Optimizing a Candidate Therapeutic Antibody to Optimize Pharmacokinetics using Informed Library Design and Precise Synthesis

ABSTRACT

When panning for antibodies against epitopes that impose a strong selective pressure for non-specific binders, the resulting molecules may exhibit poor binding specificity and unsatisfactory pharmacokinetic (PK) profiles. Highly-charged epitopes are a key example, where charge-based interactions saturate the binding pool. A leading pharmaceutical company was developing a high-affinity antibody for the highly-charged interleukin-21 receptor (IL-21R), an important player in the immune system's cytokine response[1]. By leveraging Twist Bioscience's Combinatorial Variant Library (CVL) Technology in combination with structure-guided library design and model-based selection, the company was able to enrich functional antibody variants with desirable characteristics and identify a stable lead with high-affinity for IL-21R.

INTRODUCTION

The commercial and therapeutic success of an antibody depends on both its pharmacodynamic (PD) and pharmacokinetic (PK) properties. Optimized PD can be pursued with high-throughput screening technologies like phage display, which allow researchers to rapidly and iteratively screen large-scale (up to 10^{10}) antibody libraries for high-affinity antigen binding, thermal stability, and other desirable properties. However, promising antibody candidates with strong PD profiles may still have poor PK properties that result in rapid clearance from the body and potentially significant off-target effects leading to toxicity[1].

Poor PK is a hurdle to successful antibody development, particularly when targeting highly-charged epitopes. Biopanning for antibodies against charged targets can inadvertently select for antibodies that exhibit strong binding solely through charge-based interactions. Such antibodies are likely to be unusable for further pharmaceutical development as they may also exhibit off-target binding to other charged epitopes in the body, such as the common cell membrane components heparin and sialic acid[1].

A leading pharmaceutical company was recently faced with such a challenge. The team aimed to improve on a previously identified antibody, MJ4-2, to develop a high-affinity therapeutic antibody targeting IL-21R, a negatively-charged protein found on the surface of several immune cells[2]. Previous work by the company showed that enrichment for antibodies with positively-charged complementarity-determining regions (CDRs) resulted in high-affinity leads with poor PK[3,4]. This application note describes how the company leveraged a combination of structure-guided design, computational modeling, and Twist CVLs to overcome the complex charge-affinity relationship and generate a high-affinity anti-IL-21R antibody with improved biophysical properties[5].

WORKFLOW

To overcome the challenge of antibody charge and poor pharmacokinetics, the researchers followed a 7-step workflow:

1. **Soft mutagenesis and affinity screening:** The team used mutagenic primers to create large-scale libraries of antibodies based on MJ4-2. Next Generation Sequencing (NGS) was used to catalog each antibody library. Libraries were screened for affinity to biotinylated human IL-21R (hIL-21R) using phage display.
2. **Pharmacokinetic analysis:** Select antibodies with affinity for hIL-21R were generated as IgG antibodies in HEK-293 cells and their PK properties were analyzed. Specifically, ELISA were used to assess antibody binding to negatively charged off-target-bait antigens. Self-association was assessed using an affinity-capture self-interaction nanoparticle spectroscopy assay.
3. **Antibody-antigen crystal structure analysis:** Co-crystal structures were solved for the candidate antibody in complex with the extracellular domain of the IL-21R antigen to understand charge-based interactions and affinity.
4. **Development of linear regression model:** Informed by co-crystal modeling data, NGS data was then revisited to select antibody variants possessing mutations that reduced charge or improved antigen affinity for testing in biophysical liability assays. A linear regression model was developed to predict which mutational profiles were most likely to lead to favorable biophysical properties.
5. **Generation of rational combinatorial variant libraries:** Guided by co-crystal data and modeling predictions, a CVL was designed containing mutations across all CDRs, and synthesized by Twist Bioscience. Antibodies from the CVL library were subject to the same selection and analysis processes as described in steps 1 and 2.
6. **Refinement of linear regression model:** Data from biophysical liability assays using Twist CVL-derived antibodies was then used to refine the linear regression model.
7. **Identification of high-affinity IL-21R antibody with reduced off-target binding:** Informed by the updated linear regression model, the team screened NGS data from the combinatorial variant library to identify antibody candidates whose mutational profile was predicted to reduce off-target binding while maintaining high-affinity for IL-21R.

For a detailed description of the methods, please refer to Campbell *et al.*, 2021 (ref 5).

RESULTS

Antibody deselection failed to generate quality leads

A parent antibody MJ4-2 was chosen that demonstrated binding affinity for the target IL-21R, but also showed significant off-target binding due to the antibody's highly-positive charge. To build on this promising lead, the team generated 15 antibody libraries in which each antibody possessed a series of consecutive mutations in variable heavy (VH) or variable light (VL) CDR sequences. Several rounds of deselection screening and panning were employed to proactively filter the libraries for antibodies that show off-target binding while selecting for anti-IL-21R binders.

However, resultant hits showed a stronger positive charge than the parental molecule, a high propensity for binding to charged bait molecules DNA and insulin, as well as a higher likelihood of self-association (Figure 1). Despite competitive binding with deselection agents, the charge-based interaction with IL-21R was pervasive in selection rounds. DNA and insulin were not adequate agents for removing the strong starting charge-based affinity candidate antibodies exhibited for IL-21R.

Co-crystal structure modeling helps identify potential mutation hotspots

The charge-affinity relationship clearly dominates the antibody-IL-21R interaction. To better understand which amino acids were involved in antibody-antigen binding the team sequenced the output of their selection screen with NGS. Additionally, the team solved the co-crystal structure of an MJ4-2 Fab in complex with the extracellular domain of IL-21R.

It was observed that MJ4-2 was near-exclusively bound to a negatively charged domain of IL-21R (Figure 2). By combining the co-crystal structure with NGS data, the researchers identified non-binding residues that could be manipulated to potentially reduce antibody charge without sacrificing binding affinity. Rationally-selected candidates from the NGS dataset showed improved self-association scores, and showed reduced binding for DNA and insulin. While trending in the right direction, the scores were still too high.

Linear regression model homes in on better antibodies

To further inform antibody design, the team generated a linear regression model based on NGS and IgG biophysical liability assay data. The model made genotype-phenotype predictions by identifying which antibody sequences were most likely to produce high-affinity antibodies with reduced biophysical liability scores. Additional rationally-selected antibodies showed further improvements in their biophysical properties, but still failed to meet developability standards scored on association with DNA and insulin.

Creating a combinatorial variant library with Twist Bioscience

Data thus far suggested that loci spread across multiple CDR domains could be manipulated to improve antibody biophysical properties, but manipulating the charge of the antibody's paratope would likely disrupt antigen binding. Therefore, the team needed to precisely alter the antibody sequence, generating a library that introduced mutations in locations identified as being amenable

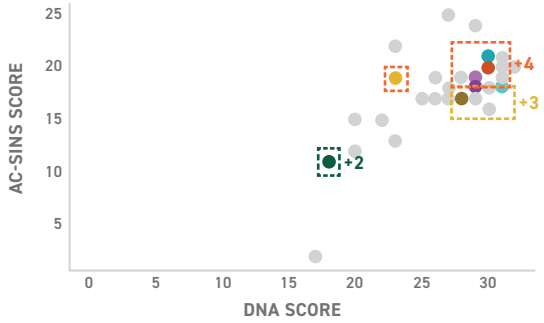

Figure 1. Deselection methods fail to improve antibody leads. After phage display and deselection of soft-mutagenesis libraries, antibody candidates with greater than two-fold increased IC50 for hIL-21R relative to the C2 parental antibody were subject to biophysical liability assays. Each antibody was scored according to their affinity for DNA and insulin (x-axis; lower is better) as well as self-association (y-axis; lower is better). Red and yellow boxes denote antibody candidates with charge indicated next to them, and green box highlights the parental C2 antibody with its charge listed next to it. Figure modified from Figure 1 in Campbell *et al.*, 2021 (ref 5)

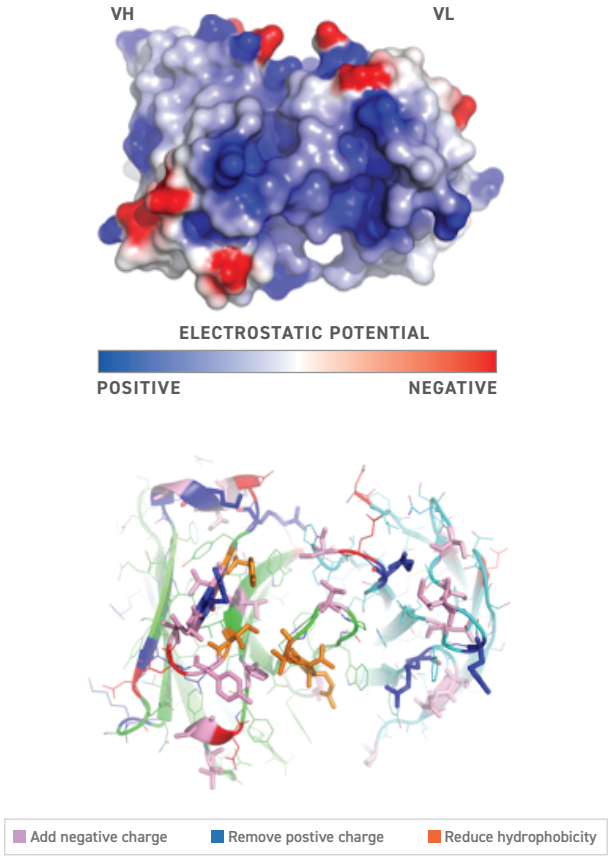

Figure 2: Structural modeling identifies key residues for manipulation. Structural analyses enabled researchers to identify potential residues that could be modified to improve antibody activity. On the top, the dominant blue coloration indicates a highly positive paratope. The structure on the bottom highlights residues that may be manipulated to reduce charge without affecting binding affinity. Figure modified from Figure 3 in Campbell *et al.*, 2021 (ref 5).

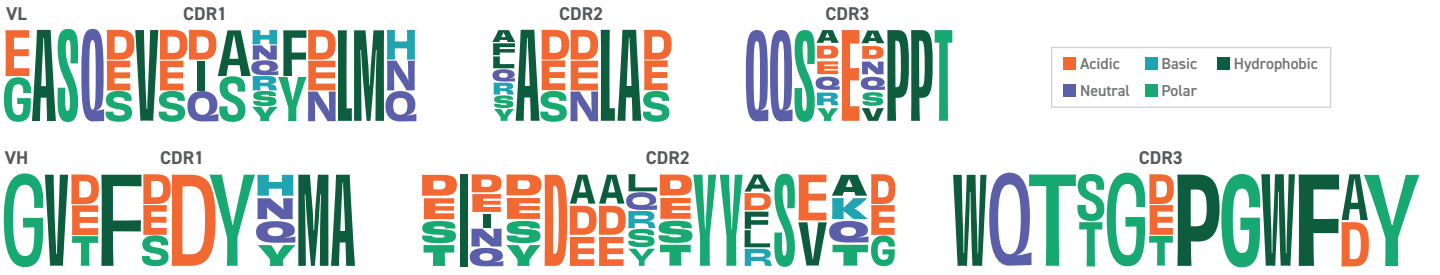

Figure 3: The CVL design built by Twist Bioscience is illustrated, with each letter corresponding to an amino acid. Letter size is proportional to its frequency within the CVL. Stacked letters indicate locations where mutagenesis was directed. Results from the linear regression model were used to select which amino acids would be tested in each location with the ultimate goal of improving antibody biophysical performance. Figure modified from Figure 4 in Campbell *et al.*, 2021 (ref 5).

	VH-CDR1	VH-CDR2	VH-CDR3	VL-CDR1	VL-CDR2	VL-CDR3	Net charge	AC-SINS	DNA Score	Insulin Score
C2 parental	GVTFSDYHMA	TIIYDAARTYYRDSVKG	WQTTGTPGWFAY	GASQSVSISRFNLMH	RASNLAS	QQSRESPPT	16	11	18	17
MAb 1	..E....N..	..ES.EE....A...QE	.....	.....	.....	...Y.A...	0	0	4	4
MAb 2	.....	.....	.....	....E.DQAQY...N	L..D..E	...Q.V...	2	-2	5	4
MAb 3	..D.....	..QD.D.LD..F..EQ.	.....	E...D.....	.....	.....	-4	-1	6	5
MAb 4	.....	.....	.....	....Y..D.HYD..N	..N....	.....D...	8	0	10	8
MAb 5	.....Y..	D..S.ED.D.....E	.....E....D.	.....	.....	.....	2	4	11	9
MAb 6	.....H....	..QS....E.....AD	.....	.....	.....	.....	12	7	10	16

	VH-CDR1	VH-CDR2	VH-CDR3	VL-CDR1	VL-CDR2	VL-CDR3	Net Charge	AC-SINS	DNA Score	Insulin Score	K_d (nM)
C2 Parental	GVTFSDYHMA	TIIYDAARTYYRDSVKG	WQTTGTPGWFAY	GASQSVSISRFNLMH	RASNLAS	QQSRESPPT	16	10	31	23	4.99
mAb1-Rational	.....	.....ED.....	.....	.....	.....	.....	12	3	11	12	32.3
mAb2-Rational	.....	.....ED.....	.....	.....D...	.....	.....	10	1	7	7	81.23
mAb3-Rational	.....	.....A.....	.....	.....T.....	.....	.....	12	3	11	13	33.35
mAb4-Rational	.....	.....E.....	.....	.....D...	.....	.....	12	1	10	12	81.6
mAb5-Rational	.....	.....D.....	.....	.....T.....	.....	.....H...	12	2	10	11	13.61

Figure 4: Twist CVL generated high affinity antibodies with improved biophysical score. Representative antibodies were selected from the Twist CVL for biophysical analyses (**a**) which revealed decreased propensity for self-association and decreased affinity for charge bait molecules DNA and Insulin. This data was then used to refine the linear regression model (**b**) to help select antibodies from the CVL that are most likely to succeed in biophysical analyses (**c**). Analyses revealed an antibody (**d**; red box) with significantly improved properties that retained high affinity for IL-21R (K_d). Figure modified from Figure 4 in Campbell *et al.*, 2021 (ref 5).

to negative charge introduction, positive charge removal or reduction of hydrophobicity to either decrease antibody charge or support antigen binding.

Traditional mutagenesis methods that use randomness to generate variation would not be accurate enough to generate such a precise library. In order to efficiently create a combinatorial library of select amino acids at tailored positions variants, the team turned to Twist Bioscience's CVL technology. Twist Bioscience created CVLs containing 107 and 108 antibody candidates designed with multiple mutations spread across every CDR in the VH and VL domains respectively (Figure 3).

Following panning by phage display, select antibodies from the Twist CVL were tested in biophysical liability assays and showed significantly improved scores. Both Self association scores and DNA/insulin binding scores were passable for further development, however, these candidates showed reduced binding affinity for hIL-21R.

Biophysical liability data was fed into the linear regression model which was subsequently used to identify antibodies from the Twist CVL library predicted to retain binding affinity for IL-21R while still improving biophysical liability scores. Among these rationally-selected antibodies, one showed double-digit nanomolar binding for hIL-21R, along with suitable charge, self-association and DNA/Insulin binding scores (Figure 3). Together, this data suggests that this antibody is likely to bind strongly to hIL-21R with reduced off-target binding, ultimately translating to longer half-life in the body and better pharmacokinetics.

CONCLUSION

A leading pharmaceutical company had identified a promising anti-IL-21R antibody candidate with high affinity but poor pharmacokinetics caused by its highly-positive charge known to increase off-target binding and accelerate antibody catabolism. Rapid clearance and off-target binding detracts from antibody developability due to an increased likelihood of unintended side-effects, a lower concentration of active antibody in circulation, and a decreased therapeutic effectiveness.

Because IL-21R's epitope has a strong negative charge, traditional phage-display panning of antibody libraries enriched for positively-charged antibodies with poor pharmacokinetics. In order to

optimize this lead, they would need to take a considered approach to reduce antibody charge in locations that don't significantly influence antibody-antigen binding. Linear regression-based modeling of pharmacokinetic and NGS data, combined with and co-crystal models of protein binding suggested that biophysical properties may be improved by introducing variants into multiple CDR domains simultaneously.

Creating a library of specific and precisely chosen combinatorial variants required Twist Bioscience's CVL synthesis technology—a DNA synthesis process that deliberately inserts variants according to predetermined criteria, enabling elimination of sequence bias, prevention of undesirable motifs, and allowance for codon optimization. Twist CVLs are characterized by precision and uniformity, two key features when performing antibody optimization.

Biophysical analysis of Twist CVL antibodies that were predicted to have improved performance revealed multiple candidates with greater target specificity and a comparable affinity to the parent compound, while showing reduced binding to off-target bait. With this library, promising candidates have been identified that have improved biophysical properties and serve as a foundation for further therapeutic development.

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Contact one of our Library design specialists and start creating your precisely designed Library to meet your project needs. Email us at **library@twistbioscience.com**

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