Poster 0463

Structure of VYD2311 & in vitro neutralisation against a panel of contemporary SARS-CoV-2 variants

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KEY FINDINGS

- Affinity optimisation of pemivibart led to generation of VYD2311, a half-life extended monoclonal antibody with enhanced neutralising activity against SARS-CoV-2 variants
- VYD2311 RBD co-structure demonstrated conservation of critical interaction determinants and mechanism of binding
- VYD2311 demonstrated sustained neutralisation against all variants tested, including LP.8.1

INTRODUCTION[1]

- Given the evolution of SARS-CoV-2 variants that display resistance to monoclonal antibody (mAb) therapies, the development of next-generation mAbs with activity against circulating variants is needed to continue to protect certain immunocompromised populations
- VYD2311 is a recombinant human monoclonal IgG1λ antibody that is derived from pemivibart (VYD222)
- In order to derive VYD2311, pemivibart went through a yeast binding affinity maturation to improve neutralizing activity
- Affinity maturation identified ADI-90030 as lead candidate
- Following half life optimization of ADI-90030, VYD2311 was selected for therapeutic development
- VYD2311 targets the SARS-CoV-2 spike protein receptor binding domain (RBD), thereby inhibiting virus attachment to the human ACE2 receptor on host cells
- Here, we characterize the structure and in vitro neutralising potency of VYD2311 against a panel of SARS-CoV-2 variants

METHODS[2,3]

Structure

- The VYD2311 Fab:HK.3 RBD complex was formed by mixing Fab with RBD in a 1:1.2 molar ratio of Fab to antigen and allowing the mixture to incubate overnight on ice
- The complex was then purified using SEC and concentrated to 13.5 mg/mL, in 20 mM Tris-HCl pH 7.4 and 150 mM NaCl
- The complex crystallised in a sitting drop vapor diffusion setup in 0.15 M magnesium acetate, 0.1 M citrate pH 5.5-6.0 and 16-18% v/v PEG Smear Broad (Molecular Dimensions defined mix of PEGs)
- After transferring the crystals to a suitable cryoprotectant, they were flash cooled in liquid nitrogen and exposed to synchrotron radiation at station BioMAX, MAX IV, Lund, Sweden
- X-ray diffraction data was processed with EDNA using XDS and Aimless
- The structure was determined via molecular replacement using the Phaser software

Pseudovirus Neutralisation

- SARS-CoV-2 pseudovirus neutralisation assays were performed using the PhenoSense SARS-CoV-2 Neutralising Antibody Assay (Labcorp Monogram Biosciences)
- Pseudoviruses bearing SARS-CoV-2 variant spike proteins were produced by co-transfecting HEK293 cells with codon-optimized spike sequence expression vectors and an HIV genomic vector containing a firefly luciferase reporter gene replacing the HIV envelope gene
- To test antibody neutralisation, a predetermined amount of pseudovirus was incubated with titrating amounts of test mAb for 1 hour at 37 °C before inoculating HEK293 cells expressing hACE2 and TMPRSS2. After 3 days luciferase activity was assessed
- Percent neutralisation was calculated using the formula 100% x {1 – (RLU virus + sample + cells) / (Avg RLU virus + diluent + cells)}. Neutralisation IC_{50} values were determined based on a four parameter logisitic regression of mAb concentration versus % inhibition

RESULTS[2,3]

Structure

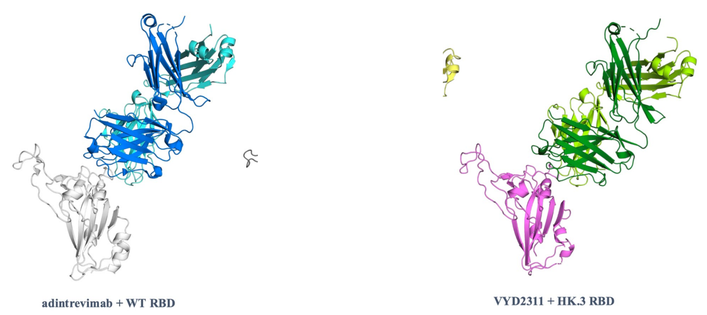
- Alignment of the VYD2311:HK.3 structure to the adintrevimab:wild type (WT) RBD structure (PDB ID 7u2d) shows strong structural overlap (**Figure 1**)
- The VYD2311 epitope overlaps with the receptor binding site consistent with a mechanism of action of direct inhibition of ACE2 binding (**Figure 2**)

Neutralisation

- VYD2311 neutralised the SARS-CoV-2 pseudovirus variants that were tested, with a potency ranging from 0.0017-0.0420 µg/mL, including WT (D614G), Delta, BA.1, and BA.2 variants, as well as current emerging or dominant variants such as JN.1, KP.3, KP.3.1.1, XEC, and LP.8.1 (**Table 1, Figure 3**)

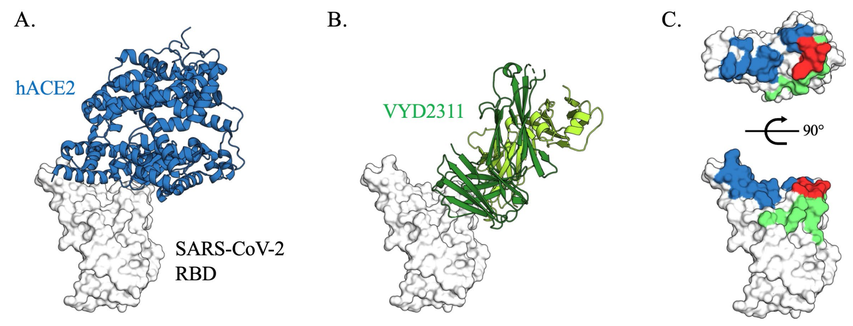
RESULTS - Figures and Tables[2]

Figure 1: VYD2311 Shares the Adintrevimab and Pemivibart Binding Motif



The X-ray crystal structure of the VYD2311 Fab domain (dark green/light green) bound to HK.3 RBD (pink) was aligned to the previously solved structure of adintrevimab Fab domain (blue/cyan) bound to WT RBD (white), PDB ID 7u2d. The VYD2311:HK.3 RBD structure displays a high degree of similarity to the adintrevimab:WT RBD structure with Cα root mean square deviation (RMSD) of only 0.45 Å^2.

Figure 2: Structural Representation of the VYD2311 Epitope Compared to hACE2 Binding Site on RBD



(A) The crystal structure of SARS-CoV-2 RBD (white surface) bound with hACE2 (blue cartoon) (PDB ID: 6m0j). (B) The crystal structure of VYD2311 (green cartoon) from the complex bound to HK.3 RBD is shown aligned to the RBD domain (white surface) from the hACE2 + RBD complex. Alignment was achieved by aligning the HK.3 RBD domain from the VYD2311 complex structure to the RBD domain of the hACE2 complex with a Cα RMSD of 0.47 Å^2. (C) Positions within 5 Å of hACE2 bound to SARS-CoV-2 RBD are shown as a blue surface, those within 5 Å of VYD2311 bound to RBD are shown as a green surface. Positions that fall within 5 Å of both hACE2 and VYD2311 are colored red and include positions 498, 500, 501, 502, and 505.

Figure 3: VYD2311 Neutralisation of SARS-CoV-2 Pseudoviruses[3]

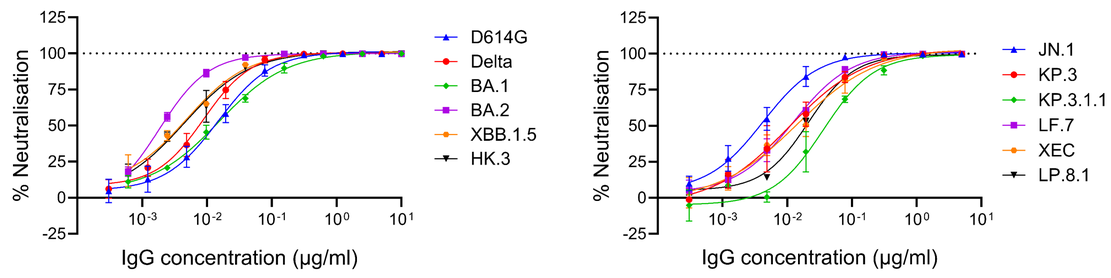


Table 1: VYD2311 Half Maximal Inhibitory Concentration (IC_{50}) Values against SARS-CoV-2 Pseudovirus variants[3]

Variant	Mean IC_{50} (µg/mL)	Variant	Mean IC_{50} (µg/mL)
WT (D614G)	0.0132	JN.1	0.0048
Delta	0.0068	KP.3	0.0117
BA.1	0.0130	KP.3.1.1	0.0420
BA.2	0.0017	LF.7	0.0109
XBB.1.5	0.0037	XEC	0.0142
HK.3	0.0040	LP.8.1	0.0189

CONCLUSIONS

- VYD2311 neutralised clinically relevant and recently emergent SARS-CoV-2 variants tested in pseudovirus assays
- The antiviral potency of VYD2311 against SARS-CoV-2 variants tested may offer the ability to deliver clinically meaningful titers and supports further investigation using more patient-friendly routes of administration such as intramuscular delivery

DISCLOSURES

Funding for this research was provided by Invivyd, Inc.
DC, BW, AK, JH, CP, RM, BW, PH, FG, and RA are employees of Invivyd and may own stock.

REFERENCES

1. VYD2311-DOF-001
2. VYD2311-DOF-002
3. VYD2311-DOF-003

Poster Presented at ECCMID 2025; April 11-15, Veinna, Austria

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