Pairing in vivo diversities with a yeast-based platform for integral membrane protein-specific antibody discovery

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BACKGROUND

Integral membrane proteins continue to be a hurdle for antibody therapeutic discovery. By combining a yeast-based, B-cell platform with diversities derived from humanized transgenic murine or wildtype mice, and llamas, we have developed a high-throughput methodology for the discovery of membrane-obligate-target (MOT) specific antibodies. In the absence of soluble recombinant antigen, this platform enables the discovery of large panels of clonally-diverse, high-affinity, target-specific IgGs or Heavy Chain-only Antibodies (HCAbs).

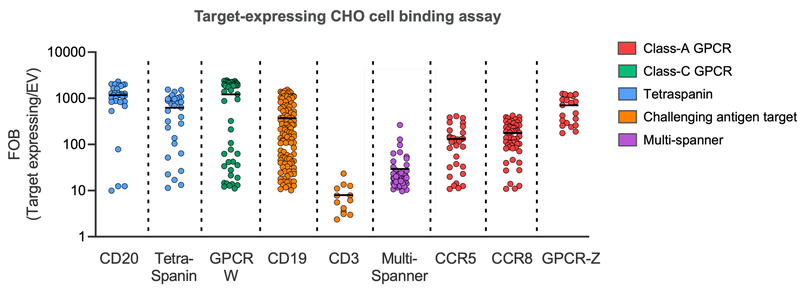

Figure 1: Discovery of mAbs against different types of MOTs, including GPCRs and tetraspanins.
Summation of multiple membrane-obligate projects where the Y-axis displays fold-over-background (FOB) binding signal of antibodies used to stain target-expressing CHO cells versus Empty Vector (EV) CHO cells at 100 nM staining concentration

METHODS

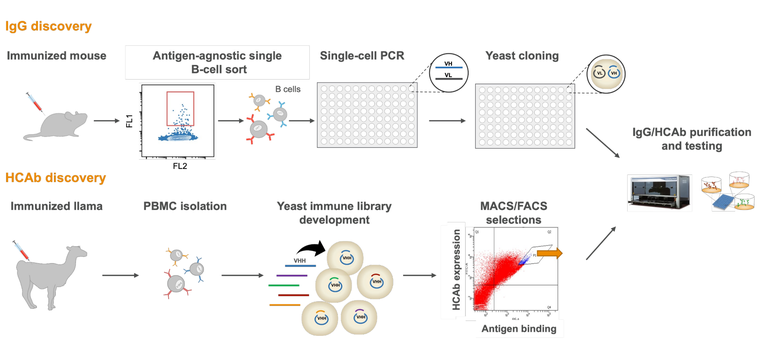

Figure 2: Adimab pairs immunized animal diversities with our engineered yeast-based platform for the rapid discovery of membrane protein-specific mAbs.

RESULTS

CCR8 mAb Discovery and Characterization

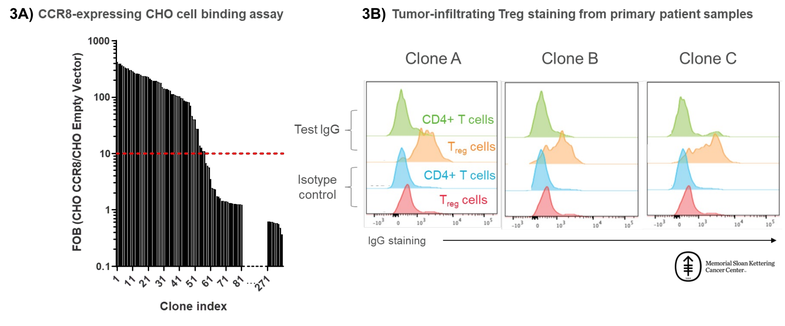

Figure 3: Efficient recovery of many CCR8-specific antibodies using Adimab's MOT platform.
3A) 57 CCR8-specific antibodies recovered following workflow. **3B)** Representative histograms of individual IgG clones stained on tumor infiltrating T cells taken from human donors at 100 nM primary staining.

'GPCR-A' Immune Library HCAb Discovery

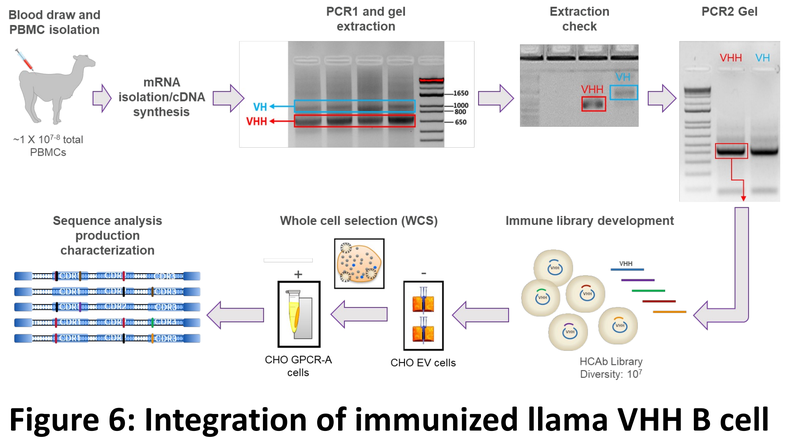

Figure 6: Integration of immunized llama VHH B cell diversities for the generation of yeast immune libraries for selection against MOT "GPCR-A".
Discovered VHH regions are cloned into yeast-specific acceptor vectors that contain a modified hinge fused with the human IgG1 Fc domain to create the HCAb format.

CCR8 mAb Discovery and Characterization

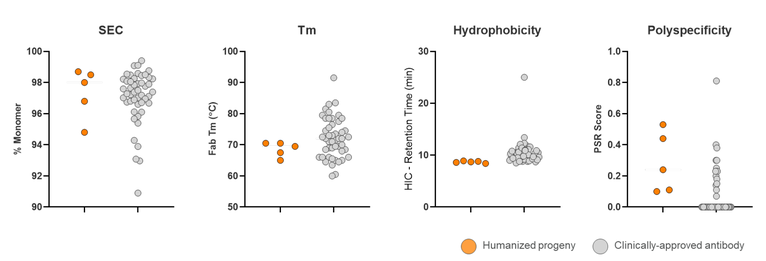

Figure 4: Humanized CCR8-specific IgGs displayed favorable developability profiles.
Results of Size Exclusion Chromatography (SEC), melting temperatures (Tm), Hydrophobic Interaction Chromatography (HIC), and Poly-Specificity Reagent (PSR) binding, respectively. Antibodies were HEK-produced and compared against clinically-approved antibody developability data (Jain, et al., 2017)

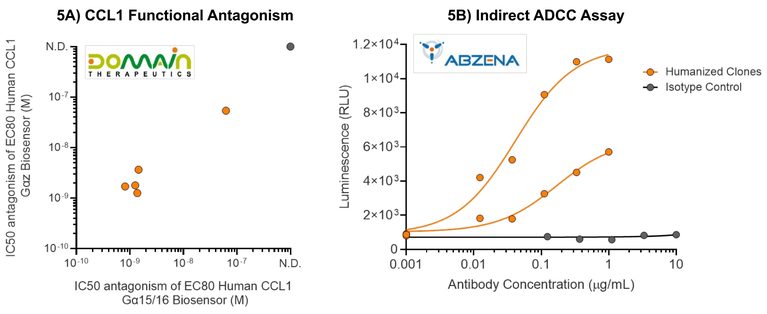

Figure 5: CCR8 mAbs antagonize CCL1 and display ADCC activity.
5A) GPCR Biosensor BRET-based assay which detects CCR8 signaling when in the presence of its natural ligand, CCL1. **5B)** Results of indirect Antibody Dependent Cell-mediated Cytotoxicity (ADCC) assay in which genetically-modified effector cells express luciferase upon antibody-dependent recognition of antigen expressed on target cells

'GPCR-A' Immune Library HCAb Discovery

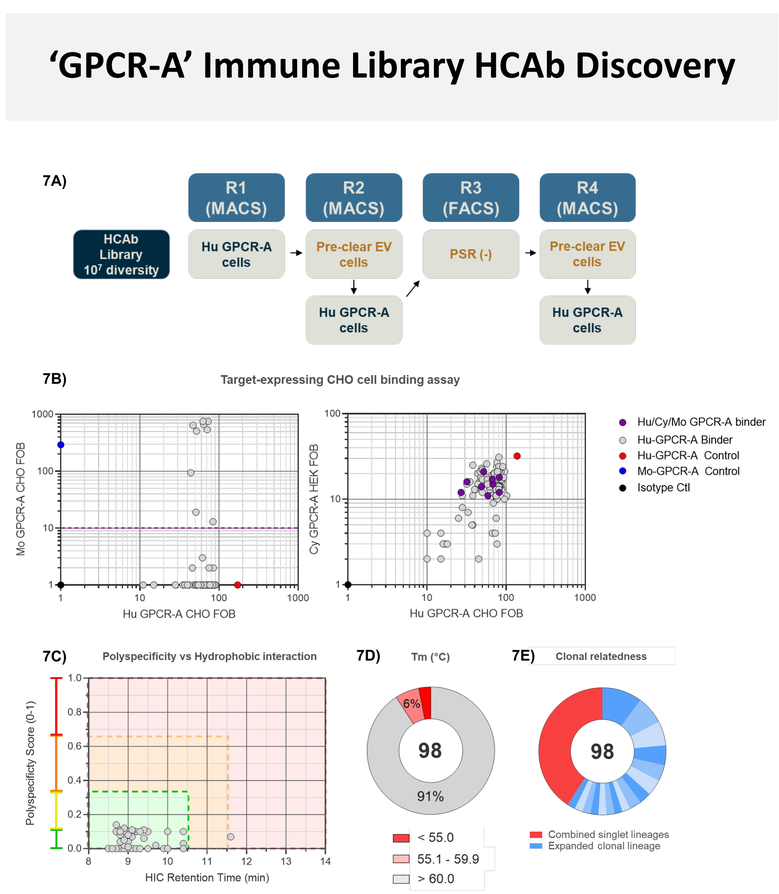

Figure 7: Triple cross-reactive GPCR-A-specific HCAbs were successfully isolated using Adimab's MOT platform.
7A) Four-round immune library selection scheme. **7B)** 98 unique HCAb isolated against GPCR-A were assayed at 100 nM for binding against cell lines expressing Hu, Cy, and Mo GPCR-A. **7C and 7D)** Selection output developability analysis of polyspecificity, HIC retention time, and Tm. **7E)** Clonal lineage analysis comparing sequence relatedness in GPCR-A specific HCAb output

CONCLUSIONS

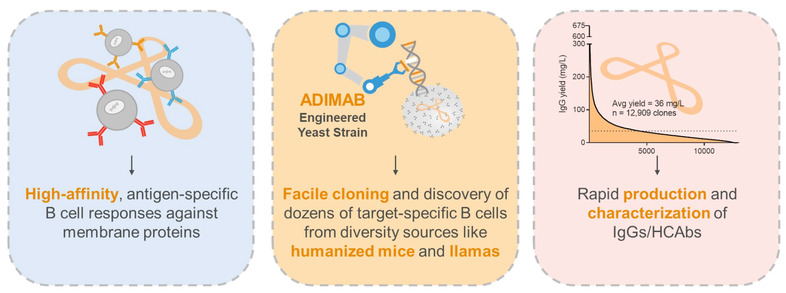

Figure 8: Adimab's yeast-based platform, paired with immunized in vivo diversities, can solve many of the challenges of membrane-protein antibody discovery

REFERENCES

(1) Jain, Tushar et al. "Biophysical properties of the clinical-stage antibody landscape." *PNAS,* vol. 114,5 (2017): 944-949.

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