Discovery of Novel Complement component 5a receptor 1 Antagonistic Antibodies Using a Target-Specific Generated Phage Library

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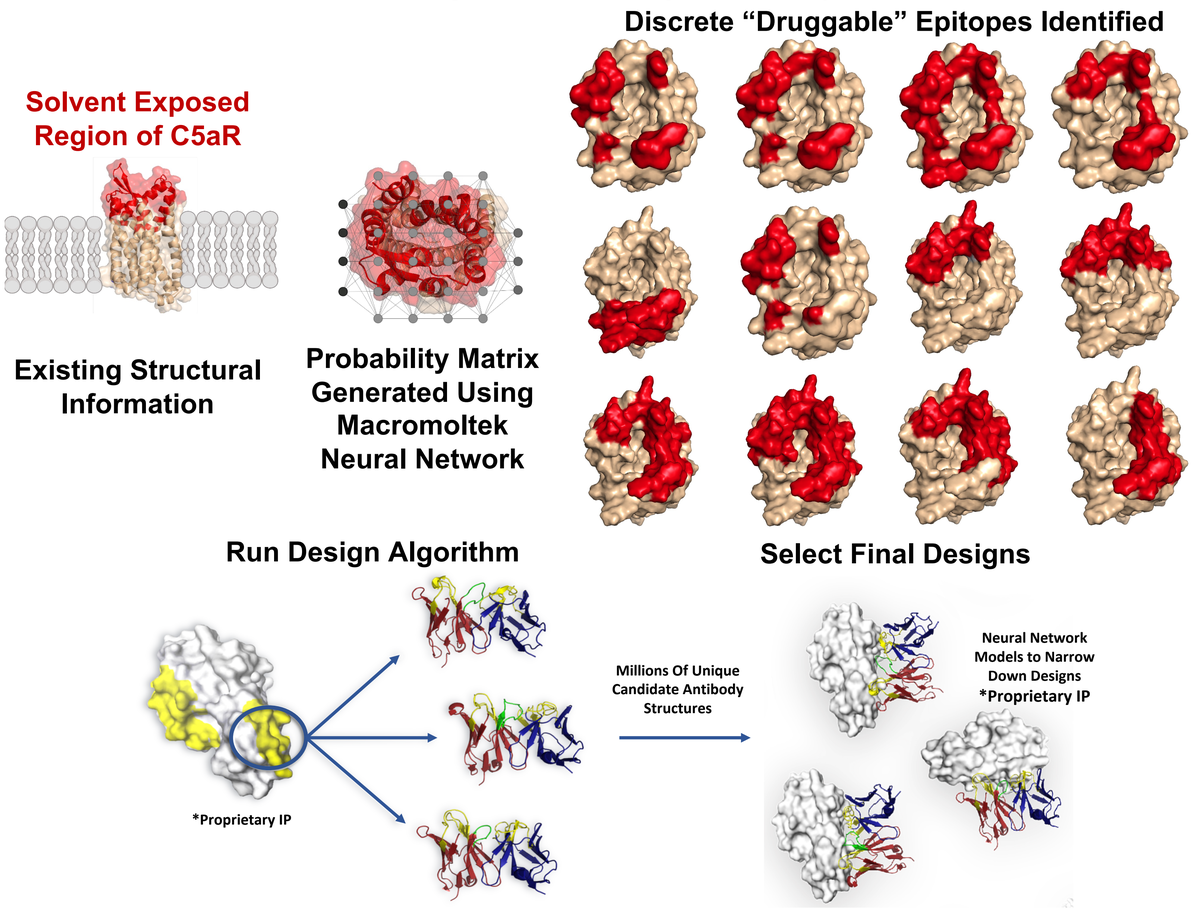
Abstract

G-Coupled Protein Receptors (GPCR) remain one of the most attractive but more challenging therapeutic targets for antibody discovery. Complement component 5a receptor 1 (C5aR1) is a GPCR of interest with potential therapeutic applications in immune-oncology and inflammation. This GPCR is involved with the complement cascade and in innate immunity. Stimulation of C5aR1 by its ligand, C5a, promotes tumorigenesis by suppressing T-cell mediated anti-tumor activity while blockade of C5aR signaling has been shown to attenuate tumor growth. In a combined approach with Macromoltek, we leverage Twist's DNA synthesis and library assembly technology with structural-based machine learning to design a targeted anti-C5aR discovery library to identify several highly potent and selective lead antibodies against C5aR. Several of the candidates identified from this study are also shown to have functionally antagonistic effects on C5aR1-mediated signaling in cell-based assays. This work demonstrates how the combination of highly specific DNA synthesis, coupled with informed design, can be paired to successfully prosecute challenging targets for novel therapeutic discovery.

Library Design and Construction

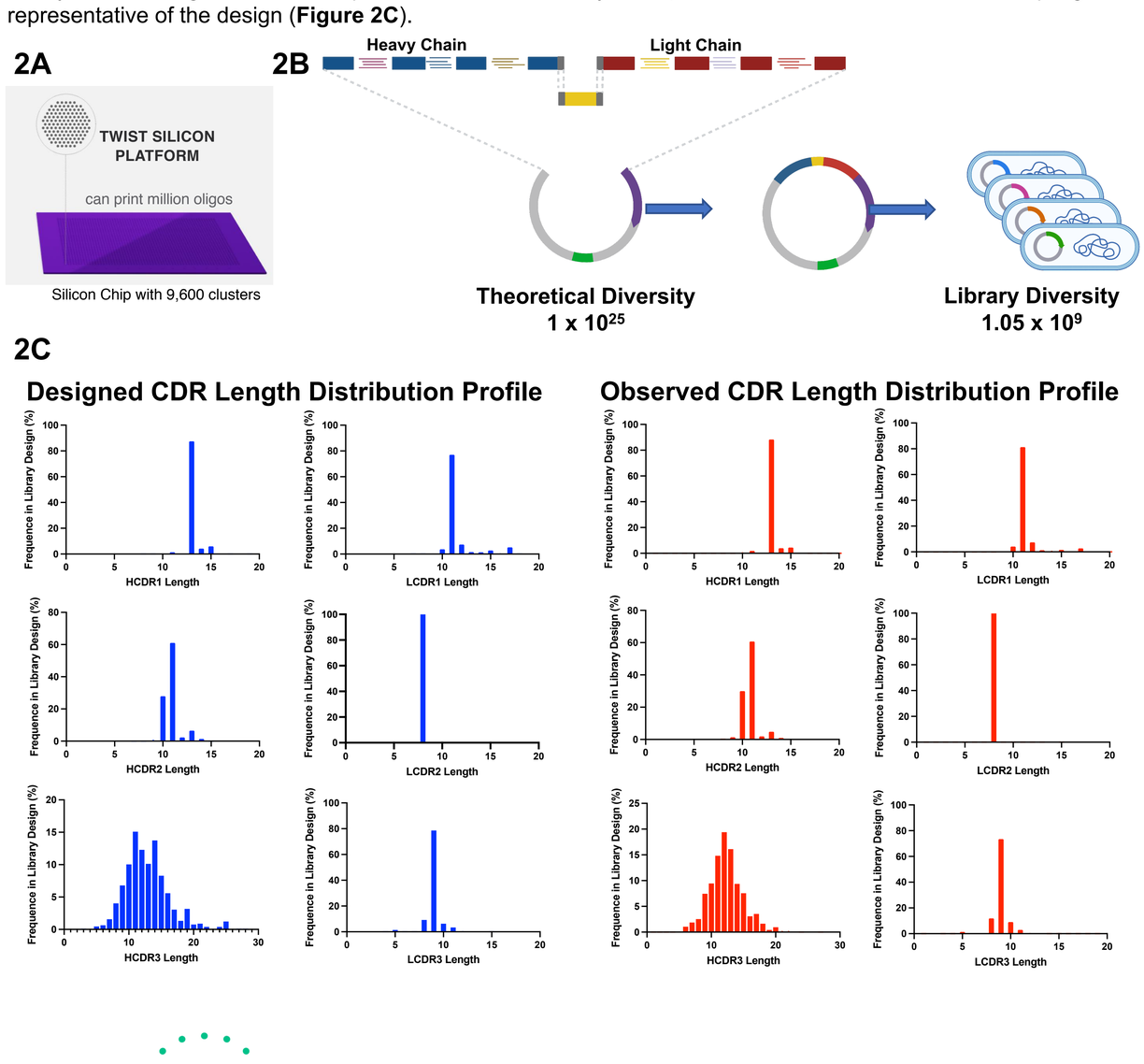
The design of a C5aR1 focused library utilized existing structural information to predict solvent exposed regions of the receptor (**Figure 1A**). The exposed surface was then divided into smaller epitopes using a neural network trained on a structural repository of antibody-antigen interaction to identify epitopes with high-probability of antibody interaction (**Figure 1B**). Using these discrete epitopes, an algorithm generated a large pool of millions of candidate antibody structures with unique binding loops. This collection of structures was finally triaged down to ~10,000 structures using neural network models which selects candidates with favorable biophysical characteristics (**Figure 1C**).

Design of C5aR1 Targeted Library



Library Synthesis and Assembly

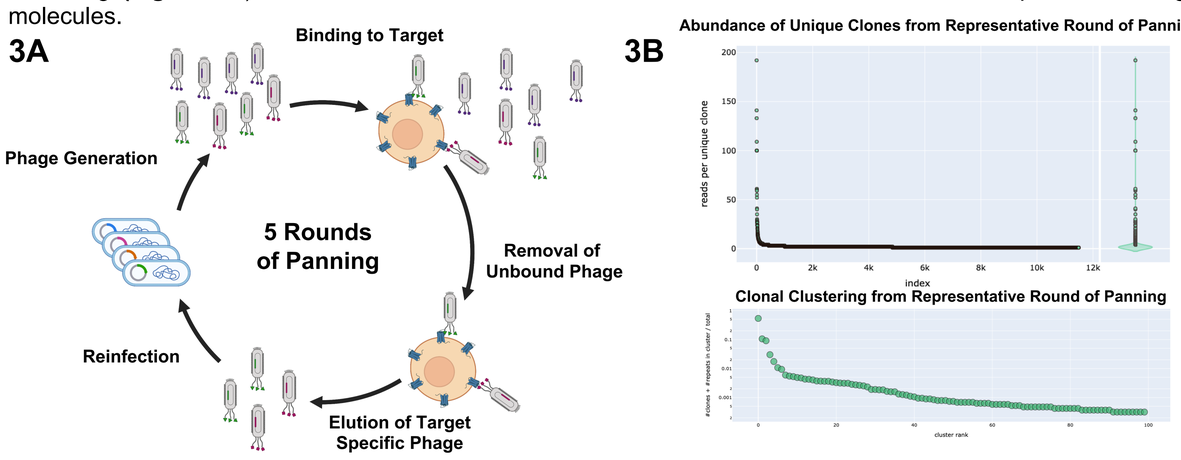
The C5aR focused phagemid library was designed using the predicted CDRs from the ~10,000 candidates using Twist's synthetic DNA printing capabilities (**Figure 2A**). The assembled scFv phagemid library was constructed using combinatorial assembly and represented a sampling of the overall theoretical diversity **(Figure 2B**). The library's CDR length distribution profile was assessed by NGS, which showed the observed sampling was representative of the design (**Figure 2C**).



Antibody Discovery and Characterization

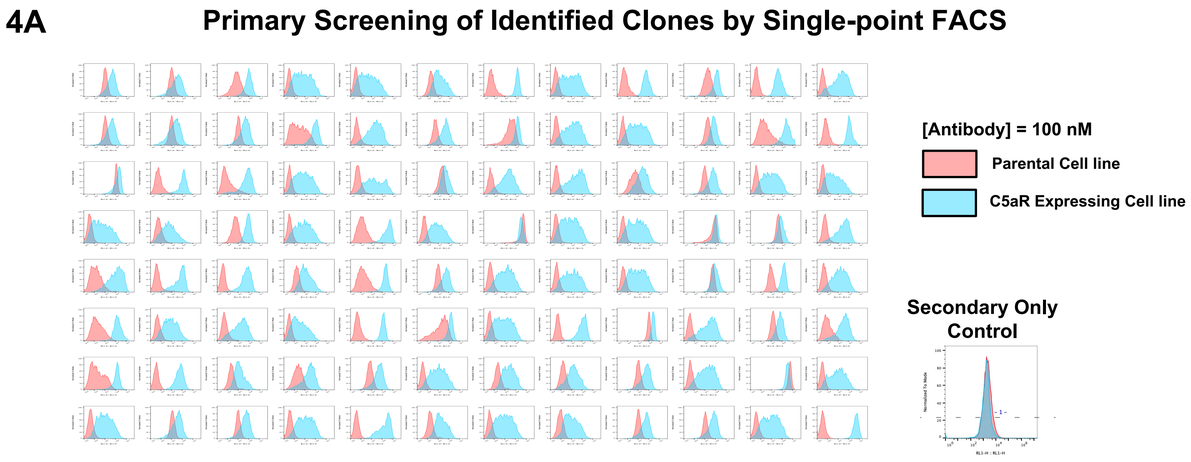
Panning Campaign and Clone Identification by NGS Sequencing

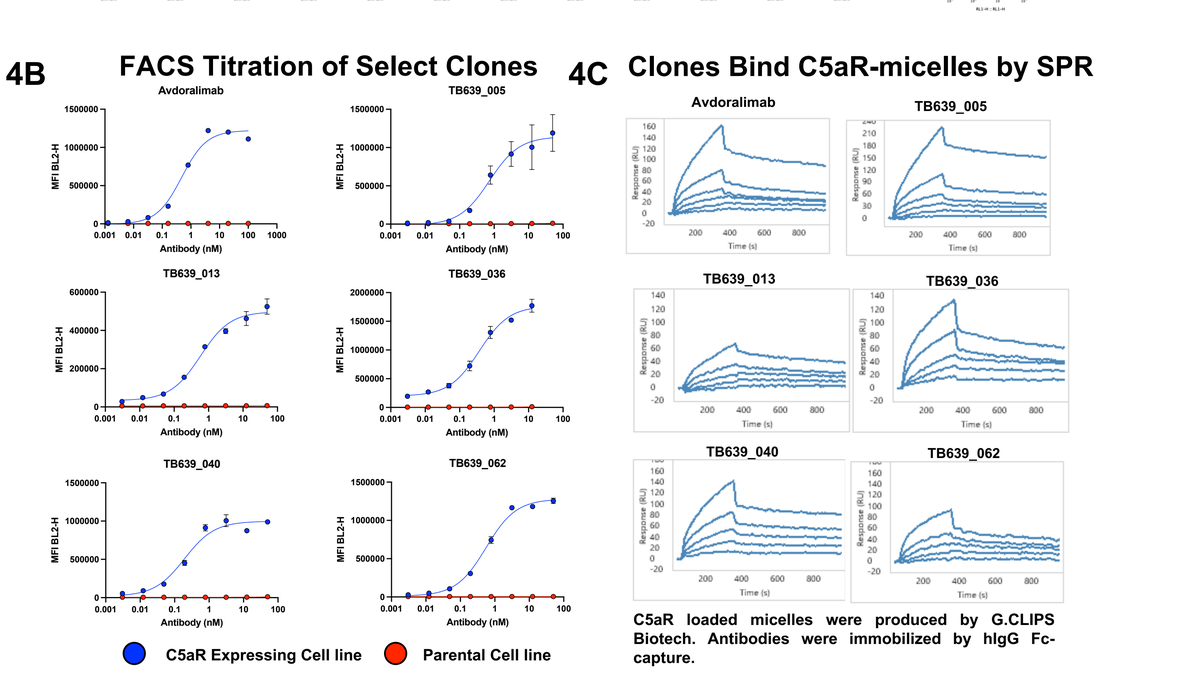
A cell-based campaign was conducted with 5 rounds of panning. Phage pools were depleted on parental cells prior to incubation with the C5aR expressing cell line **(Figure 3A**). Phage pools from later rounds of panning were characterized by long-read NGS to get VH and VL paired sequencing. Clones were selected to move into IgG reformatting using Twist's bioinformatic pipelines looking at abundance of clones, enrichment across rounds, and clustering **(Figure 3B**). A total of 96 clones with 45 different HCDR3's were selected and produced as IgG molecules.

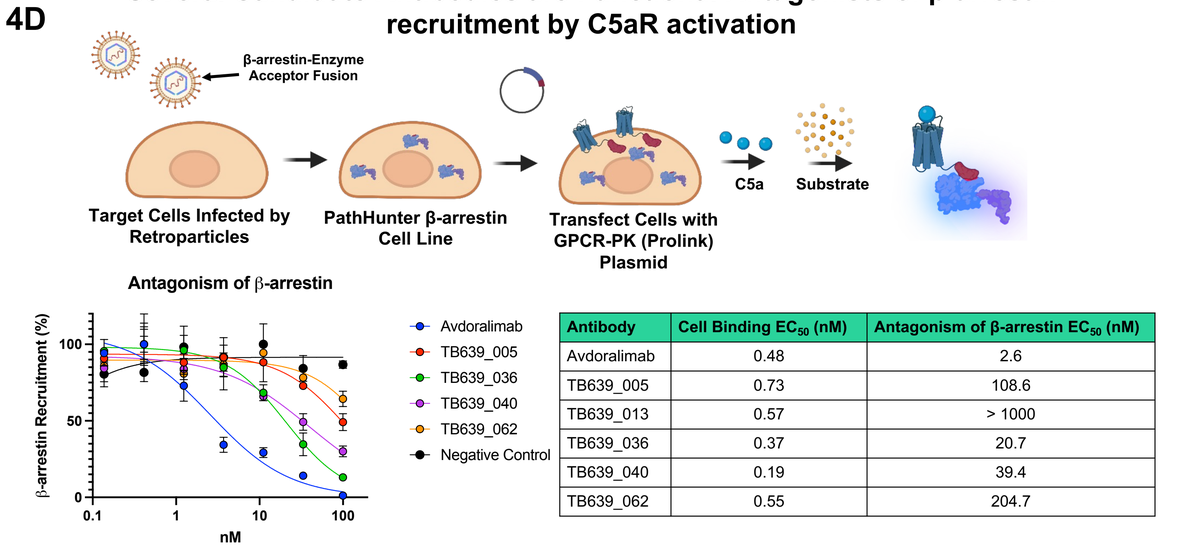


Primary Screening and Secondary Confirmation Assays

Primary screening of IgG candidates was performed by single-point FACS at 100 nM (**Figure 4A**)**.** Clones which exhibited an MFI ratio greater than 3 compared to the parental cell line were considered a positive hit. Of the 96 clones reformatted 90% met this positive hit criteria. Titration of top clones show potent and specific binding to the stable expressing cell line, with several having EC_{50}'s comparable to the clinical control Avdoralimab (**Figure 4B**)**.** Clones were further validated by Carterra SPR using C5aR loaded micelles from G.CLIPS Biotech (**Figure 4C**)**.** Finally, clones were evaluated in a cell-based assay for antagonism of C5aR (**Figure 4D**)**.** Several clones identified block C5a stimulation of C5aR and β-arrestin recruitment.







Antibody	Cell Binding EC_{50} (nM)	Antagonism of β-arrestin EC_{50} (nM)
Avdoralimab	0.48	2.6
TB639_005	0.73	108.6
TB639_013	0.57	> 1000
TB639_036	0.37	20.7
TB639_040	0.19	39.4
TB639_062	0.55	204.7

Summary

A C5aR focused antibody discovery library was produced in collaboration with Macromoltek using neural network machine learning and Twist's synthetic DNA capabilities. Using this library, a number of specific and potent lead molecules were identified. Importantly, a number of these were functional antagonists of C5aR. Using this approach, high quality target-specific libraries can be generated to enable drug discovery for challenging therapeutic targets.

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