In vitro and *in vivo* characterization of a broadly neutralizing anti-SARS-CoV-2 antibody isolated from a semi-immune phage display library

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INTRODUCTION

Neutralizing antibodies targeting the receptor-binding domain (RBD) of SARS-CoV-2 are among the most promising strategies to prevent and/or treat COVID-19. However, as SARS-CoV-2 has evolved into new variants, most of the neutralizing antibodies authorized by the US FDA and/or EMA to treat COVID-19 have shown reduced efficacy or have failed to neutralize the variants of concern (VOCs), particularly B.1.1.529 (Omicron). Previously, we reported the discovery and characterization of antibodies with high affinity for SARS-CoV-2 RBD Wuhan (WT), B.1.617.2 (Delta), and B.1.1.529 (Omicron) strains (Antibodies. 11 (1): 13, 2022). One of the antibodies, called IgG-A7, also blocked the interaction of human angiotensin-converting enzyme 2 (hACE2) with the RBDs of the three strains, suggesting it may be a broadly SARS-CoV-2 neutralizing antibody. In this work we complete the physicochemical characterization of the IgG-A7 antibody previously reported by our work group and we evaluate its neutralizing activity against IgG-A7 in *in vitro* and *in vivo* infection models.

RESULTS

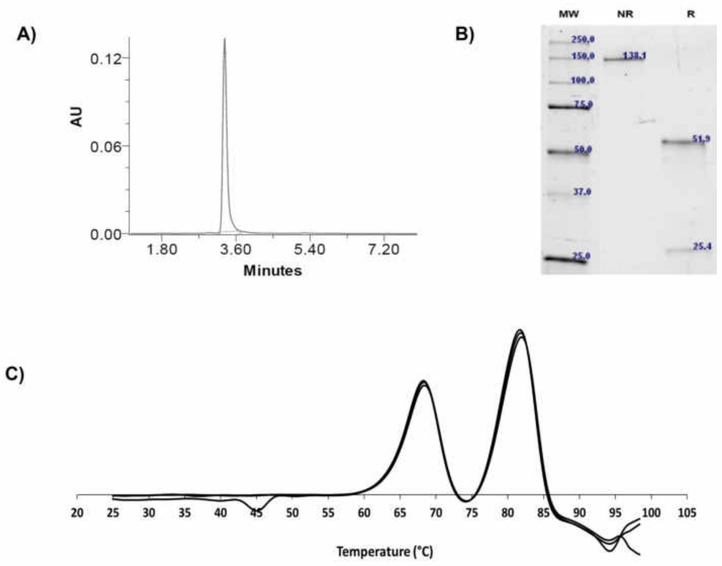


Figure 1. Physicochemical characterization of IgG-A7. (**A**) Analytical SEC shows close to 100% monomeric content. (**B**) SDS-PAGE under non-reducing conditions indicates a single band at 138 kDa and two bands: 51.9 kDa (heavy chain) and 25.4 kDa (light chain) under reducing conditions showing. (**C**) Protein Thermal Shift™ assay shows two unfolding transitions: 68.5 C and 82.1 C.

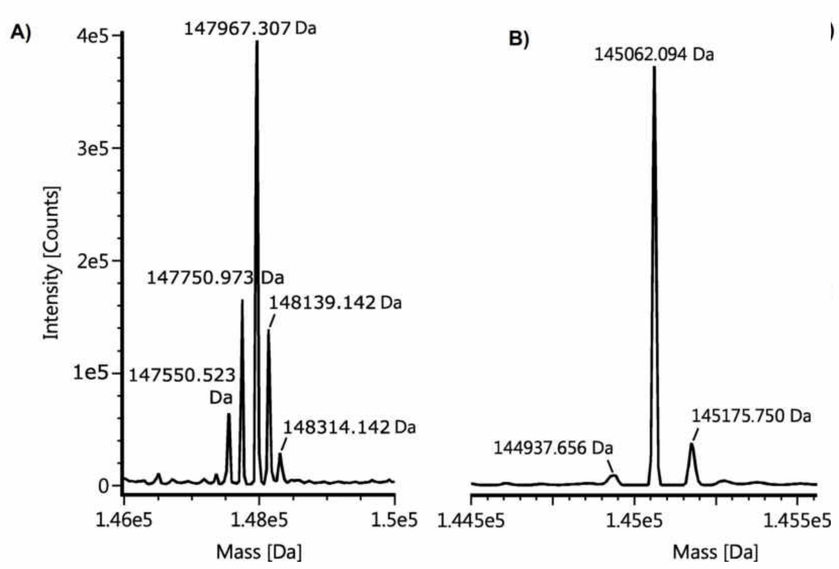


Figure 2. Mass Spectrometry analysis of IgG-A7, a broadly SARS-CoV-2 neutralizing antibody. Main isoforms of the intact (A) and deglycosylated (B) molecules.

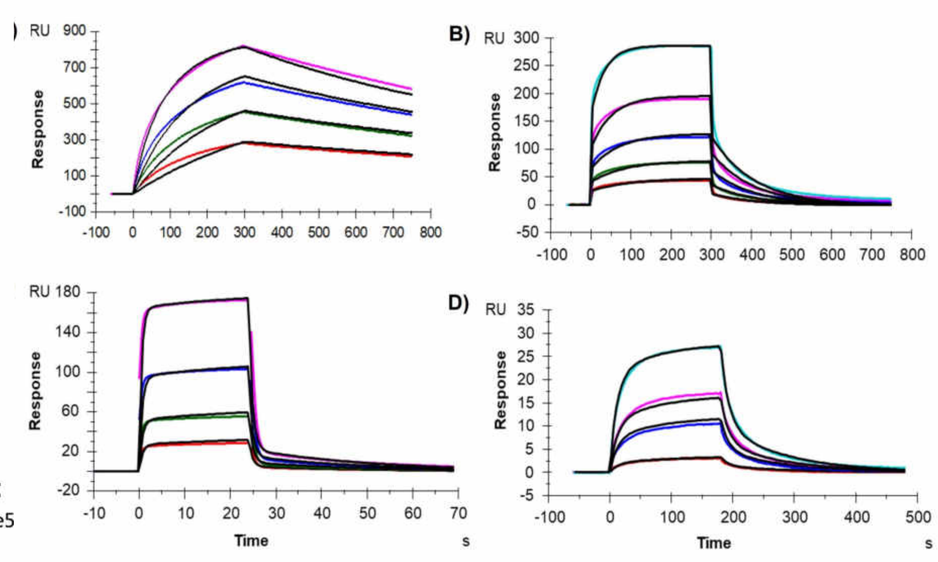
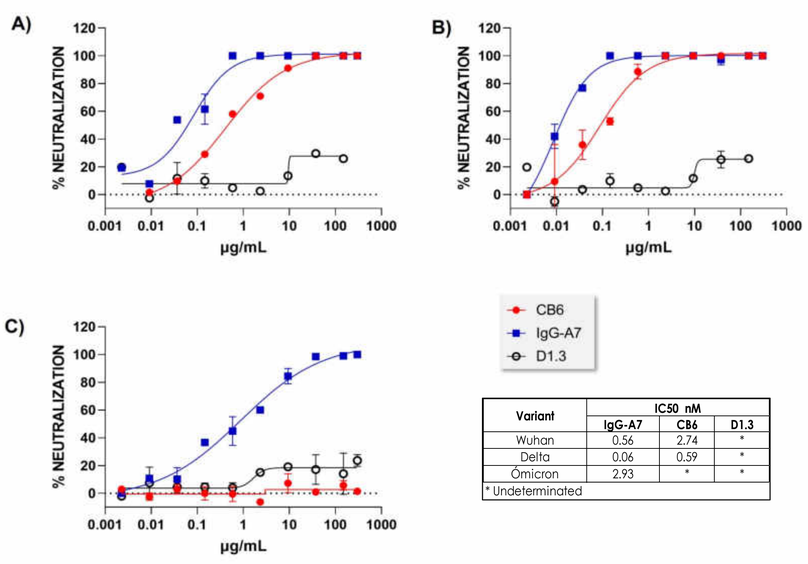


Figure 3. Binding of IgG-A7 to (A) FcγRIA, (B) FcγRIIA, (C) FcγRIIIA and (D) FcRn.



Variant	IC50 nM		
	IgG-A7	CB6	D1.3
Wuhan	0.56	2.74	*
Delta	0.06	0.59	*
Ómicron	2.93	*	*

* Undeterminated

Figure 4. Dose-response neutralization curves of IgG-A7, CB6 and D1.3 (negative control) in PRNT for different variants of SARS-CoV-2. (A) WT, (B) Delta, and (C) Omicron. The Table on the left-bottom corner reports the NC50 values obtained by fitting the raw data to a four-parameter dose-response curve in GraphPad Prism 9.3.1.

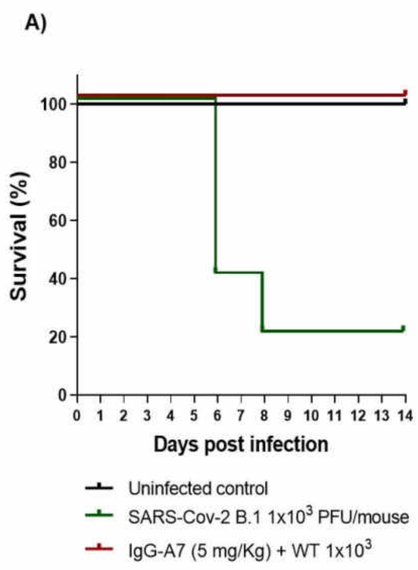
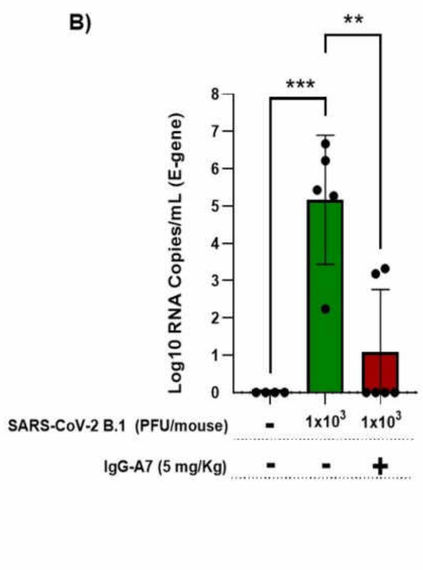


Figure 5. IgG-A7 protection of K18-ACE2 mice expressing hACE2 infected with SARS-CoV-2 WT. The efficacy of IgG-A7 was assessed through survival (A) and viral load (B) analyses. Statistics were performed through Kaplan Meier survival analysis (Chi2 = 11.30, p = 0.0035) while viral load was performed by one-way ANOVA (F2,12 = 16.27) with Dunnett's post hoc test (** p < 0.01; *** p < 0.001).



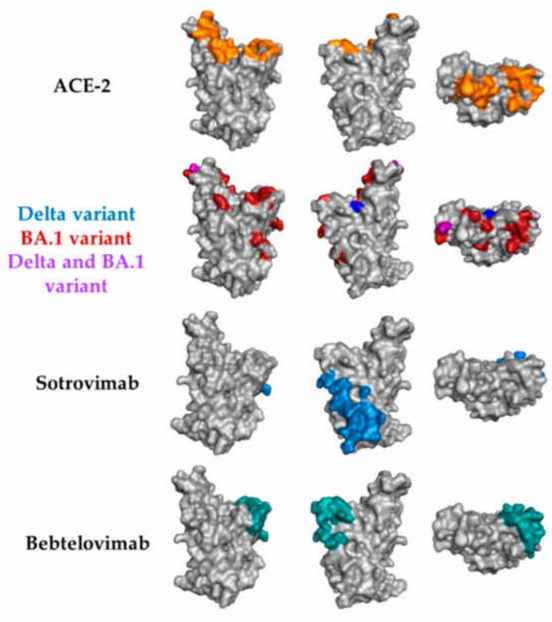


Figure 6. Connolly surface of the RBD side and top views mapping the residues involved in the interaction with hACE2 (top), mutations of the Delta and Omicron (BA.1) variants with respect to the RBD WT and epitopes of sotrovimab and figures were generated with PyMOL Molecular Graphics System version 2.4.1 using the PDB ID: 6VW1 for the RBD:hACE2 interface and mutations of Delta and Omicron, PDB ID: 7SOC for the epitope of sotrovimab and PDB ID: 7MMO for the epitope of bebtelovimab.

Table 1. Summary of IgG-A7 developability profile.

Attribute	Units	IgG-A7	Success Criteria
Expression yield	mg/L	20	12–16
Endotoxin	UE/mL	<0.125	<15
Purity (SEC)	%	100	>95
	kDa	205	~150
Integrity (SDS-PAGE)	Heavy chain (kDa)	51.9	~50
	Light chain (kDa)	25.4	~25
	Whole molecule (kDa)	138.1	~150
Thermal stability	Tm1 (°C)	68.5	68
	Tm2 (°C)	82.1	68–83
Intact Mass (Main peak)	Da [ppm]	147,967	Should correspond to the calculated mass based on the amino acid sequence plus glycans
Deglycosylated (Main peak)	Da [ppm]	145,062	Should correspond to the calculated mass based on the amino acid sequence
Fcγ receptors (reference values are those measured with a similar capture reagent, i.e., anti-HIS antibody)	FcγRI (µM)	0.02	0.0009–0.052
	FcγRIIA (µM)	0.42	4.20–6.00
	FcγRIIIA (µM)	0.38	0.089–2.166
FcRn	µM	2.74	0.9–4.3

CONCLUSION

Herein, we show that IgG-A7 efficiently neutralizes all the three SARS-CoV-2 strains in plaque reduction neutralization tests (PRNTs). In addition, we demonstrate that IgG-A7 fully protects K18-hACE2 transgenic mice infected with SARS-CoV-2 WT in a similar manner to that reported for other anti-SARS-CoV-2 antibodies. Taken together, our findings indicate that IgG-A7 could be a suitable candidate for development of antibody-based drugs to treat and/or prevent SARS-CoV-2 VOCs infection.

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