SARS-CoV-2 Omicron and Pan-Variant Neutralization Activity of Ensovibep: a DARPin® Therapeutic Candidate for Treatment of Covid-19

Abs # 209

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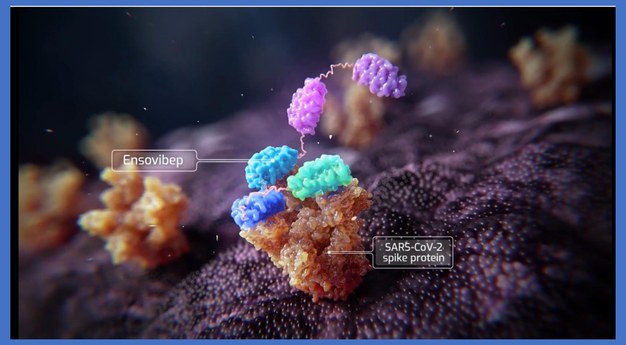
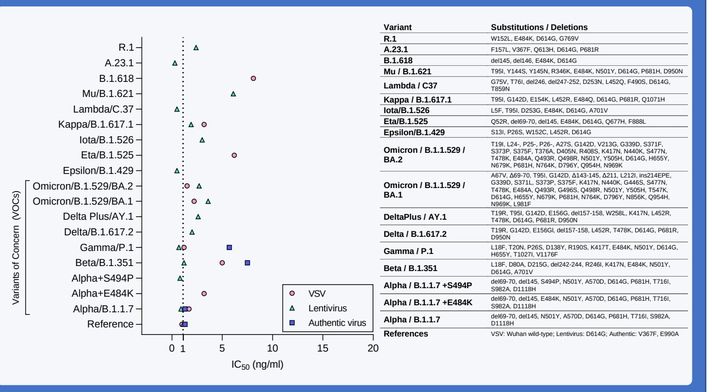
BACKGROUND - The omicron variant of the SARS-CoV-2 virus highlights how a pandemic can change course. Omicron's increased transmission and ability to evade natural or vaccine-induced immunity developed against earlier variants was a strong reminder of the power of viral evolution. Therapies with potential for pan-variant potency are a key component of effective pandemic management.

ENSOVIBEP is a first-in-class anti-SARS-CoV-2 DARPin therapeutic candidate that uses three distinct DARPin domains (R1, R2, R3) with similar paratopes to cooperatively bind to different regions of the receptor binding domain (RBD) of the SARS-CoV-2 spike protein trimer, thereby preventing interaction with the host ACE2 receptor. The multi-specific attachment imparted by the RBD-binding DARPin domains limits the impact of spike protein mutations on antiviral potency. We present here data supporting the pan-variant potency of ensovibep.



Depiction based on structural data showing ensovibep RBD-binding DARPin domains (cyan, blue, green) binding to the RBD of the SARS-CoV-2 spike protein trimer. The two additional DARPin domains (purple) bind to human serum albumin (HSA, not shown for clarity) that provide half-life extension in circulation.



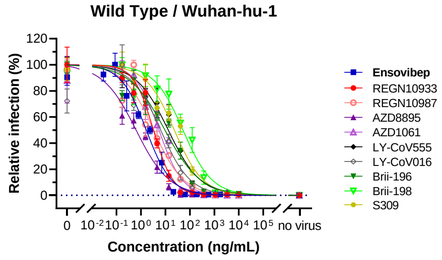
Variant	Substitutions / Deletions
R.1	W152L, E484K, D614G, G769V
A.23.1	F157L, V367F, Q613H, D614G, P681R
B.1.618	del145, del146, E484K, D614G
Mu / B.1.621	T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H, D950N
Lambda / C37	G75V, T76I, del246, del247-252, D253N, L452Q, F490S, D614G, T859N
Kappa / B.1.617.1	T95I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H
Iota/B.1.526	L5F, T95I, D253G, E484K, D614G, A701V
Eta/B.1.525	Q52R, del69-70, del145, E484K, D614G, Q677H, F888L
Epsilon/B.1.429	S13I, P26S, W152C, L452R, D614G
Omicron / B.1.1.529 / BA.2	T19I, L24-, P25-, P26-, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron / B.1.1.529 / BA.1	A67V, del69-70, T95I, G142D, del143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
DeltaPlus / AY.1	T19R, T95I, G142D, E156G, del157-158, L452R, T478K, D614G, P681R, D950N
Delta / B.1.617.2	T19R, G142D, E156G, del157-158, L452R, T478K, D614G, P681R, D950N
Gamma / P.1	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
Beta / B.1.351	L18F, D80A, D215G, del242-244, R246I, K417N, E484K, N501Y, D614G, A701V
Alpha / B.1.1.7 +S494P	del69-70, del145, S494P, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
Alpha / B.1.1.7 +E484K	del69-70, del145, E484K, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
Alpha / B.1.1.7	del69-70, del145, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
References	VSV: Wuhan wild-type; Lentivirus: D614G; Authentic: V367F, E990A

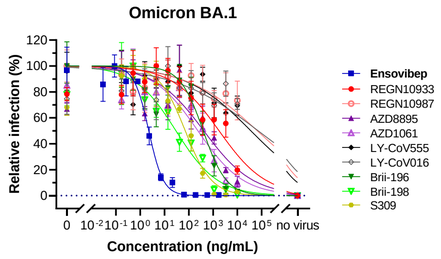
Neutralization by ensovibep in VSV, lentivirus and authentic virus assays across a range of variants of concern and variants of interest identified during the 2020-2021 SARS-CoV-2 coronavirus pandemic. Notably, ensovibep potency (IC_{50}) against all tested variants remains in the 1-10 ng/mL range; less than an order of magnitude difference from the reference/Wuhan WT virus. (Rothenberger *et al* .2021: https://doi.org/10.1101/2021.02.03.429164)

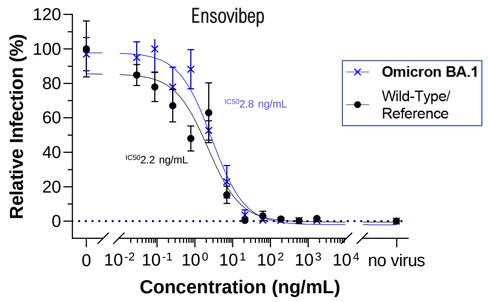
Compound	Wild Type IC_{50} (ng/mL)	Omicron BA.1 IC_{50} (ng/mL)	Omicron BA.1 Fold change to WT
Ensovibep	1.6	2.2	1.4
REGN10933	3.2	>1000	>100
REGN10987	3.3	>1000	>100
LY-CoV555	13	>1000	>100
LY-CoV016	6.4	>1000	>100
S309	23	72	3.1
AZD8895	0.6	415	>100
AZD1061	5.5	237	43
Brii-196	9.5	392	41
Brii-198	52	30	0.6

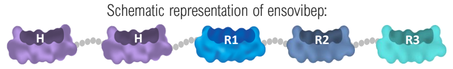
IC_{50}: green: <10 ng/mL; orange: 10-100 ng/mL; dark orange: 100-1000 ng/mL; red: >1000 ng/mL
fold change to wt: green: <10-fold; orange: 10-100-fold; red: >100-fold

Titration curves (right panels; mean ±SEM) and IC_{50} values (above) for VSV-pseudotype neutralization assays with wild-type and Omicron BA.1 variant spike protein. Ensovibep was tested together with a panel of clinically validated monoclonal antibodies. The table provides the numeric IC_{50} values as well as the fold change with respect to the wild-type.

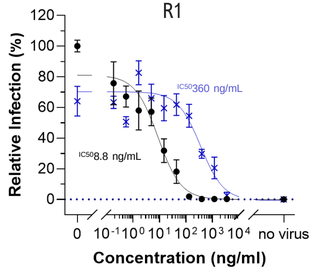


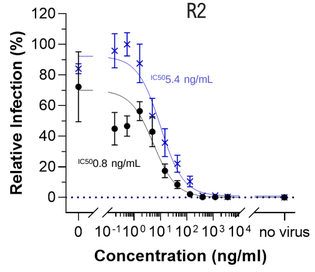


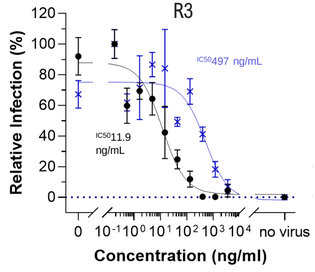




Entity	Wild-Type IC_{50} (ng/mL)	Omicron BA.1 IC_{50} (ng/mL)	Omicron BA.1 Fold change to WT
Ensovibep	2.2	2.8	1.3
R1	8.8	359.6	40.7
R2	0.8	5.4	9.9
R3	11.93	496.8	41.6





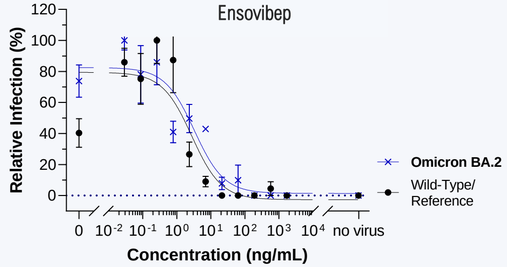


Titration curves and IC_{50} values for VSV-pseudotype neutralization assays of ensovibep and the individual ensovibep DARPin domains against WT and BA.1 variant spike proteins of SARS-CoV-2. Some reduction in potency was seen for each individual DARPin domain (R1, R2, R3), however the tri-specific ensovibep containing all three domains retained potency.

METHODS - Plasmid pCAGGS containing the Wuhan-hu-1 spike protein of SARS-CoV-2 was used as wild-type (WT), reference strain, and spike protein mutants were generated using standard PCR molecular biology techniques.

The pseudotype system was based on the recombinant VSV*ΔG-Luc virus with G replaced by genes encoding green fluorescent protein and luciferase.

The neutralization assay was conducted using serial dilutions of ensovibep (or histidine tagged ensovibep) in quadruplicates in DMEM-2 % [vol/vol] FCS supplemented with 20 µM human serum albumin mixed with an equal volume of DMEM-2% FCS containing 250 infectious units (IU) per well of SARS-CoV-2 pseudoviruses. After incubation (1 h at 37 °C), Vero E6 cells were inoculated and further incubated at 37 °C for 16 h and washed. Analysis were undertaken using the ONE-Glo™ luciferase assay system. Raw data (relative light unit values) were exported to GraphPad Prism v8.4.3. IC_{50} was modelled with a nonlinear regression fit with settings for log (inhibitor) vs normalized response curves. Data points are plotted by the mean ± SEM (standard error of mean) of quadruplicate data.



Above - Titration curves for VSV-pseudotype neutralization assays for ensovibep against SARS-CoV-2 WT and Omicron BA.2 variant spike proteins showing potency is maintained against BA.2. Below - Table of the IC_{50} values and fold change.

Entity	Wild-Type IC_{50} (ng/mL)	Omicron BA.2 IC_{50} (ng/mL)	Omicron BA.2 Fold change to WT
Ensovibep	2.8	3.3	1.2

CONCLUSIONS – The cellular neutralization potency of ensovibep is maintained across SARS-CoV-2 variants, including both BA.1 and BA.2 Omicron lineages. These findings highlight the multi-specific and cooperative binding characteristics of ensovibep, which was designed with the intent develop a durable treatment that could continue to bind to the spike protein of a rapidly evolving virus. Ensovibep continues to be investigated in clinical trials.

DISCLOSURES - This work was supported independently by investigators at the US Food and Drug Administration, Center for Biologics Evaluation and Research as part of Therapeutics Research Team for the US government COVID-19 response efforts. CGK and KR are employees of Novartis Institutes for Biomedical Research and Novartis Pharma AG, respectively. MW, FM, AB, SM, & MTS are employees of and hold ownership interest in Molecular Partners AG.