular transmembrane protease serine 2 (TMPRSS2) primes SARS-CoV-2 for entry, and a serine protease inhibitor blocks SARS-CoV-2 infection of lung cells [1]. The cellular glycosaminoglycans (GAGs) also play a role in SARS-CoV-2 viral entry as the SARS-CoV-2 S1/S2 cleavage site includes a heparin-binding motif. Heparan sulfate proteoglycans bind to the spike protein on a site adjacent to the ACE2 binding sire and act as a co-factor for ACE2 binding to S1 [11,16]. Thus, a variety of host cell receptors act as attachment factors and/or co-factors to facilitate viral entry in tissues where they are expressed.

The role of neuropilins

More recently, neuropilin 1 (NRP1) has been shown to act as a facilitator of viral entry [13,14]. Co-expression of TMPRSS2 with either ACE2 or NRP1 potentiates the infection, with ACE2 together with TMPRSS2 being twice as efficient as NRP1 with TMPRSS2. Maximal levels of infection are observed when all three are co-expressed [14]. Here, we provide context to critical recent work on the role of NRPs in SARS-CoV-2 cell entry and potential impact of NRP-based therapeutic targeting aiming to reduce infection. Tissue specificity plays an important role, as viral entry depends on local expression of key host receptors (Figs 1 and 2). SARS-CoV-2 impacts the brain and the central nervous system and relieves pain in patients [17]. Some of the early COVID-19 symptoms, such as disruption of olfaction, appear upon SARS-CoV-2 human respiratory tract infection. Yet, TMPRSS2 is not significantly expressed in neurons and olfactory epithelium [14]. NRP1 is abundantly expressed in the mouse and human endothelium, sensory olfactory neurons, gastrointestinal, respiratory, and olfactory epithelium, in particular in the epithelial cells facing the nasal cavity [14]. To determine whether SARS-CoV-2 utilizes NRP1 for virus entry, Cantuti-Castelvetri *et al.* [14] systematically examined *in vitro* cultured HEK-293T cells and identified that TMPRSS2 expressed alone does not enable viral entry and ACE2 expressed alone allows some level of infection, but co-expressed with NRP1, infection levels increased significantly further observed. Using autopsies of COVID-19 patients and available expression data, Cantuti-Castelvetri *et al.* [14] also found that NRP1 is expressed at significantly higher protein and mRNA levels compared to ACE2 and TMPRSS2 in several human tissues (Figs 1 and 2).

A particular region of the NRP1 structure is important to spike protein interaction, as established by mutagenesis experiments on various residues on the NRP1 b1 extracellular domain [13]. A C-terminal peptide of S1 region of the Spike protein is liberated following processing by Furin enabling binding to the b1 domain of NRP1. The Spike CendR motif becomes available only after processing of S1 into the S1 and S2 polypeptides. Daly *et al.* co-expressed fluorescently tagged NRP1 and viral S1 protein and found that the two proteins interact. They further mutated one (R685) or all residues in the R(R/K)(L/A)(R) motif on the viral S1 protein commonly recognized by cell surface proteins (also known as the Cend residues) and discovered that these residues enable S1-NRP1 interactions and access to the host cells [13] and that this interaction enhances SARS-CoV-2 infectivity. Triple mutagenesis of NRP1 b1 domain residues S346A, E348A, and T349A disrupted the utility of the CendR binding pocket as a substrate for the S1 protein [14]. Furthermore, recombinant wild-type soluble NRP1 (containing only the extracellular domains of NRP1), and not the b1/b2 mutated NRP1, blocked entry of virus particles to cells [14]. They provided further evidence that NRP1 uses its extracellular b1 domain to interact with the spike protein as mutating residues (D320 on this pocket on NRP1 and T316 on the Cend

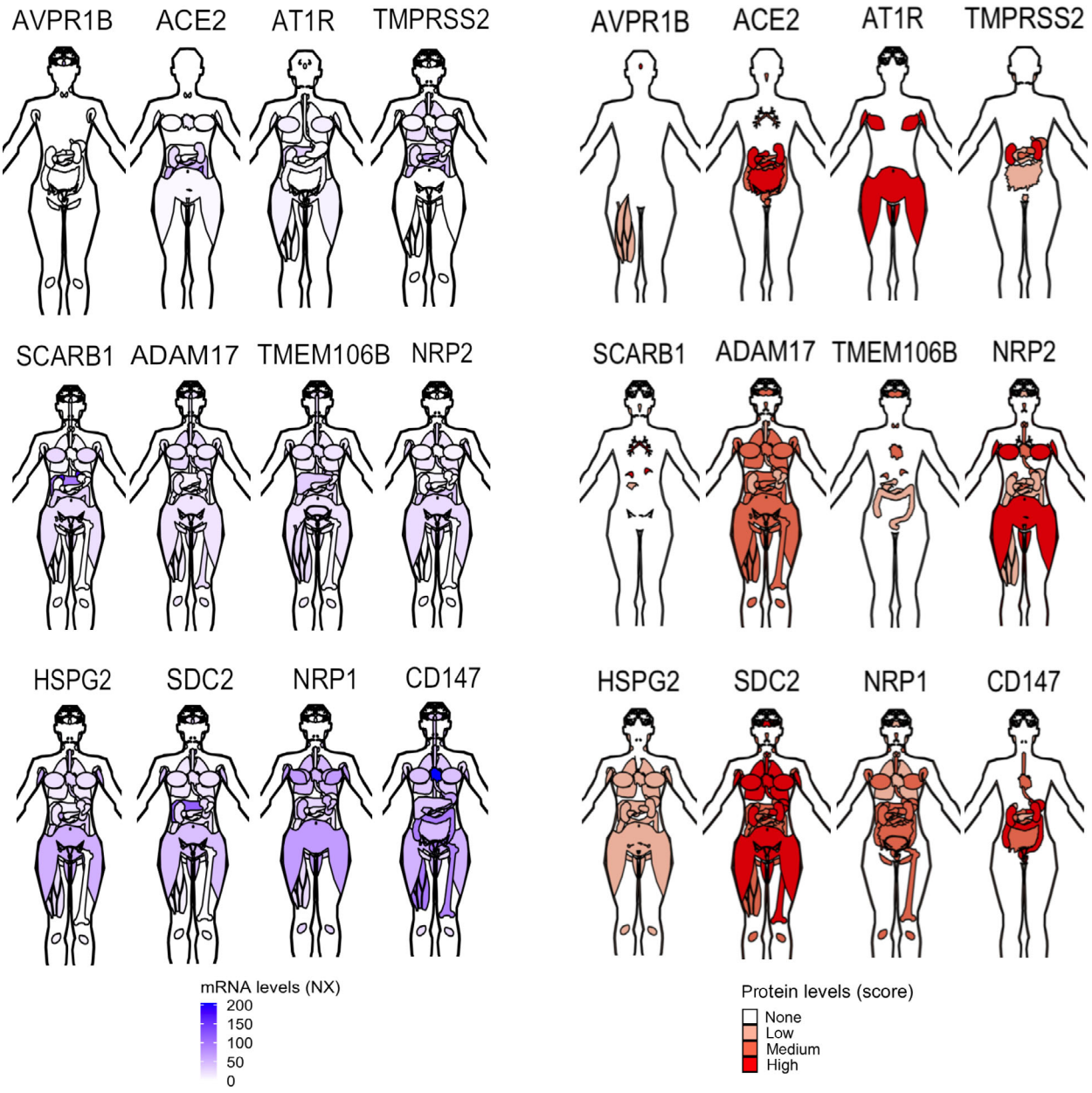

Fig. 1. Female organ and tissue-specific protein (in red) and mRNA (in blue) expression patterns for human angiotensin-converting enzyme 2 (ACE2), neuropilin1 (NRP1), neuropilin2 (NRP2), transmembrane protease serine 2 (TMPRSS2), heparan sulfate proteoglycan 2 (HSPG2) and syndecan 2 (SDC2), transmembrane protein 106B (TMEM106B), ADAM metallopeptidase domain 17 (ADAM17), HDL-scavenger receptor B type 1 (SCARB1), angiotensin II receptor type 1 (AT1R), vasopressin V1b receptor (AVPR1B), and basigin (CD147). Genes/proteins are ordered by the number of organs or tissues expressing the mRNA. NRP1 and NRP2 are expressed in a large number of organs in both protein and mRNA forms. Tissues not shown lack detectable mRNA or protein expression. The anatograms were created in R using *gganatogram* [32]. RNA expression summary shows the consensus RNA-data based on normalized expression (NX) data from three different sources: internally generated Human Protein Atlas (HPA) RNA-seq data, RNA-seq data from the Genotype-Tissue Expression (GTEx) project, and CAGE data from FANTOM5 project. Protein expression scores are based on a best estimate of the 'true' protein expression from a knowledge-based annotation. Data source: https://www.proteinatlas.org/ [33]. The expression levels of a number of these genes may be further elevated in COVID-19 patients [20] and in individuals with other morbidities [21,34].

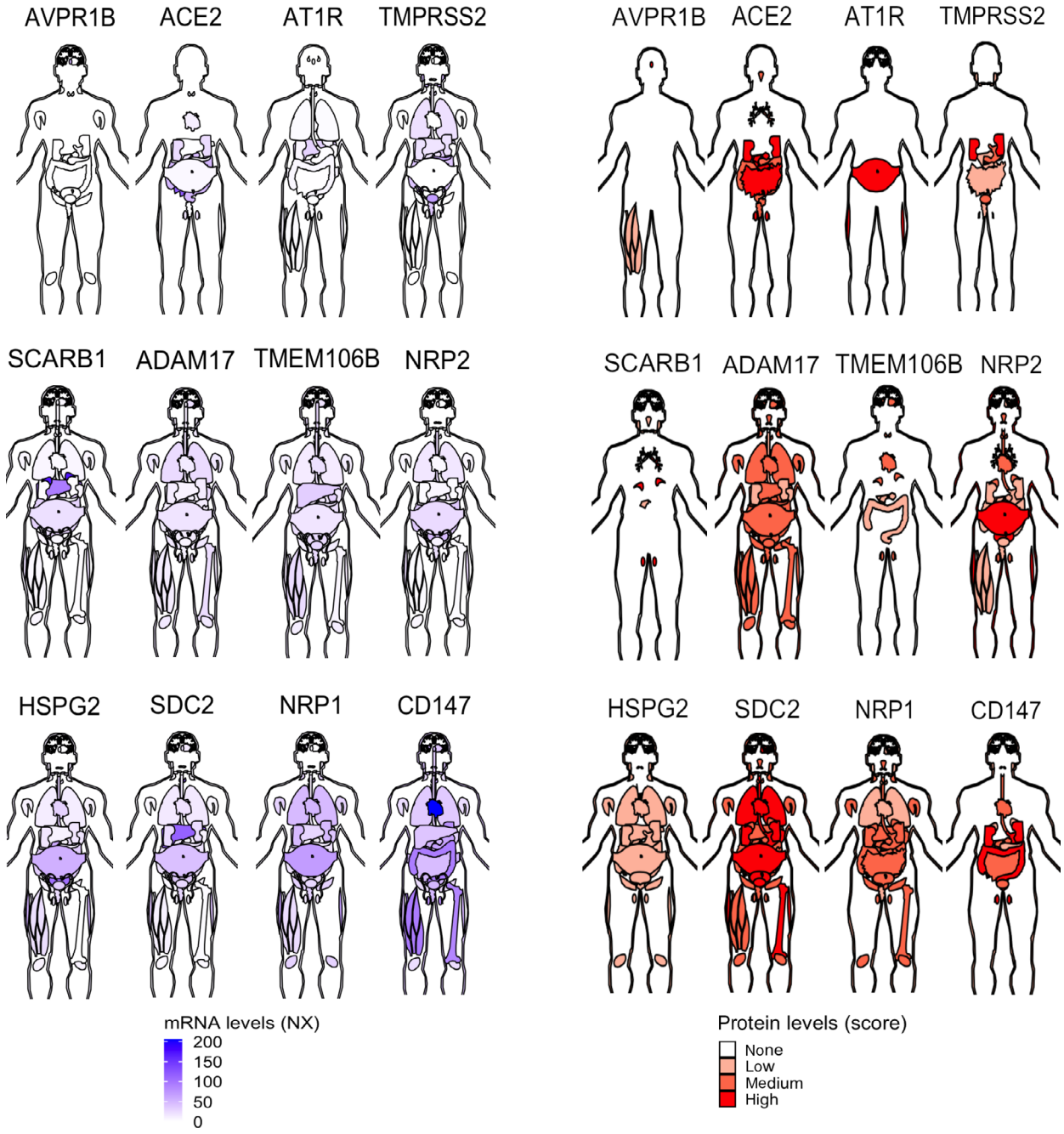

Fig. 2. Male organ and tissue-specific protein (in red) and mRNA (in blue) expression patterns for human angiotensin-converting enzyme 2 (ACE2), neuropilin1 (NRP1), neuropilin2 (NRP2), transmembrane protease serine 2 (TMPRSS2), heparan sulfate proteoglycan 2 (HSPG2) and syndecan 2 (SDC2), transmembrane protein 106B (TMEM106B), ADAM metallopeptidase domain 17 (ADAM17), HDL-scavenger receptor B type 1 (SCARB1), angiotensin II receptor type 1 (AT1R), vasopressin V1b receptor (AVPR1B), and basigin (CD147). NRP1 and NRP2 are expressed in a large number of organs in both protein and mRNA forms. Tissues not shown lack detectable mRNA or protein expression. See Fig. 1 caption for more details.

motif on viral S1 protein), impaired viral entry to a number of cell types *in vitro*. The authors further found that NRP1 b2 domain stabilizes the interaction [13]. Daly *et al*. also showed that S1 lacking the CendR motif to some extent retains the ability to bind NRP1, suggesting that there could be a second, yet to be defined, sequence in S1 that interacts with NRP1. Thus, future work characterizing the molecular bases

of the S1-NRP1 association may facilitate design of new molecules that alone, or in combination with available ones, could specifically target the interaction between NRP1 and S1 (but not that of NRP1 with endogenous ligands).

The spike protein S1 binding site on NRP1's b1 domain is also long known as the docking site for multiple other ligands such as isoforms of the angiogenic cytokines VEGF-A, VEGF-B, PlGF, VEGF-C, and VEGF-D [18]. Binding of these ligands (and of the S1 protein) to NRP1 is blocked when the binding pocket on NRP1 b1 is mutated [13,14,17]. Further, binding of the S1 protein or NRP1 antagonist EG00229 inhibits binding of ligands to NRP1 b1 [17]. The competition of S1 protein with NRP1 ligands such as VEGF-A for binding and disruption in VEGF/NRP1 signaling pathway likely contributes to silencing of pain and olfactory sensory mechanisms in COVID-19 patients [14,17]. These structural findings all suggest potential for major side effects for therapeutic targeting of neuropilins in patients. NRPs act as major co-receptors for VEGF receptors, which play a critical role in angiogenesis [19].

Disrupting the CendR-peptide binding to NRPs via agents may disrupt viral entry but present multiple side effects. For example, there is a second neuropilin cell-surface protein in humans, NRP2, which also recognizes the Cend motif on the spike S1 protein [13]. Compared to NRP1, NRP2 is expressed at lower levels in most tissues but is expressed at considerably higher levels compared to ACE2 in all tissues (Figs 1 and 2) [14]. In the lung, both NRP1 and NRP2 are abundantly expressed with highest expression in endothelial cells and detectable levels in all pulmonary cells [14,20]. Gene expression analysis of lung tissue from COVID-19 patients has revealed an upregulation of NRP1 and NRP2 [20] and ACE2 [21]. Cantuti-Castelvetri *et al*. point to protein and mRNA staining of tissue alongside sequencing results showing the relative NRP1 and NRP2 expression in multiple cell and tissue types. Yet, the exact quantity of NRP proteins, their interaction partners, and spatial organization on the cell surface not only in cells cultured *in vitro* but also in normal and pathological tissue conditions and in different healthy persons and COVID-19 patients remains unclear. SARS-CoV-2 has multi-organ (e.g., cardiovascular) involvement in tissues, with extensive vascular damage [22], where NRP1s are also significantly expressed [14]. Yet, the antibodies used in the NRP1-spike protein interactions (those developed by both Daly *et al*. and Cantuti-Castelvetri *et al*.) only antagonize NRP1 [13,14], and thus, separate antibodies would need to be engineered and administered to target NRP2. The antibodies engineered and tested by both groups against NRP1 b1/b2 domains only modestly inhibited host cell SARS-CoV-2 infection under cell culture conditions [13,14]. In order to fully inhibit viral entry mechanisms by targeting host cell receptors, a cocktail of antibodies (against ACE2, NRP1, NRP2, and other receptors) may be required (Fig. 3).

Targeting neuropilins to inhibit viral entry

Considerable mechanistic data currently available on NRP interactions and physiological and pathophysiological roles point to potential side effects of anti-NRP therapy [23]. These data and previous clinical trials using anti-NRP1 antibodies suggest how NRP-based interventions may interfere with normal cellular and tissue function. NRPs, their ligands, and co-receptors VEGFRs are abundantly expressed at high protein and mRNA levels in a wide variety of human tissues, such as fetal tissues, the placenta, and corpus luteum undergoing active angiogenesis. VEGF-A binding to NRPs and VEGFRs plays an important role in wound healing, ovulation, menstruation, maintenance of blood pressure and pregnancy as well as in immune cells and their signaling is implicated in a host of vital physiological functions such as neuronal growth, immune response, angiogenesis, and vascular permeability [23,24]. Potential approaches to inhibit NRPs, such as NRP1 knock down or NRP1 blockade, can significantly impact the normal functions of a number of cell types such as endothelial cells [25]. Use of soluble NRP1 that can bind to the viral S1 protein can in turn produce off target effects by disrupting normal function of VEGFRs, and their ligands such as PlGFs and VEGFs (a survival factor for endothelial cells) in many tissues. Targeting the NRP-S1 protein interactions using soluble ACE2 or soluble NRP could deplete ligand pools necessary for normal tissue function.

NRP1 targeting by antibodies such as MNRP1685A or via oligopeptide/peptidomimetics has been recognized as a potential anti-cancer strategy that can induce off-target effects [26] and inhibit vital physiological angiogenesis [17,26,27] and the same can occur if anti-NRP antibodies are used to inhibit COVID-19 infection. Intravenous delivery of anti-angiogenic molecules such as antibodies targeting NRP b1/b2 domains in tissue targeted by SARS-CoV-2 can impact neovascularization in those tissue, adjacent, and distant tissues for instance during ovulation and follicular angiogenesis. Ischemic tissue is particularly sensitive to anti-angiogenic therapy. Targeting NRP1 clinically can thus impact vital normal angiogenesis and immune

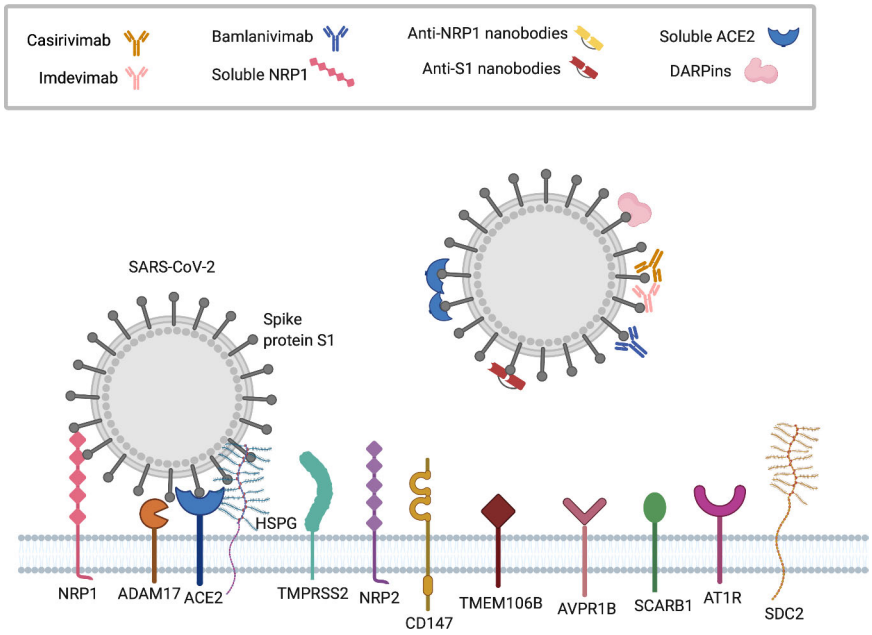

Fig. 3. Multiple host-expressed receptors interact with the SARS-CoV-2 S1 protein. Therapeutics targeting these interactions could include anti-S1 or anti-receptor antibodies, and soluble proteins, and may prevent or diminish early infection. However, we must also consider the potential other effects of targeting endogenously expressed receptors, whether ubiquitous or tissue-specific. Figure was created in BioRender.

response in multiple tissues and organs for instance during female ovulation and menstruation by inhibiting angiogenesis or causing abnormal vessel structure (enlarged or thin vessels), growth (fewer or poorly formed vessels), or functionality. Patients with co-existent disease who have ischemic tissue (such as ischemic limb in patients with peripheral artery disease and ischemic myocardium in patients with coronary artery disease) where angiogenesis is vital may also be impacted by anti-NRP therapy. Anti-NRP1 treatment can also induce membrane NRP1 shedding resulting in elevated soluble NRP1 in circulation [28], which could further complicate side effects. Time-limited therapy may have the potential to minimize side effects. Even when time-limited, however, anti-NRP therapies may well induce widespread side effects if administered systematically (e.g., orally or intravenously reaching a wide range of tissues). Future clinical trials may help clarify this issue. Thus, questions remain on whether NRPs are promising targets for the expensive and time-consuming therapeutic design and clinical trial efforts in the upcoming months and what the short- and long-term side effects of drugs targeting NRPs will be in patients of different health backgrounds.

While vaccines are critical in battle against SARS-CoV-2 infection, treatments for patients at early and late stages of infection will remain important in short- and long-term. Despite the excitement generated upon identifying the role of neuropilins in SARS-CoV-2 infection, potent therapeutic interventions such as antibodies and biologics against membrane receptors are rarely free of potential serious side effects. Further, antibodies targeting NRP1 offer small inhibition of viral infection under cell culture conditions [13,14]. Targeted delivery of NRP1-targeting therapies to key tissues rather than systemic delivery (e.g., inhalable anti-NRP1 nanobodies) could be a viable solution. Small inhibitory effects provided by anti-NRP1 antibodies also suggest that multiple mechanisms may help the virus in cell entry, thus targeting NRP1 using peptides or monoclonal antibodies may do more harm and not have considerable beneficial therapeutic outcomes as a clinical pursuit. Virus-neutralizing antibodies targeting the S1 protein in SARS-CoV-2 have shown promise in masking S1 protein, preventing infection, reducing the viral load in clinical trials are underway or planned for the upcoming months [29], paving the way to virus-side rather than host-side targeting. A cocktail of two monoclonal antibodies Casirivimab (REGN10933) and Imedevimab (REGN10987) bind noncompetitively to the critical receptor binding domain of the virus's spike protein, which diminishes the ability of mutant viruses to escape treatment and protects against spike variants that have arisen in the human population [30]. Potent

DARPins (designed ankyrin repeat proteins) have also gained attention as COVID-19 therapeutics [31]. The MP0420 DARPin binds three epitopes on the S1's receptor-binding domain (RBD); MP0423 targets one epitope on the spike's RBD, one on its S1 N-terminal domain and another on its S2 domain.

Conclusion

In summary, while neuropilins are an exciting potential target for SARS-CoV-2 therapeutics, we should tread carefully interfering with so central and ubiquitous a receptor, with key roles in vascular and neuronal development and dynamics, and in pathologies ranging from cancer to ischemic disease. However, understanding the involvement of NRP1 in viral entry, and how the structure of NRP1 governs this, may help in design and testing of other effective therapeutic antiviral approaches.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SS and FMG both reviewed the literature and wrote the manuscript.

References

1 Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A *et al.* (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181**, 271–280.e8.

2 Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A & Li F (2020) Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci USA* **117**, 11727–11734.

3 Seyran M, Takayama K, Uversky VN, Lundstrom K, Palù G, Sherchan SP, Attrish D, Rezaei N, Aljabali AAA, Ghosh S *et al.* (2020) The structural basis of accelerated host cell entry by SARS-CoV-2. *FEBS J* **288**, 5010–5020.

4 Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O & Pöhlmann S (2014) TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J Virol* **88**, 1293–1307.

5 Wei C, Wan L, Yan Q, Wang X, Zhang J, Yang X, Zhang Y, Fan C, Li D, Deng Y *et al.* (2020) HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. *Nat Metab* **2**, 1391–1400.

6 Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai J-P, Zhou R, Chan K-H, Zhao H, Zhu L *et al.* (2021) Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. *Cell* **184**, 2212–2228.e12.

7 Hudák A, Letoha A, Szilák L & Letoha T (2021) Contribution of syndecans to the cellular entry of SARS-CoV-2. *Int J Mol Sci* **22**, 5336.

8 Kai H & Kai M (2020) Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors—lessons from available evidence and insights into COVID-19. *Hypertens Res* **43**, 648–654.

9 Wang R, Simoneau CR, Kulsuptrakul J, Bouhaddou M, Travisano KA, Hayashi JM, Carlson-Stevermer J, Zengel JR, Richards CM, Fozouni P *et al.* (2021) Genetic screens identify host factors for SARS-CoV-2 and common cold coronaviruses. *Cell* **184**, 106–119.e14.

10 Wang K, Chen W, Zhang Z, Deng Y, Lian J-Q, Du P, Wei D, Zhang Y, Sun X-X, Gong L *et al.* (2020) CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther* **5**, 283.

11 Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, Narayanan A, Majowicz SA, Kwong EM, McVicar RN *et al.* (2020) SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell* **183**, 1–15.

12 Gao J, Mei H, Sun J, Li H, Huang Y, Tang Y, Duan L, Liu D, Wang Q, Gao Y *et al.* (2021) Neuropilin-1 mediates SARS-CoV-2 infection in bone marrow-derived macrophages. *bioRxiv* [PREPRINT].

13 Daly JL, Simonetti B, Antón-Plágaro C, Kavanagh Williamson M, Shoemark DK, Simón-Gracia L, Klein K, Bauer M, Hollandi R, Greber UF *et al.* (2020) Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* **270**, 861–865.

14 Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, van der Meer F, Kallio K, Kaya T, Anastasina M *et al.* (2020) Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* **370**, 856–860.

15 Asselta R, Paraboschi EM, Mantovani A & Duga S (2020) ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging* **12**, 10087–10098.

16 Mycroft-West CJ, Su D, Pagani I, Rudd TR, Elli S, Guimond SE, Miller G, Meneghetti MCZ, Nader HB, Li Y *et al.* (2020) Heparin inhibits cellular invasion by SARS-CoV-2: structural dependence of the

interaction of the surface protein (spike) S1 receptor binding domain with heparin. *Thromb Haemost* **120**, 1700–1715.
17 Moutal A, Martin LF, Boinon L, Gomez K, Ran D, Zhou Y, Stratton HJ, Cai S, Luo S, Gonzalez KB *et al.* (2020) SARS-CoV-2 spike protein co-opts VEGF-A/neuropilin-1 receptor signaling to induce analgesia. *Pain* **162**, 243–252.
18 Parker MW, Guo H-F, Li X, Linkugel AD & Vander Kooi CW (2012) Function of members of the neuropilin family as essential pleiotropic cell surface receptors. *Biochemistry* **51**, 9437–9446.
19 Plein A, Fantin A & Ruhrberg C (2014) Neuropilin regulation of angiogenesis, arteriogenesis, and vascular permeability. *Microcirculation* **21**, 315–323.
20 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A *et al.* (2020) Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* **283**, 120–128.
21 Pinto BGG, Oliveira AER, Singh Y, Jimenez L, Gonçalves ANA, Ogava RLT, Creighton R, Schatzmann Peron JP & Nakaya HI (2020) ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. *J Infect Dis* **222**, 556–563.
22 Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, Chilla S, Heinemann A, Wanner N, Liu S *et al.* (2020) Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med* **383**, 590–592.
23 Guo H-F & Vander Kooi CW (2015) Neuropilin functions as an essential cell surface receptor. *J Biol Chem* **290**, 29120–29126.
24 Hey-Cunningham AJ, Markham R, Fraser IS & Berbic M (2013) Dysregulation of vascular endothelial growth factors and their neuropilin receptors in the eutopic endometrium of women with endometriosis. *Reprod Sci* **20**, 1382–1389.
25 Sulpice E, Plouët J, Bergé M, Allanic D, Tobelem G & Merkulova-Rainon T (2008) Neuropilin-1 and neuropilin-2 act as coreceptors, potentiating proangiogenic activity. *Blood* **111**, 2036–2045.
26 Weekes CD, Beeram M, Tolcher AW, Papadopoulos KP, Gore L, Hegde P, Xin Y, Yu R, Shih LM, Xiang H *et al.* (2014) A phase I study of the human monoclonal anti-NRP1 antibody MNRP1685A in patients with advanced solid tumors. *Investig New Drugs* **32**, 653–660.
27 Wang L, Feng Y, Xie X, Wu H, Su XN, Qi J, Xin W, Gao L, Zhang Y, Shah VH *et al.* (2019) Neuropilin-1 aggravates liver cirrhosis by promoting angiogenesis via VEGFR2-dependent PI3K/Akt pathway in hepatic sinusoidal endothelial cells. *EBioMedicine* **43**, 525–536.
28 Lu Y, Xiang H, Liu P, Tong RR, Watts RJ, Koch AW, Sandoval WN, Damico LA, Wong WL & Meng YG (2009) Identification of circulating neuropilin-1 and dose-dependent elevation following anti-neuropilin-1 antibody administration. *mAbs* **1**, 364–369.
29 Schoof M, Faust B, Saunders RA, Sangwan S, Rezelj V, Hoppe N, Boone M, Billesbølle CB, Puchades C, Azumaya CM *et al.* (2020) An ultra-high affinity synthetic nanobody blocks SARS-CoV-2 infection by locking Spike into an inactive conformation. *Science* **370**, 1473–1479.
30 Hansen J, Baum A, Pascal KE, Russo V, Giordano S, Wloga E, Fulton BO, Yan Y, Koon K, Patel K *et al.* (2020) Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010–1014.
31 Walser M, Rothenberger S, Hurdiss DL, Schlegel A, Calabro V, Fontaine S, Villemagne D, Paladino M, Hospodarsch T, Neculcea A *et al.* (2020) Highly potent anti-SARS-CoV-2 multi-DARPin therapeutic candidates. *bioRxiv* [PREPRINT].
32 Maag JLV (2018) gganatogram: an R package for modular visualisation of anatograms and tissues based on ggplot2. *F1000Res* **7**, 1576.
33 Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A *et al.* (2015) Tissue-based map of the human proteome. *Science* **347**, 1260419.
34 Goulter AB, Goddard MJ, Allen JC & Clark KL (2004) ACE2 gene expression is up-regulated in the human failing heart. *BMC Med* **2**. https://doi.org/10.1186/1741-7015-2-19