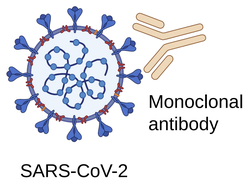
Optimized ACE2-Fc fusion proteins with picomolar pan-neutralization of SARS-CoV-2 variants, including, JN.1 and KP.2

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INTRODUCTION





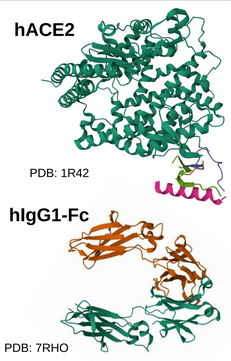
Prevention of high-risk groups from:

- SARS-CoV-2 infection
- COVID-19 adverse events

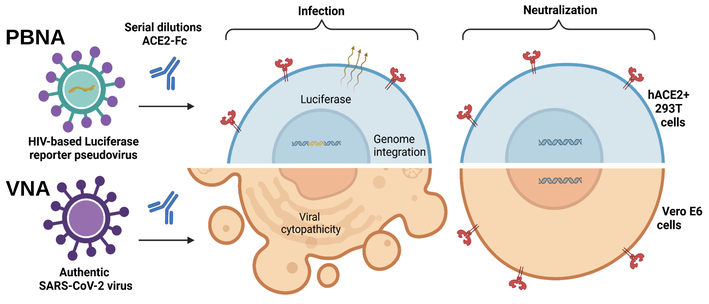
Although **neutralizing monoclonal antibodies** have been approved for clinical use in **immunocompromised populations**, the emergence of new **SARS-CoV-2** variants is associated with an evasion from acquired immunity that renders these therapies **obsolete**. Since **ACE2** is the natural viral host receptor, designing an **ACE2-based decoy molecule** could provide neutralizing activity against all SARS-CoV-2 variants for prophylactic purposes. Here, we engineered **ACE2-Fc molecules** with enhanced neutralization (picomolar activity) against all tested variants.

METHODS

- Design of recombinant ACE2-Fc molecules based on the fusion of **human ACE2 peptidase domain** and **human IgG1 Fc domain**.



- Addition of ACE2-inactivating (H345A and H505A) and *in silico*-predicted mutations (**M1**: T27F, K31W, S43M, Q81S) in the ACE2-binding interface to enhance its affinity for the **Spike receptor-binding domain** (RBD).
- **Pseudovirus** (PBNA) and **virus-based** (VNA) **neutralization assays** against pre-Omicron and Omicron-derived SARS-CoV- 2 variants.



RESULTS

The optimization of ACE2-based Fc fusion proteins through *in silico*-predicted binding mutations, the use of flexible linkers, and an increased valency allows the neutralization of all pre-Omicron and Omicron-derived SARS-CoV-2 variants with picomolar activity.

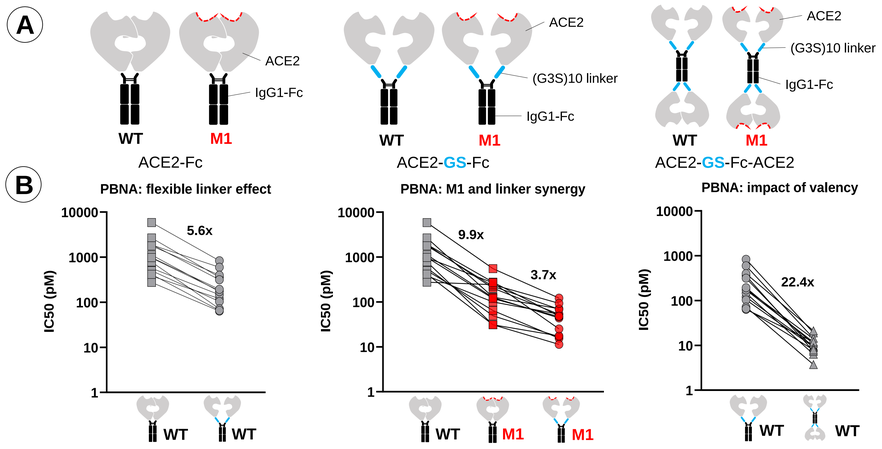


Figure 1. Neutralizing optimization of ACE2-Fc molecules. A) Structural designs of ACE2-Fc molecules achieved through the combination of three approaches: *in silico*-predicted mutations (M1), (G3S)10 flexible linker, and the increase of binding valency. B) Analysis by PBNA of the designed ACE2-Fc molecules and the impact of each optimization approach against a panel of SARS-CoV-2 variants.

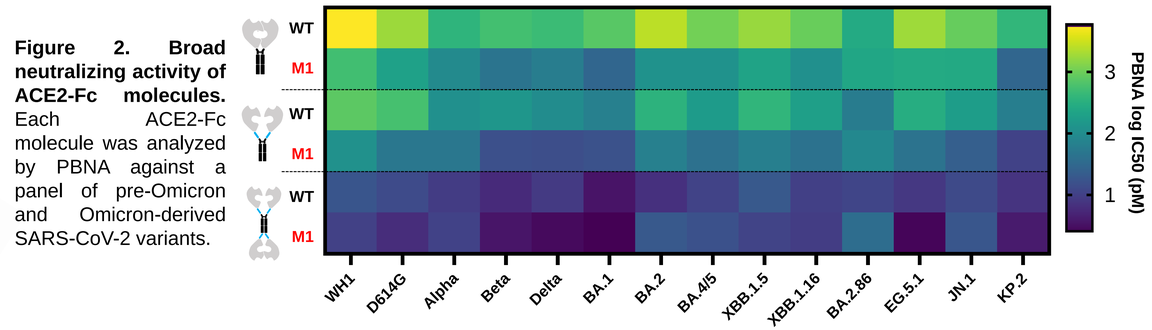


Figure 2. Broad neutralizing activity of ACE2-Fc molecules. Each ACE2-Fc molecule was analyzed by PBNA against a panel of pre-Omicron and Omicron-derived SARS-CoV-2 variants.

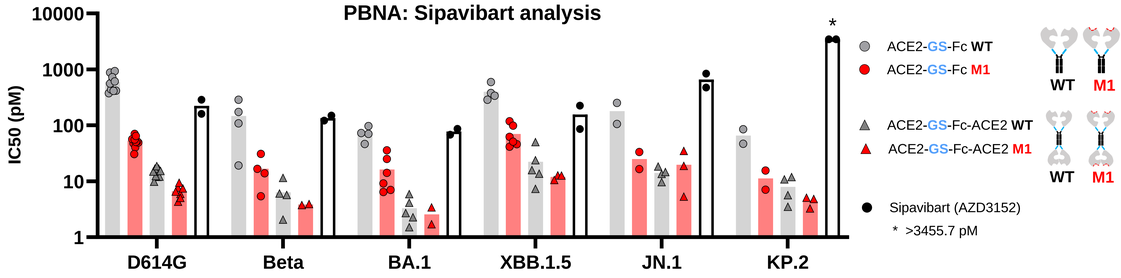


Figure 3. Comparison of the neutralizing activity with the monoclonal antibody Sipavibart (AZD3152). Sipavibart was evaluated under identical PBNA conditions against relevant SARS-CoV-2 variants and compared to the optimized ACE2-Fc molecules that previously exhibited better neutralization.

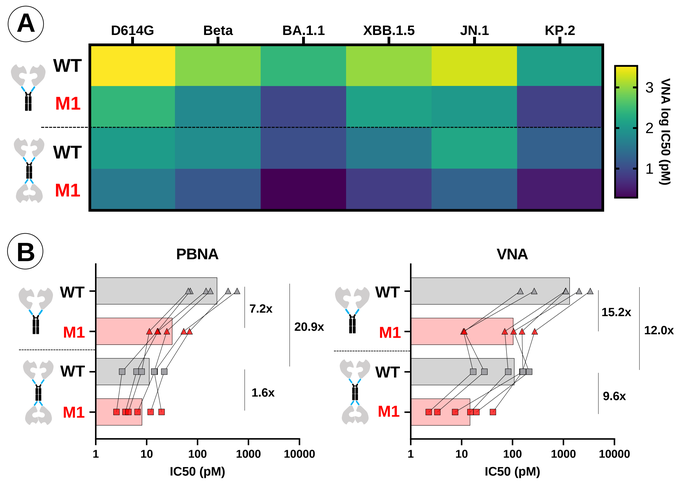


Figure 4. Neutralizing activity of ACE2-Fc molecules against authentic SARS-CoV-2 viruses. A) Optimized ACE2-Fc molecules were analyzed via VNA against D614G, Beta, BA.1.1, XBB.1.5, JN.1 and KP.2. B) Comparison of neutralization data obtained by PBNA and VNA.

CONCLUSIONS

- The introduction of ***in silico*-predicted mutations** at the binding interface, the **flexible linker,** and the **increased valency** all contributed to **enhance the neutralizing potency** against SARS-CoV-2. Furthermore, the combination of M1 mutations and the flexible linker had an **additive effect**.
- **ACE2-Fc molecules** effectively neutralized all tested pre-Omicron and Omicron-derived SARS-CoV-2 variants. In comparison, **Sipavibart** exhibited a loss of neutralizing activity against recently circulating variants such as JN.1 and KP.2.
- Optimized ACE2-Fc molecules exhibited comparable neutralizing activity against SARS-CoV-2 pseudoviruses and authentic viruses. However, ACE2-Fc molecules **mutated** at the binding interface showed **greater benefit against authentic viruses**.

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