CASE STUDY

Discovery of diverse GPCR-targeted antibodies using integrated technologies

AUTHORS

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BACKGROUND

GPCRs are difficult targets.

Despite being high-value therapeutic targets, G protein-coupled receptors (GPCRs) have been largely intractable to conventional antibody discovery technologies.[1] Due to the deep complexity in GPCR structure and function, there are challenges at each stage of discovery.

Generation of robust, target-specific immune responses is limited by high cross-species homology and a small number of available epitopes.[1] In addition, target-binding hits are extremely rare, reducing the chances of finding functional and developable leads.[2]

AIM

Find diverse GPCR-targeted antibodies.

Here, we present a case study in which we leveraged our discovery and development engine to find diverse antibodies against an established GPCR target, chemokine receptor 8 (CCR8).

CCR8 is highly and selectively expressed on tumor-associated regulatory T cells in multiple cancer types.[3] Antibodies against CCR8 represent a promising therapy to treat solid tumors that have progressed on standard immune checkpoint inhibitors.[4,5,6]

To be effective, antibodies against CCR8 should be human/humanized and show favorable developability properties, highly specific binding, and potent antibody-dependent cellular cytotoxicity (ADCC) reporter activity.

METHODS

Amplify diversity, enrich for potency, and fine-tune leads.

A combination of protein and DNA immunization campaigns was used to generate robust immune responses. We captured hundreds of diverse antibodies using deep single-cell screening. High-throughput function-based screening was then used to down-select antibodies based on ADCC reporter activity. Selected antibodies were optimized for developability using structure-guided protein engineering technologies paired with high-throughput antibody assessment.

RESULTS

A diverse panel of anti-CCR8 antibodies optimized for developability and function.

Selected molecules displayed desired properties, including:

- **Diversity:** derived from two animal species, including two humanized mouse strains
- **Potency:** strong ADCC reporter activity that is similar to a clinical benchmark
- **Developability:** favorable biophysical properties that have been de-risked by pre-emptive remediation

272 diverse CCR8-binding hits

Immunization and deep single-cell screening technologies were used to find hundreds of diverse sequences.

Figure 1. 272 diverse CCR8-specific antibodies were identified from immunization and deep single-cell screening. (A) Two strains of humanized mice and two strains of wild-type rats were immunized using recombinant protein- or genetic-based formulations. Antibody titers were measured by flow cytometry using cells expressing human CCR8. **(B)** Single-cell screening assays were used to screen 2.6 million single cells and identify CCR8-specific antibodies. Representative output images of a CCR8-specific hit in a single chamber from our single-cell screen are shown. Antibodies that bind to CCR8-expressing cells were detected using fluorescence microscopy and images were analyzed using machine vision. **(C)** Antibody sequence diversity was visualized using Celium™, and 272 CCR8-binding antibodies were selected for high-throughput expression based on clonal diversity. Clusters represent clonal families based on V genes, J genes, and CDR3 lengths.

194 functional anti-CCR8 antibodies

High-throughput functional screening and liability assessment guided our down-selection and pre-emptive remediation strategy.

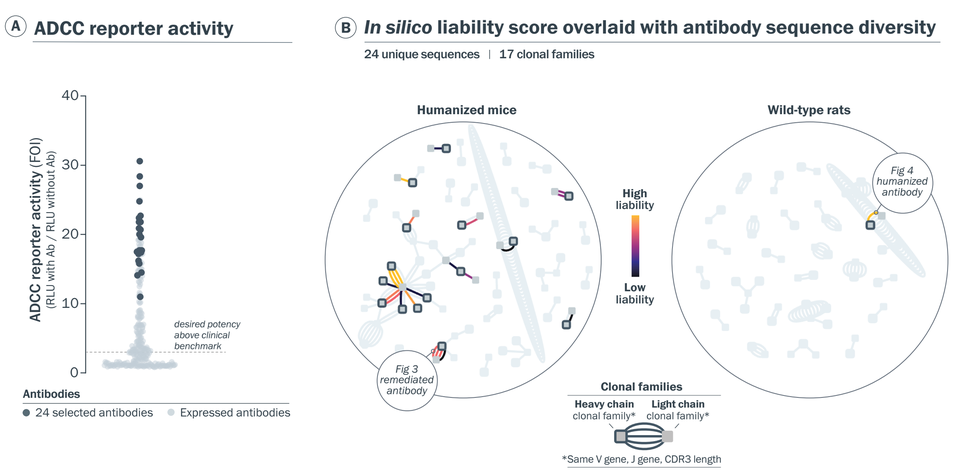

Figure 2. From 194 functional antibodies, 24 highly potent candidates were selected for high-throughput characterization and liabilities were mapped to guide pre-emptive remediation. (A) 194 of 272 expressed antibodies met the desired functional activity, measured in an ADCC reporter assay with activity quantified using luminescence. **(B)** Potential sequence liabilities of the 24 selected antibodies were identified *in silico* and mapped using proprietary bioinformatics pipelines. Antibody clonal diversity and liabilities were visualized using Celium™.

RLU: Relative luminescence units; FOI: Fold of induction.

24 highly potent antibodies optimized for developability

Remediation of sequence liabilities in a humanized mouse-derived antibody.

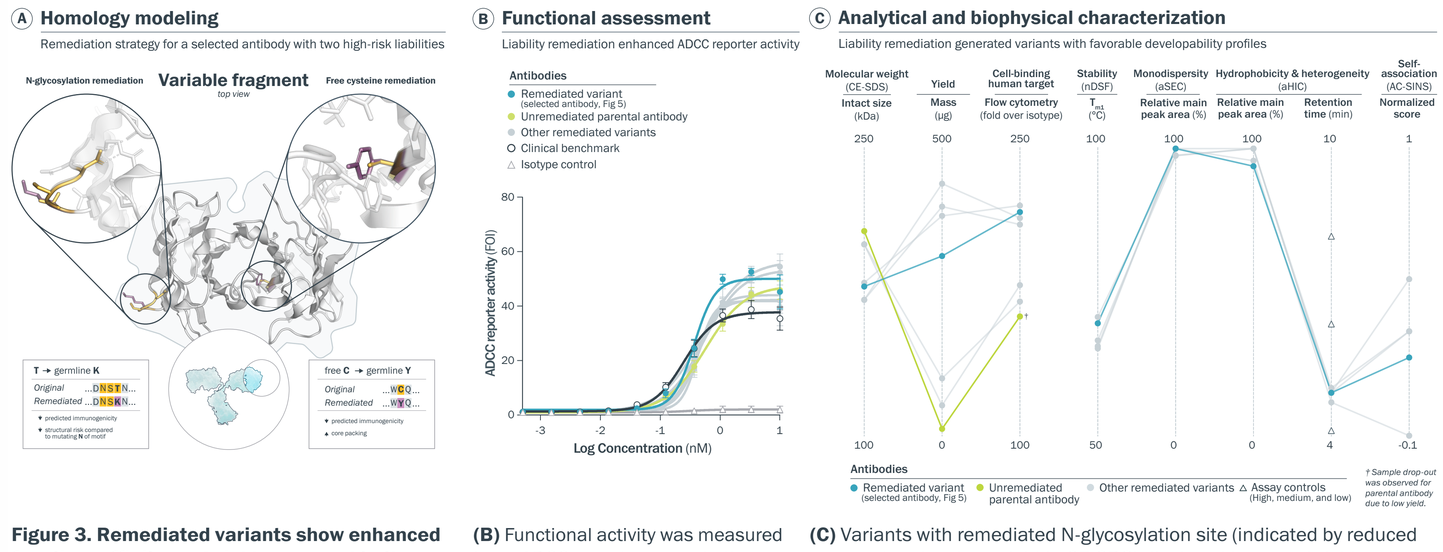

Figure 3. Remediated variants show enhanced function, affinity, and yield compared to the parental antibody. (A) Homology modeling of a humanized mouse-derived antibody guided the remediation strategy.

(B) Functional activity was measured in an ADCC reporter assay in three-fold serial dilutions starting at 10 nM and data were normalized to an isotype control; activity was quantified using luminescence.

(C) Variants with remediated N-glycosylation site (indicated by reduced apparent molecular weight in CE-SDS) displayed improved yield and cell-binding compared to the parental antibody. Extended developability profiles of remediated variants were favorable, including desired stability, high monodispersity, and low self-association.

Structure-guided humanization of a highly potent rat-derived antibody.

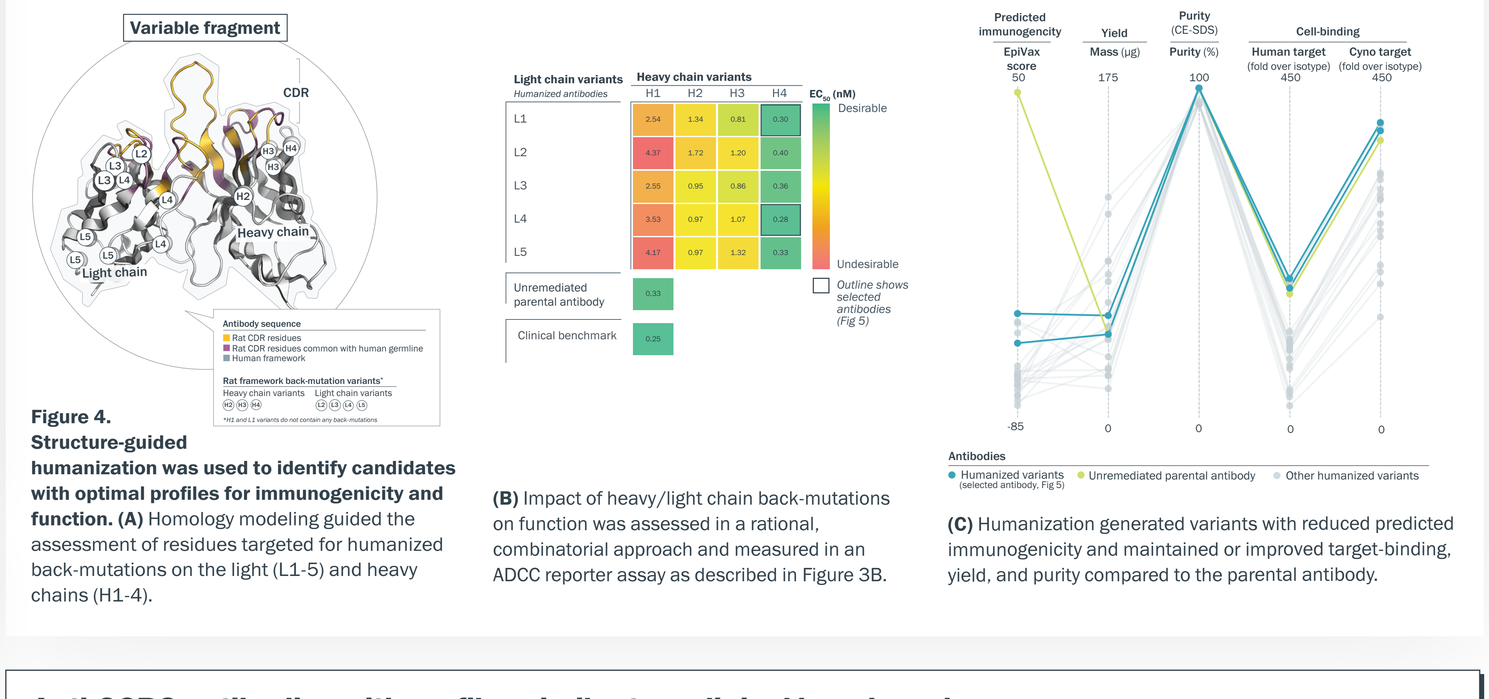

Figure 4. Structure-guided humanization was used to identify candidates with optimal profiles for immunogenicity and function. (A) Homology modeling guided the assessment of residues targeted for humanized back-mutations on the light (L1-5) and heavy chains (H1-4).

(B) Impact of heavy/light chain back-mutations on function was assessed in a rational, combinatorial approach and measured in an ADCC reporter assay as described in Figure 3B.

(C) Humanization generated variants with reduced predicted immunogenicity and maintained or improved target-binding, yield, and purity compared to the parental antibody.

Anti-CCR8 antibodies with profiles similar to a clinical benchmark

A panel of wild-type and remediated antibodies was selected based on:

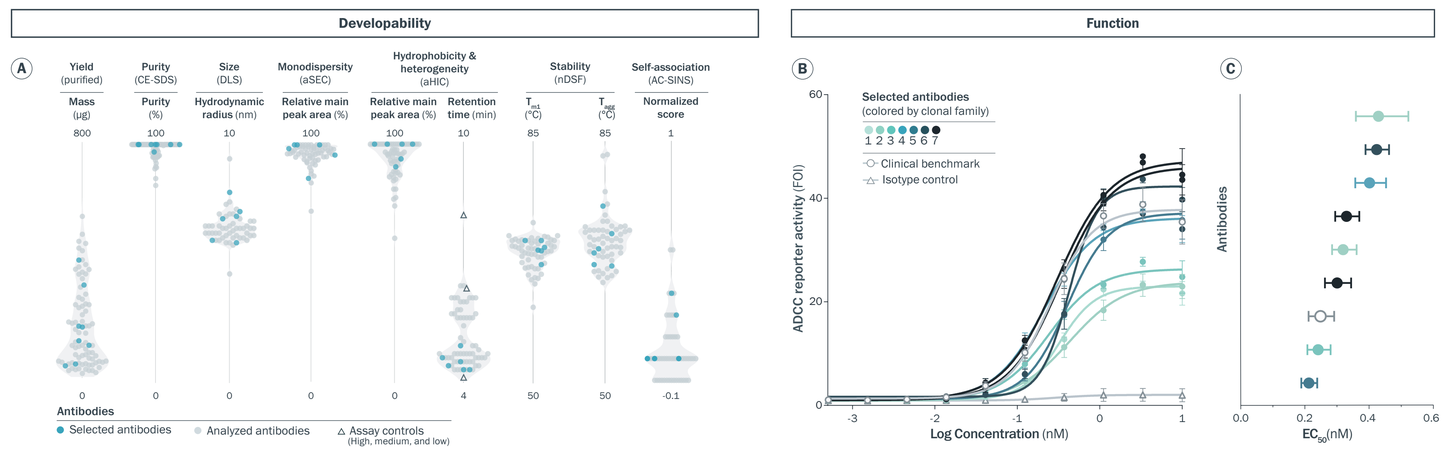

Figure 5. Strategic selection of diverse, developable antibodies with a range of potencies. (A) Selected antibodies have favorable developability profiles, including desired purity, monodispersity, relative surface hydrophobicity and heterogeneity, stability, hydrodynamics, and self-association with scores normalized to high and low controls.

(B) Selected antibodies were comparable to a clinical benchmark molecule in preliminary ADCC assessment. Functional activity was measured with an ADCC reporter assay as described in Figure 3B.

(C) ADCC assay EC_{50} values and 95% confidence intervals are shown.

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