

Commentary

Decoy ACE2-expressing extracellular vesicles that competitively bind SARS-CoV-2 as a possible COVID-19 therapy

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The novel strain of coronavirus that appeared in 2019, SARS-CoV-2, is the causative agent of severe respiratory disease, COVID-19, and the ongoing pandemic. As for SARS-CoV that caused the SARS 2003 epidemic, the receptor on host cells that promotes uptake, through attachment of the spike (S) protein of the virus, is angiotensin-converting enzyme 2 (ACE2). In a recent article published by Batlle et al. (*Clin. Sci. (Lond.)* (2020) **134**, 543–545) it was suggested that soluble recombinant ACE2 could be used as a novel biological therapeutic to intercept the virus, limiting the progression of infection and reducing lung injury. Another way, discussed here, to capture SARS-CoV-2, as an adjunct or alternative, would be to use ACE2⁺-small extracellular vesicles (sEVs). A competitive inhibition therapy could therefore be developed, using sEVs from engineered mesenchymal stromal/stem cells (MSCs), over-expressing ACE2.

It was recently proposed that an excess of soluble angiotensin-converting enzyme 2 (ACE2) would neutralise SARS-CoV-2 by binding the spike (S) protein and blocking virus–host cell membrane fusion [1]. The current paper proposes an improvement to the concept of competitively inhibiting SARS-CoV-2 binding to ACE2⁺ type II alveolar cells, by using small extracellular vesicles (sEVs) engineered to bind virus. As for soluble ACE2, such a sEV-mediated therapy would also prevent ACE2 down-regulation on alveolar type II cells and thus rescue their activity [2]. ACE2-mediated regulation of the renin-angiotensin pathway would thus be maintained, which could otherwise cause severe lung injury [3]. By protecting alveolar type II cells, SARS-CoV-2-binding sEVs would also ensure continued production of alveolar surfactant needed to maintain elasticity and as these cells have a progenitor function of alveolar type I cells [4], thereby maintain their repair and ensure effective gas exchange.

sEVs have much potential in disease therapy ranging from their use as drug delivery vehicles, whether carrying chemotherapeutic agents [5] or siRNA [6], to mesenchymal/stromal stem cell (MSC)-derived sEVs in ameliorating acute organ injury [7]. To achieve targeting, when used as delivery vehicles, sEVs have been engineered to express ligands that interact with receptors found on target cells [8]. However, MSC-EVs naturally target injured tissue, and early preclinical success is now being translated into the clinical setting [9]. By sharing surface receptor proteins with their parental cell, sEVs may act as decoys, for example antagonising inflammatory cytokines such as TNF- α [10]. This can extend to the decoy functions host cell EVs show for intracellular pathogens [11] and more specifically as with CD4⁺ T-cell-derived sEVs, to their ability to bind HIV [12,13].

In work carried out almost a decade ago, an increased level of leucocyte-EVs was observed at the onset of Acute Respiratory Distress Syndrome (ARDS). Deemed to be of prognostic value, it was also presumed to have a protective role [14]. More recently, the isolation of EVs from hyperoxia-induced cultured, primary alveolar type II cells has been described [15]. Whether in ARDS patients being given mechanical

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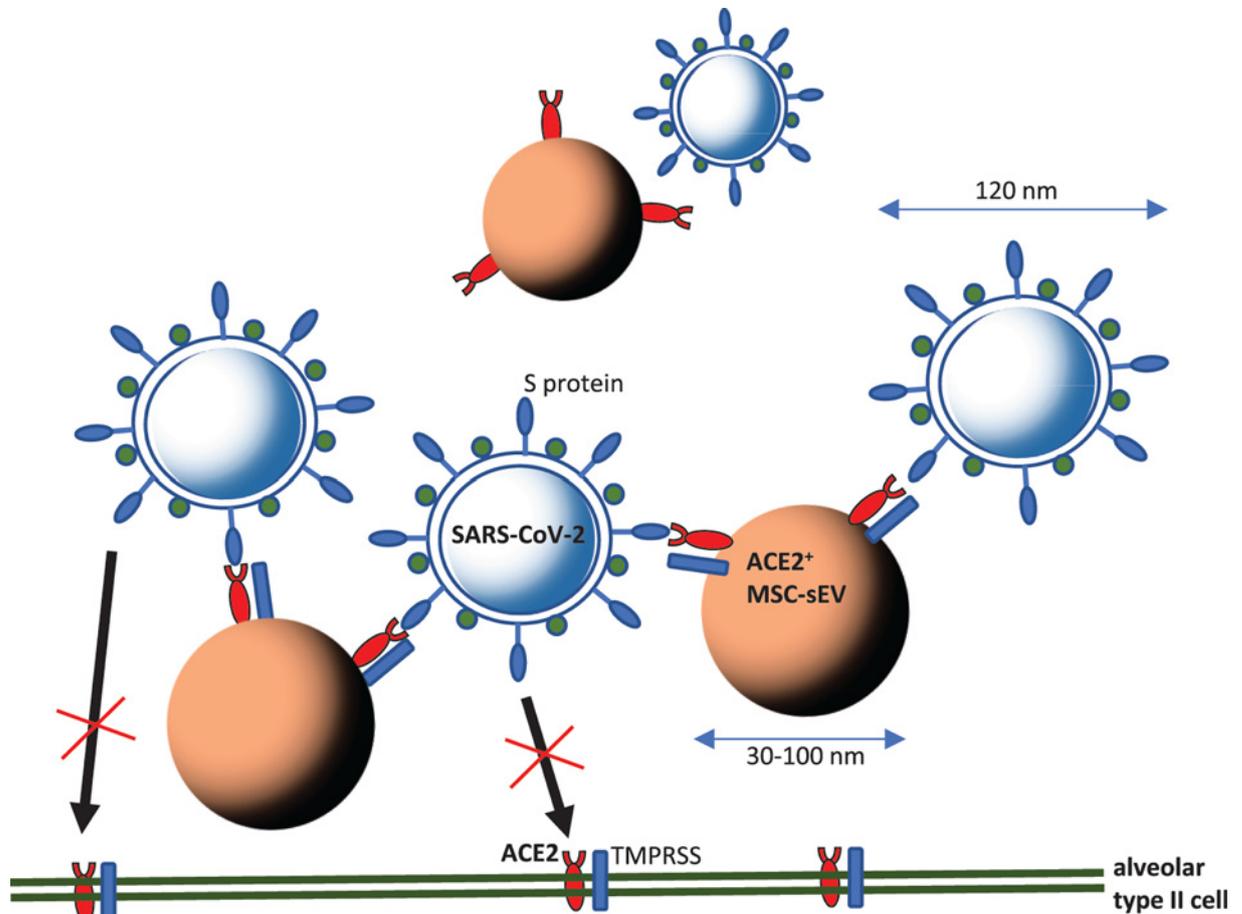


Figure 1. Schematic for ACE2-expressing small EVs binding SARS-CoV-2

Binding of SARS-CoV-2 S protein through ACE2 expressed on MSC-derived sEVs to competitively inhibit binding to ACE2 on alveolar type II cells and thereby limit infection. This could be tested using a human ACE2 transgenic mouse model and as a preliminary proof of concept study using sEVs from the tumour cell line, A549, a known model of alveolar type II cells [26] and thus a ready source of ACE2⁺ sEVs.

ventilation and oxygen, such EVs expressing ACE2, might play a decoy function for SARS-CoV-2, is open to conjecture.

There remains however, the prospect of enhancing any such decoy effect with sEVs, for example derived from MSC cells, and engineered to overexpress ACE2, that could competitively inhibit the binding of SARS-CoV-2 to ACE2-expressing alveolar type II cells (Figure 1). In the lungs of severe COVID-19 patients where ARDS promotes a hyperinflammatory condition [16], using engineered MSC-sEVs would have additional benefits [17]. Just as apoptotic cell clearance occurs in chronically inflamed lungs [18], ACE2⁺ MSC-sEVs (and associated virus) with similar ‘eat-me’ signals will also be phagocytosed [19,20]. Furthermore, they will promote increased phagocytic activity of neutrophils/monocytes, resulting in decreased lung injury [21], promote anti-inflammatory effects [17], and convert immune cells into a more immunosuppressive phenotype. [17]. MSC-EVs have also been shown to promote recovery of patients with ARDS [22] and administration of ACE2⁺ MSC-sEVs, beside their decoy function for SARS-CoV-2, would likely translate into useful therapies for ARDS [23] and so COVID-19 ARDS [24]. By way of further example, the immunomodulatory capacity of MSCs, intravenously transplanted into seven patients with COVID-19, was recently assessed. The MSCs which were ACE2⁻, and so non-infectible by SARS-CoV-2, promoted recovery from advanced COVID-19 pneumonia. They regulated the inflammatory response, reducing pro-inflammatory TNF- α , increased anti-inflammatory IL-10 and promoted tissue repair [25], thus further supporting the additional benefits, besides blocking infection of ACE2-expressing cells, of using MSC-derived sEVs to treat COVID-19.

A human recombinant soluble ACE2 (hrsACE2; APN01) has been shown to be safe in ARDS patients [27,28] resulting in reduced levels of the peptide angiotensin II and concentrations of IL-6 and on that basis is currently being trialled in COVID-19 patients [8]. In the meantime, hrsACE2 has shown efficacy in reducing early entry of SARS-CoV-2 in Vero-E6 cells and blood vessel and kidney organoids, although lung organoids were not yet tested [29]. To use ACE2 embedded in the membrane of sEVs, as an adjunct or alternative to soluble ACE2, would likely increase the effectiveness and improve delivery of such inhibition therapy. Besides competitively binding SARS-CoV-2, multivalent ACE2⁺ MSC-sEVs would have the advantage of doing so with increased binding avidity, therefore matching the likely high stoichiometry of association between several S proteins per virion and ACE2-expressing target cell membranes. They would also not be as readily degraded by proteases in the lung as would inhaled therapeutic proteins [30] and not be as expensive to develop [31].

Intranasal delivery, as we described earlier in a mouse model of hypoxia–ischaemia brain injury [32] and in a bleomycin-induced lung injury model in mice [33], in this case using ACE2⁺ MSC-sEVs, in the human ACE2 transgenic mouse model [34] or golden hamster model [35], should now be given a high priority, as a competitive inhibition therapy against SARS-CoV-2.

Competing Interests

The author declares that there are no competing interests associated with the manuscript.

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Abbreviations

ACE2, angiotensin-converting enzyme 2; ARDS, Acute Respiratory Distress Syndrome; COVID-19, coronavirus disease 2019; EV, extracellular vesicle; hrsACE2, human recombinant soluble ACE2; MSC, mesenchymal/stromal stem cell; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

References

- 1 Battle, D., Wysocki, J. and Satchell, K. (2020) Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin. Sci. (Lond.)* **134**, 543–545, <https://doi.org/10.1042/CS20200163>
- 2 Verdecchia, P., Cavallini, C., Spanevello, A. and Angeli, F. (2020) The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Int. Med.* **76**, <https://doi.org/10.1016/j.ejim.2020.04.037>
- 3 Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B. et al. (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436**, 112–116, <https://doi.org/10.1038/nature03712>
- 4 Barkauskas, C.E., Crouce, M.J., Rackley, C.R., Bowie, E.J., Keene, D.R., Stripp, B.R. et al. (2013) Type 2 alveolar cells are stem cells in adult lung. *J. Clin. Invest.* **123**, 3025–3036, <https://doi.org/10.1172/JCI68782>
- 5 Yong, T., Zhang, X., Bie, N., Zhang, H., Zhang, X., Li, F. et al. (2019) Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy. *Nat. Commun.* **10**, 3838, <https://doi.org/10.1038/s41467-019-11718-4>
- 6 Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Likhani, S. and Wood, M.J. (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* **29**, 341–345, <https://doi.org/10.1038/nbt.1807>
- 7 Park, K.S., Bandeira, E., Shelke, G.V., Lasser, C. and Lotvall, J. (2019) Enhancement of therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. *Stem Cell Res. Ther.* **10**, 288
- 8 Zhang, H., Penninger, J.M., Li, Y., Zhong, N. and Slutsky, A.S. (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* **46**, 586–590, <https://doi.org/10.1007/s00134-020-05985-9>
- 9 Akyurekli, C., Le, Y., Richardson, R.B., Fergusson, D., Tay, J. and Allan, D.S. (2015) A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. *Stem Cell Rev. Rep.* **11**, 150–160, <https://doi.org/10.1007/s12015-014-9545-9>
- 10 Duong, N., Curley, K., Brown, A., Campanelli, A., Do, M.A., Levy, D. et al. (2019) Decoy exosomes as a novel biologic reagent to antagonize inflammation. *Int. J. Nanomed.* **14**, 3413–3425, <https://doi.org/10.2147/IJN.S196975>
- 11 Inal, J.M., Ansa-Addo, E.A. and Lange, S. (2013) Interplay of host-pathogen microvesicles and their role in infectious disease. *Biochem. Soc. Trans.* **41**, 258–262, <https://doi.org/10.1042/BST20120257>
- 12 Dias, M. V.S., Costa, C.S. and daSilva, L. L.P. (2018) The ambiguous roles of extracellular vesicles in HIV replication and pathogenesis. *Front. Microbiol.* **9**, 2411, <https://doi.org/10.3389/fmicb.2018.02411>
- 13 de Carvalho, J.V., de Castro, R.O., da Silva, E.Z., Silveira, P.P., da Silva-Januario, M.E., Arruda, E. et al. (2014) Nef neutralizes the ability of exosomes from CD4⁺ T cells to act as decoys during HIV-1 infection. *PLoS ONE* **9**, e113691, <https://doi.org/10.1371/journal.pone.0113691>
- 14 Guervilly, C., Lacroix, R., Forel, J.M., Roch, A., Camoin-Jau, L., Papazian, L. et al. (2011) High levels of circulating leukocyte microparticles are associated with better outcome in acute respiratory distress syndrome. *Crit. Care* **15**, R31, <https://doi.org/10.1186/cc9978>
- 15 Moon, H.G., Cao, Y., Yang, J., Lee, J.H., Choi, H.S. and Jin, Y. (2015) Lung epithelial cell-derived extracellular vesicles activate macrophage-mediated inflammatory responses via ROCK1 pathway. *Cell Death Dis.* **6**, e2016, <https://doi.org/10.1038/cddis.2015.282>

- 16 Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S. and Manson, J.J. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* **395**, 1033–1034, [https://doi.org/10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0)
- 17 Harrell, C.R., Jovicic, N., Djonov, V., Arsenijevic, N. and Volarevic, V. (2019) Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. *Cells* **8**, <https://doi.org/10.3390/cells8121605>
- 18 Grabiec, A.M. and Hussell, T. (2016) The role of airway macrophages in apoptotic cell clearance following acute and chronic lung inflammation. *Semin. Immunopathol.* **38**, 409–423, <https://doi.org/10.1007/s00281-016-0555-3>
- 19 Gonda, A., Kabagwira, J., Senthil, G.N. and Wall, N.R. (2019) Internalization of exosomes through receptor-mediated endocytosis. *Mol. Cancer Res.* **17**, 337–347, <https://doi.org/10.1158/1541-7786.MCR-18-0891>
- 20 Feng, D., Zhao, W.L., Ye, Y.Y., Bai, X.C., Liu, R.Q., Chang, L.F. et al. (2010) Cellular internalization of exosomes occurs through phagocytosis. *Traffic* **11**, 675–687, <https://doi.org/10.1111/j.1600-0854.2010.01041.x>
- 21 Hao, Q., Gudapati, V., Monsel, A., Park, J.H., Hu, S., Kato, H. et al. (2019) Mesenchymal stem cell-derived extracellular vesicles decrease lung injury in mice. *J. Immunol.* **203**, 1961–1972, <https://doi.org/10.4049/jimmunol.1801534>
- 22 Shah, T., Qin, S., Vashi, M., Predescu, D.N., Jeganathan, N., Bardita, C. et al. (2018) Alk5/Runx1 signaling mediated by extracellular vesicles promotes vascular repair in acute respiratory distress syndrome. *Clin. Transl. Med.* **7**, 19, <https://doi.org/10.1186/s40169-018-0197-2>
- 23 Shah, T.G., Predescu, D. and Predescu, S. (2019) Mesenchymal stem cells-derived extracellular vesicles in acute respiratory distress syndrome: a review of current literature and potential future treatment options. *Clin. Transl. Med.* **8**, 25, <https://doi.org/10.1186/s40169-019-0242-9>
- 24 Gibson, P.G., Qin, L. and Pua, S. (2020) COVID-19 ARDS: clinical features and differences to “usual” pre-COVID ARDS. *Med. J. Aust.*
- 25 Leng, Z., Zhu, R., Hou, W., Feng, Y., Yang, Y., Han, Q. et al. (2020) Transplantation of ACE2(-) mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis.* **11**, 216–228, <https://doi.org/10.14336/AD.2020.0228>
- 26 Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W. and Todaro, G. (1976) A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. *Int. J. Cancer* **17**, 62–70
- 27 Khan, A., Benthin, C., Zeno, B., Albertson, T.E., Boyd, J., Christie, J.D. et al. (2017) A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit. Care* **21**, 234, <https://doi.org/10.1186/s13054-017-1823-x>
- 28 Zhang, H. and Baker, A. (2017) Recombinant human ACE2: acing out angiotensin II in ARDS therapy. *Crit. Care* **21**, 305, <https://doi.org/10.1186/s13054-017-1882-z>
- 29 Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R.A., Stahl, M. et al. (2020) Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* **181**, <https://doi.org/10.1016/j.cell.2020.04.004>
- 30 Woods, A., Andrian, T., Sharp, G., Bicer, E.M., Vandra, K.A., Patel, A. et al. (2020) Development of new in vitro models of lung protease activity for investigating stability of inhaled biological therapies and drug delivery systems. *Eur. J. Pharm. Biopharm.* **146**, 64–72, <https://doi.org/10.1016/j.ejpb.2019.11.005>
- 31 Lagassé, H.A., Alexaki, A., Simhadri, V.L., Katagiri, N.H., Jankowski, W., Sauna, Z.E. et al. (2017) Recent advances in (therapeutic protein) drug development. *F1000Res.* **6**, 113, <https://doi.org/10.12688/f1000research.9970.1>
- 32 Sisa, C., Kholia, S., Naylor, J., Herrera Sanchez, M.B., Bruno, S., Deregius, M.C. et al. (2019) Mesenchymal stromal cell derived extracellular vesicles reduce hypoxia-ischaemia induced perinatal brain injury. *Front. Physiol.* **10**, 282, <https://doi.org/10.3389/fphys.2019.00282>
- 33 Tan, J.L., Lau, S.N., Leaw, B., Nguyen, H. P.T., Salamonsen, L.A., Saad, M.I. et al. (2018) Amnion epithelial cell-derived exosomes restrict lung injury and enhance endogenous lung repair. *Stem Cells Transl. Med.* **7**, 180–196, <https://doi.org/10.1002/sctm.17-0185>
- 34 McCray, Jr, P.B., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L. et al. (2007) Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *J. Virol.* **81**, 813–821, <https://doi.org/10.1128/JVI.02012-06>
- 35 Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L. et al. (2020) Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature*, <https://doi.org/10.1038/s41586-020-2342-5>