Isolation and optimization of neutralizing antibodies against SARS-CoV-2 using ALTHEA Gold Plus Libraries™

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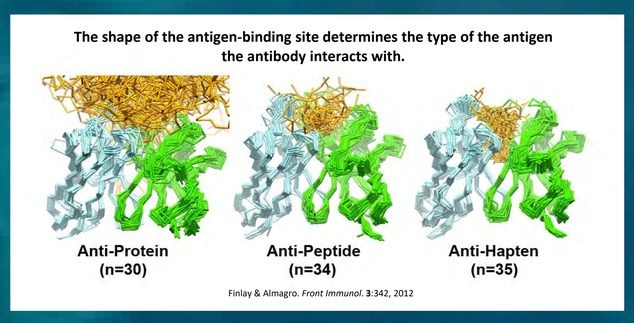
Abstract

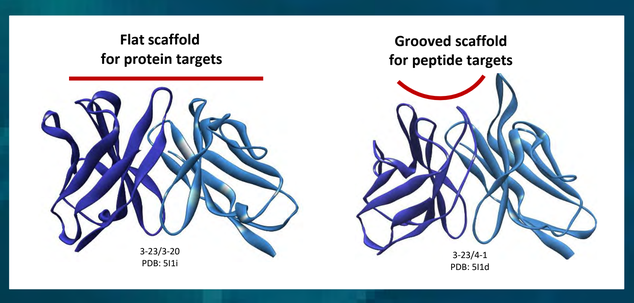
We report here the discovery and characterization of a panel of anti-SARS CoV-2 neutralizing antibodies. A first set of antibodies were obtained by panning ALTHEA Gold Plus Libraries™ with recombinant Receptor Binding Domain (RBD) of SARS CoV-2. ALTHEA Gold Plus Libraries™ are an upgrade of ALTHEA Gold Libraries™ (Valadon//Almagro. MAbs 11:516; Almagro et al. Antibodies (Basel) 8:44). This new generation of ALTHEA libraries was built on a synthetic human VH scaffold and 4 Vk scaffolds combined with natural human HCDR3 fragments obtained from a pool of 200 human donors. After three rounds of panning, 630 clones were tested for binding to RBD, yielding 27 positive and unique clones. Out of these clones, ten were converted to hIgG1 and further characterized. All the studied IgGs showed a developability profile consistent with that of therapeutic antibodies. Three clones blocked the RBD:hACE2 interaction and had KD values in the low nM range as measured by BIAcore. Two of the clones, called P5E1 and P5A10, neutralized the viral entry into Vero cells. Importantly, these clones also tightly bound the SARS CoV-2 variants of concern Delta and Delta plus.

Starting from P5E1 and P5A10, a second set of antibodies was generated by reshuffling diversity in the HCDR1 and HCDR2. After three rounds of panning with overnight incubation in the presence of soluble RBD, 20 unique clones were obtained from P5E1_CDRH1/2 library and 21 from P5EA10_CDRH1/2 library. The clones with best ELISA binding to RBD were converted to hIgG1. Two IgGs (P5E1A6 and P5A10G2) exhibited KD values of 0.8 – 0.9 nM and an increased ability to block the RBD:hACE2 interaction. Notably, P5E1A6 and P5A10G2 also showed higher neutralization potency than the parental antibodies, with IC50 of 1.54 ug/mL and 1.57 ug/mL, respectively. Epitope mapping of these two lead antibodies indicated that both molecules share contacts with residues in the RBD involved in the interaction with hACE2 but also have specific contacts for each of the antibodies. Therefore, P5E1A6 and P5A10G4 have the potential to be used as therapeutics antibodies individually or in a cocktail for preventing and/or fighting SARS-CoV-2 infection.

Developable Scaffolds with topographies to bind protein and peptide targets



ALTHEA Gold + Libraries™ scaffolds provide alternative antigen-binding site topographies: Flat suitable for protein antigens and grooved, which are biased towards the recognition of peptides.



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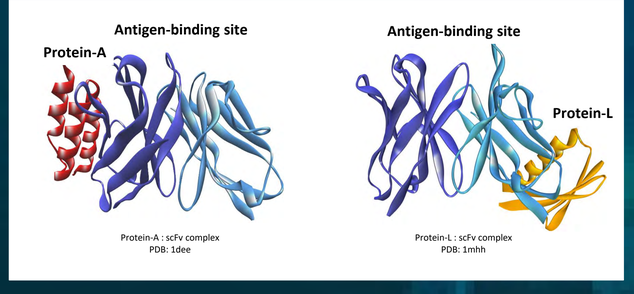

The V_H scaffolds binds Protein-A and enables filtration of well-folded antibodies. All the V_L binds Protein-L and enables assay development and identification of variants coming out of the anti-peptide libraries when used combined with the anti-protein library Antibodies.

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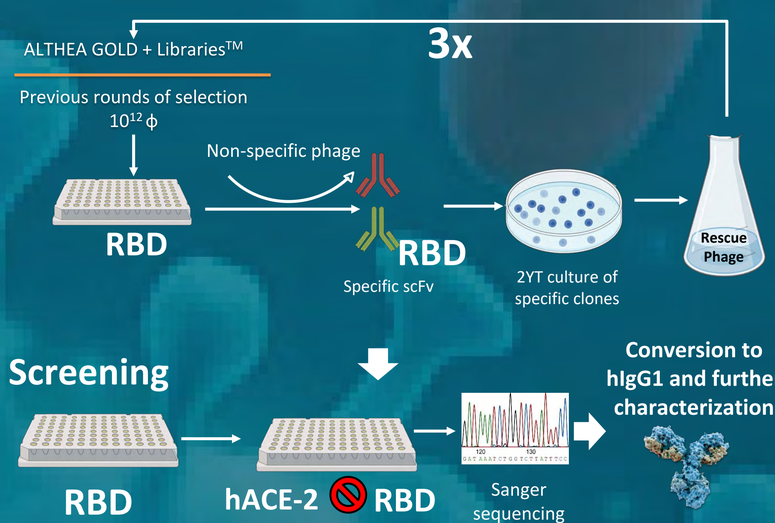
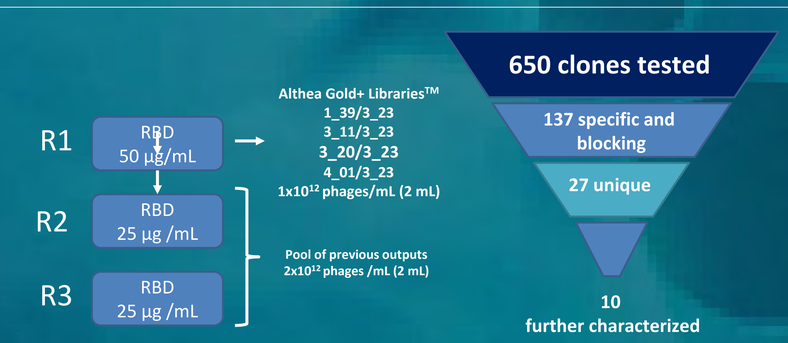
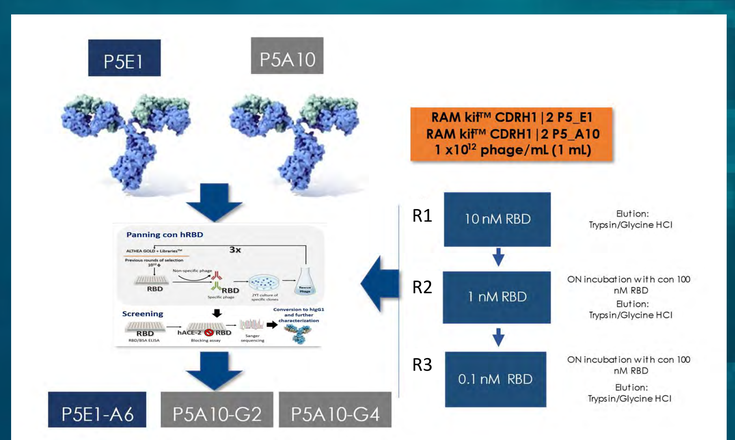
Panning con hRBD





630 clones were tested for binding to RBD, yielding 27 positive and unique clones. Ten were converted to hIgG1, expressed in HEK 293 cells and further characterized. Two of the clones, called P5E1 and P5A10 neutralized the viral entry into Vero cells and were selected for affinity maturation.

Affinity Maturation



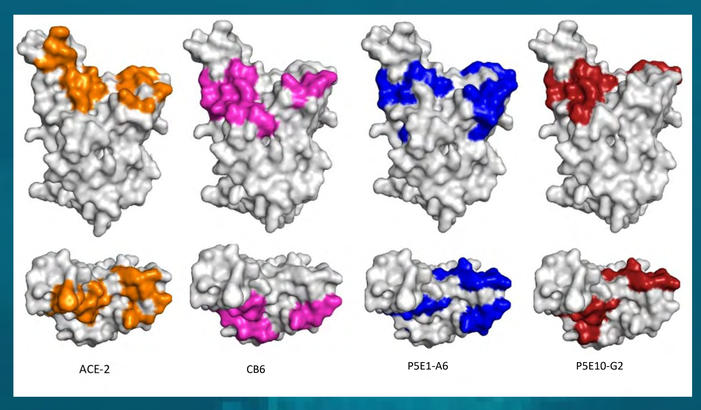
Starting from P5E1 and P5A10, a second set of antibodies was generated by reshuffling diversity in the HCDR1 and HCDR2. The clones with best ELISA binding to RBD were converted to hIgG1, expressed in HEK 293 cells and further characterized yielding two final candidates P5E1-A6 and P5A10-G2.

Developability profile (Expression in HEK 293 cells)

Assay	Units	IgG1 P5E1-A6	IgG1 P5A10-G2
Expression (HEK, 4-day culture)	mg/L	18.0	10.4
Monomeric content after Protein A purification (SEC-UPLC)	% / kDa	98.94 / 160.0	100.0 /169.0
Intact Mass (MS) [difference with theoretical mass]	Da [ppm]	147,160.96 [-12.4] 147,363.36 [16.3] 147,525.97 [19.5] 147,688.83 [24.3] 147,852.44 [34.2]	147,574.95 [26.6]
Intact Mass after degiycosylation (MS) [difference with theoretical mass]	Da [ppm]	144,477.03 [33.0] 144,583.76 [9.2] 144,678.93 [-94.4]	144,688.625 [43.6] 144,803.297 [-47.9]
Thermal Stability (Sypro-Orange assay)	Tm1 (°C)	69.57	68.52
	Tm2 (°C)	79.88	81.18
Affinity (BIAcore T200)	Ka (1/Ms)	588,248.1	1,496,462.0
	Kd (1/s)	0.000527	0.000578
	kD (nM)	0.9	0.4

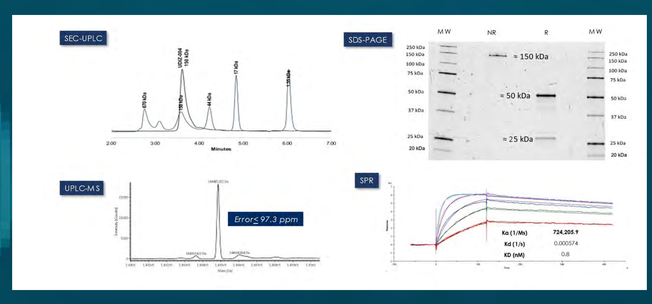
Two IgGs (P5E1-A6 and P5A10-G2) exhibited a developability profile consistent with therapeutic antibodies. KD values of 0.4 – 0.9 nM.

Epitope mapping

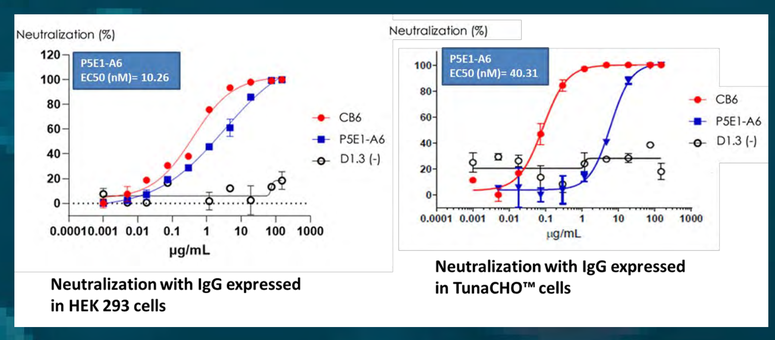


Epitope mapping of the lead antibodies P5E1-A6 and P5E10-G2, and comparison with the epitope recognized by CB6 (Shi et al. Nature 584: 1204), indicates that both molecules share contacts with residues in the RBD involved in the interaction with hACE2 and neutralization of CB6 but also have specific contacts for each of the antibodies

Production of P5E1-A6 in TunaCHO™



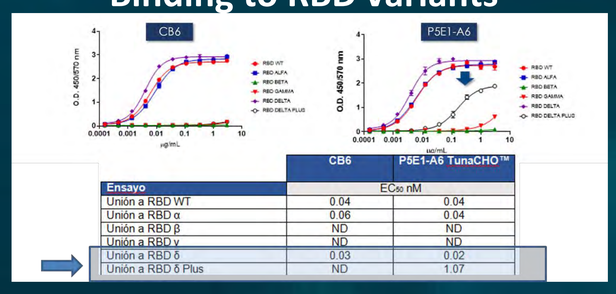
SARS-CoV2 in vitro (Wuhan isolate) neutralization



Neutralization with IgG expressed in HEK 293 cells

Neutralization with IgG expressed in TunaCHO™ cells

Binding to RBD variants



Ensayo	CB6	P5E1-A6 TunaCHO™
	EC50 nM	
Unión a RBD WT	0.04	0.04
Unión a RBD α	0.06	0.04
Unión a RBD β	ND	ND
Unión a RBD γ	ND	ND
Unión a RBD δ	0.03	0.02
Unión a RBD δ Plus	ND	1.07

The lead molecule P5E1-A6 expresses well in manufacture platform (TunaCHO™), has a developability profile consistent with a therapeutic antibody and neutralizes SARS-CoV2 in vitro, Importantly. P5E1-A6 binds the RBD variants including Delta and Delta plus, whereas the comparator antibody (CB6) does not.

Summary

- **A panel of anti-SARS CoV-2 antibodies weas isolated from ALTHEA Gold Plus Libraries™ using RBD as selector.**
- **630 clones were tested for binding to RBD, yielding 27 positive and unique clones.**
- **A second set of antibodies was generated by reshuffling diversity in the HCDR1 and HCDR2.**
- **The lead molecule P5E1-A6 has a developability profile in the manufacturing platform consistent with a therapeutic antibody, neutralizes SARS-CoV2 in vitro and recognizes the variants of concerns (Delta and Delta+).**
- **Therefore, P5E1A6 has the potential to be used as a therapeutics antibody individually or in a cocktail with the other lead antibody (P5E10-G2, described elsewhere) for preventing and/or fighting SARS-CoV-2 infection.**

A potent antibody against SARS-CoV-2 with adequate developability and functional characteristics was isolated from ALTHEA Gold Plus Libraries™. This antibodies has served as case study to develop the antibody engineering platform at UDIBI.

GlobalBio Engineering better antibodies

CONACYT

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