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Broad Efficacy of a Computationally Designed ACE2 Decoy Against SARS-CoV-2 Omicron Variants and Related Viruses In Vitro and In Vivo

Brandon Havranek
Thomas Jefferson University, brandon.havranek@students.jefferson.edu

Graeme W. Lindsey
University of Illinois at Urbana-Champaign

Yusuke Higuchi
Kyoto Prefectural University of Medicine, Kyoto, Japan

Yumi Itoh
Osaka University, Osaka, Japan

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The SARS-CoV-2 omicron variant (B.1.1.529) and its sublineages are currently the dominant variants in the United States accounting for nearly 90% of COVID-19 cases.

**Problem:** The S protein receptor-binding domain (RBD), located on the S1 subunit of the S protein, binds the human angiotensin-converting enzyme 2 (hACE2) leading to S1 shedding and proteolytic processing of S2 that is important for membrane fusion and release of viral RNA. Various neutralizing therapeutics including protein minibinders, peptides, monoclonal antibodies, and nanobodies have been developed to block the critical interaction between the RBD and hACE2. However, these therapies are often developed against the S protein of wildtype or a specific variant of SARS-CoV-2, making them highly susceptible to mutational escape.

**Solution:** A strategy employed by our group includes using sACE2 (soluble dimeric ACE2) that contains both the protease and dimerization domains with enhanced RBD affinity to outcompete native ACE2 expressed on host cells, acting as a "decoy" to block the interaction between the RBD and hACE2 (Figure 1). sACE2 has moderate affinity for the S protein (~20 nM). Therefore, sACE2 must be engineered (by introducing affinity enhancing mutations) to bind with tighter affinity to outcompete membrane bound ACE2-S interaction and rival the potency of mAbs. These sACE2 derivatives maintain close similarity to the native ACE2 receptor making them extremely resistant to virus escape. Any mutation in the RBD that limits binding to the sACE2 derivative will likely have reduced binding towards native ACE2 receptors potentially making the virus unfit to propagate.

**Objectives**

- Develop a broad-spectrum pan-coronavirus affinity-enhanced ACE2 decoy therapeutic that can protect against both future SARSCoV-2 variants and developing SARSCoV-2-associated viruses that can cross over from animals to humans in the future.

**Background**

The SARS-CoV-2 Spike protein is composed of two subunits: S1 and S2. The S1 subunit contains an N-terminal domain (NTD) and a receptor-binding domain (RBD), and the S2 subunit contains an angle domain (TAD) and a fusion peptide (FPF).

**Computation**

The receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein is highly conserved. The RBD is composed of three highly conserved domains, D1, D2, and D3, which are involved in binding to the human ACE2 receptor. The ACE2 receptor is a type I transmembrane protein that binds to the SARS-CoV-2 Spike protein through its RBD domain. The RBD of the Spike protein is composed of two α-helices, H1 and H2, which form a deep cleft that is highly conserved across different coronaviruses.

**Methods**

- MD simulations: Amber10.0 software.
- Protein design: Rosetta software using "Conformal Move" protocol.
- Flow cytometry: Cytometric analysis on a BD Accuri C6 using instrument software.
- Figures: Figure generated using Adobe Illustrator and Photoshop.
- Statistical analysis: Student's t-test and one-way ANOVA with Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001.

**Results**

1. We compared the binding affinity of wildtype and mutant RBDs to ACE2 using a computational design tool. The wildtype RBD showed the highest binding affinity to ACE2, while the mutant RBDs showed significantly lower binding affinity.

2. We observed that the mutant RBDs were less susceptible to mutational escape compared to wildtype RBD, indicating that the decoy strategy could be effective in blocking the virus.

3. We also observed that the decoy strategy could be effective in blocking the virus in vitro and in vivo.

**Conclusion**

The decoy strategy developed using sACE2 has the potential to be an effective therapeutic for the SARS-CoV-2 infection. Further studies are needed to validate the therapeutic potential of this strategy in clinical trials.