Discovering High-Affinity, Functional Anti-GLP-1R Antibodies from a Synthetic Human Combinatorial Variant Library

ABSTRACT

G protein-coupled receptors (GPCRs) are exciting, high value drug targets for treating some of the world's most pervasive diseases. However, GPCRs are challenging targets for antibody discovery because of their conformational flexibility, limited extracellular surfaces and low antigenicity. This application note highlights the discovery of over a dozen high-affinity antibodies against the GPCR glucagon-like peptide-1 receptor (GLP-1R) from a computationally designed and precisely synthesized Combinatorial Variant Library of GPCR-binding motifs. The lead candidate, TB01-3, displays potent antagonism of GLP-1R and superior pharmacokinetic properties *in vivo*. These data demonstrate that Twist Biopharma antibody libraries can yield high-quality antibodies against hard-to-drug targets such as GPCRs.

INTRODUCTION

GPCRs are attractive therapeutic targets due to their involvement in a wide variety of physiological processes, including metabolism, inflammation, neurotransmission, and carcinogenesis. For these reasons, GPCR-targeting drugs dominate regulatory approvals and the global market share of therapeutic drugs[1,2]. In fact, GPCR-targeting drugs generated nearly $1 trillion in sales from 2011 to 2015[3]. Nevertheless, currently approved drugs only cover 16% of the human GPCRome[2], indicating that the protein family is ripe for therapeutic development.

The Family B GPCR GLP-1R is an attractive therapeutic target for conditions that cause persistent or severe hypoglycemia, including congenital hyperinsulinism, neonatal hyperinsulinism, and gastric bypass surgery[4]. Persistent or severe hypoglycemia can cause cognitive dysfunction at best and death at worst[5].

Antibodies are an exciting modality for GPCR targeting due to their acute specificity, improved pharmacokinetic and pharmacodynamic profiles compared to peptides and small molecules, and their long serum half-life. Yet, only two drugs approved by the FDA are antibodies, as GPCRs present many challenges for antibody development. GPCRs are typically buried in the membrane, leaving limited surface area on the cell-surface for antibody targeting. The extracellular loops of GPCRs further confound the discovery of strong antibody-antigen interactions due to their high conformational flexibility. Low natural expression levels and poor overexpression further exacerbate antibody discovery efforts.

Moreover, high-quality variant libraries for anti-GPCR antibody discovery remain scarce. Until very recently, access to precision library synthesis was unavailable. Discovery libraries therefore relied on random diversification processes during their construction, limiting and biasing the diversity that can be achieved.

To enable the discovery of high-quality GPCR-targeting antibodies, Twist Biopharma (a division of Twist Bioscience) designed and assembled a fully synthetic human single-chain variable fragment (scFv) library using computational modeling and large-scale DNA synthesis. This application note describes an end-to-end workflow, beginning with the design of the GPCR 2.0 scFv Library and culminating in the proof-of-concept discovery and characterization of multiple antagonistic, high-affinity, and drug-like anti-GLP-1R antibodies, as described in-depth in Liu et al. (2021; ref 6).

GPCR 2.0 SCFV LIBRARY DESIGN AND SYNTHESIS

Ligands activate GPCRs by interacting with ligand-binding pockets often found within the receptors' transmembrane domain. It was hypothesized that a long, protruding CDR3 loop is therefore needed for antibodies to target potential epitopes within these regions. When designing the GPCR 2.0 scFv Library, antibody germlines were chosen based on their ability to tolerate CDR3 stem regions greater than 21 amino acids in length[6].

Hereafter, heavy chain CDRs and light chain CDRs will be denoted *HCDR* and *LCDR* respectively. A set of 155,927 GPCR-binding motifs were identified and inserted into the aforementioned stem regions, providing a rich source of functional HCDR3 diversity. These binding motifs were identified from computational modeling of hundreds of GPCR crystal structures, including those of GPCRs in complex with peptides, ligands, and antibodies.

Limited variation was also introduced into HCDR1, HCDR2, LCDR1, LCDR2, and LCDR3. These CDR variants were selected based on an analysis of V genes from 12 human donors. Manufacturing liabilities (including isomerization, deamidation, and glycosylation sites), cryptic splice sites, and restriction sites were removed from all CDRs.

The resulting GPCR 2.0 scFv Library was fabricated on the Twist Bioscience DNA Synthesis Platform (**Figure 1**), which enables the generation of large-scale Combinatorial Variant Libraries (CVLs) with precise control over sequence variation. To build the CVL, antibody CDRs were synthesized as a pool of oligonucleotides and assembled with the selected antibody frameworks to generate full-length scFv (VH-linker-VL) genes. The resulting scFv gene pool was cloned into a phagemid display vector (encoding the M13 gene-3 minor coat protein) to create a phage library of 10^{10} size.

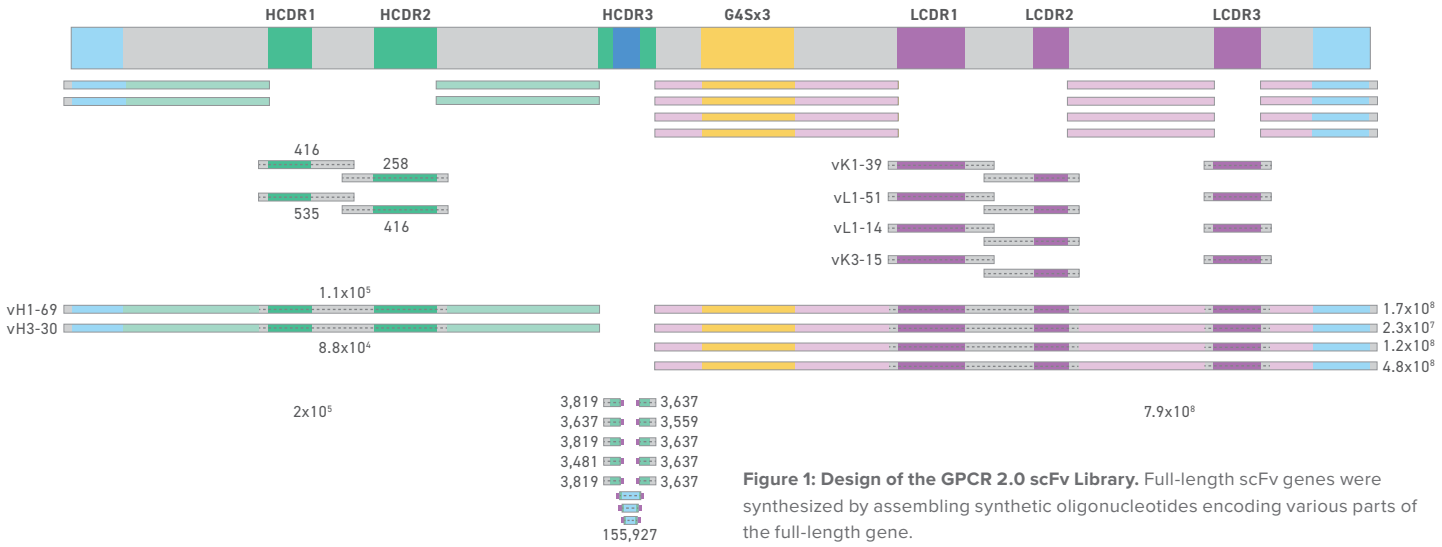

Figure 1: Design of the GPCR 2.0 scFv Library. Full-length scFv genes were synthesized by assembling synthetic oligonucleotides encoding various parts of the full-length gene.

ANTI-GLP-1R ANTIBODY DISCOVERY WORKFLOW

Refer to Liu et al (2021; ref 6) for a full description of the materials and methods used in this study.

The workflow used to discover novel anti-GLP-1R antibodies involves four primary steps, as outlined below and in **Figure 2A**.

1. **Panning:** Given the challenges associated with generating natively folded GPCR proteins, a cell-based biopanning strategy was devised. A GLP-1R stable Chinese Hamster Ovary (CHO) cell line was created by transfection with a construct encoding the full-length human *GLP1R* gene (UniProt: P43220), an N-terminal FLAG tag, and a C-terminal GFP tag (**Figure 2B**). GLP-1 R expression was confirmed by the detection of both GFP fluorescence and the cell surface expression of a FLAG tag. The GLP-1R-expressing CHO cells were used for five rounds of phage panning against the GPCR-focused library to identify potent GLP-1R binders (**Figure 2C**).
2. **Sequencing:** Iterative rounds of panning resulted in the enrichment of high-affinity clones (**Figure 3**). Approximately 1,000 clones were selected from panning rounds 4 and 5 for NGS sequencing. Of these, ~100 unique VH-VL pairs were selected for reformatting and scale-up.
3. **Reformatting & Scale-up:** scFv candidates were reformatted to full-length human IgG2, expressed in Expi293 cells, and purified at 1-ml scale for binding and functional assays.
4. **Binding & Functional Assays:** Finally, IgGs were evaluated for target binding and functionality in a series of *in vitro* and *in vivo* assays.

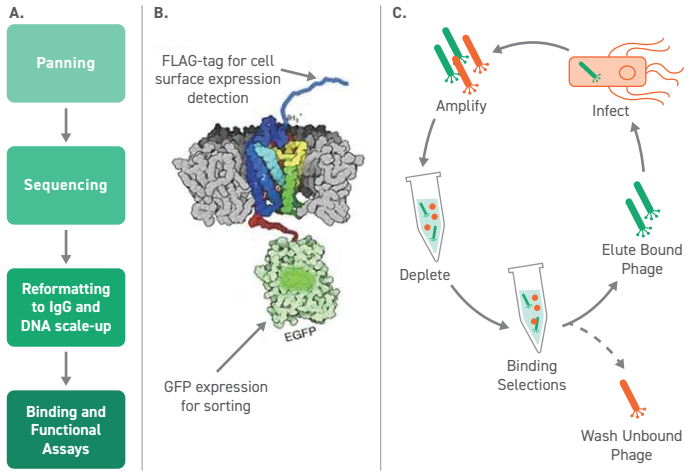

Figure 2: Antibody discovery workflow. The antibody discovery workflow (**A**) leverages a CHO cell line that overexpresses a dual-tagged GLP-1R (**B**). GLP-1 R expression was confirmed by the detection of both GFP fluorescence and the cell surface expression of a FLAG tag. The GPCR 2.0 scFv Library was panned against the GLP-1R overexpressing CHO cell line as shown in (**C**).

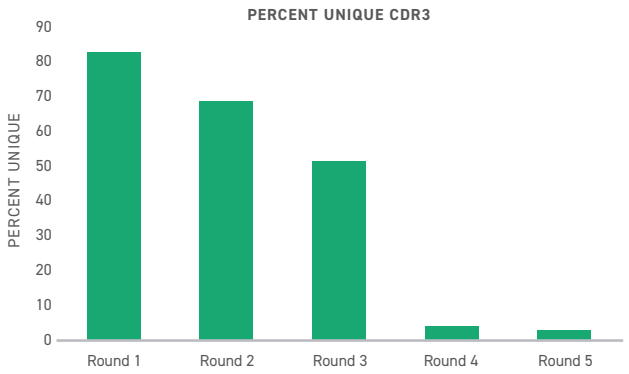

Figure 3: Clonal enrichment of VH genes over 5 successive rounds of panning. The percentage of unique HCDR3 sequences decreased after each panning round, indicating that target-specific clones can be enriched identified by our cell-based panning protocol.

RESULTS

Discovery and Characterization of GLP-1R Antagonist IgGs

Of the ~100 IgG2 clones enriched after five rounds of biopanning, 13 bound to GLP-1R expressing CHO cells without binding to wild-type CHO cells. The apparent binding affinity of these 13 hits to GLP-1R was evaluated by flow cytometry. All 13 IgG2 clones bound to GLP-1R with nanomolar affinity, including several hits (e.g., TB01-3, TB01-8, TB01-56, TB01-70) that bound more strongly than the positive control mAb-3F52 antibody (**Figure 4A**). Eight of these clones exhibited antagonistic activity in a validated GLP-1R-induced cAMP signaling assay, as demonstrated the inhibition of cAMP production stimulated GLP-1_{7-36} and exendin-4, potent peptide agonists of GLP-1R (data not shown). None of these clones displayed agonistic activity (i.e., stimulation of cAMP production).

The antagonistic activity of the most potent GLP-1R binder — TB01-3 — was further probed with a series of *in vitro* competition and functional assays. TB01-3 competed with GLP-1_{7-36} in a flow cytometry binding assay (**Figure 4B**), which suggests that TB01-3 exerts its antagonism by binding to an orthosteric site on GLP-1R. Subsequent functional assays demonstrated that TB01-3's antagonistic activity is dose-dependent and extends to other aspects of GPCR biology, such as inhibition of β-arrestin recruitment to GLP-1R (**Figure 4C, D**).

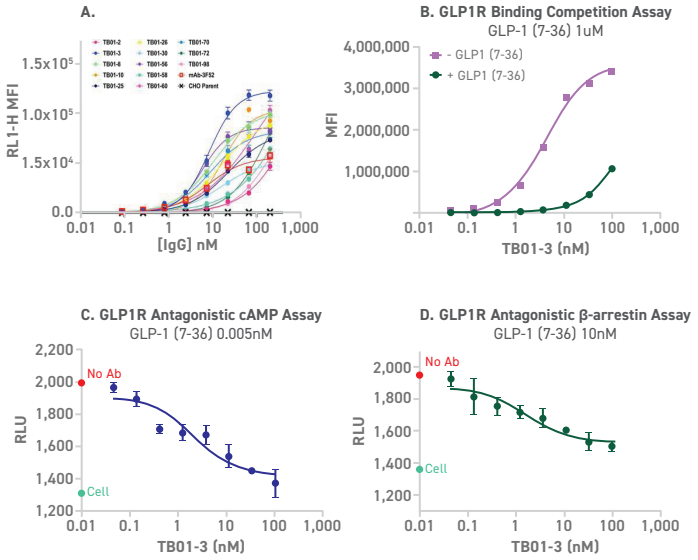

Figure 4: *In vitro* characterization of GLP-1R antagonists. (**A**) The enriched panel of 13 clones exhibited nanomolar apparent binding affinities. (**B**) TB01-3 competed with GLP-17-36 in a flow cytometry-based competition assay. TB01-3 (**C**) inhibited cAMP production and (**D**) reduced B-arrestin recruitment in GLP-1R-overexpressing CHO cells in a dose-dependent manner.

In Vivo Testing of TB01-3

Finally, the pharmacokinetics (PK) and *in vivo* functionality of TB01-3 was evaluated in rats and mice, respectively. In a 2-week PK study, TB01-3 was determined to have a half-life of ~1 week (data not shown), which is consistent with that of other antibodies and superior to that of small molecule alternatives. This half-life is also dramatically longer than that of endogenous GLP-1, which is degraded within minutes in serum.

Antagonism of GLP-1R elevates blood glucose levels by inhibiting insulin production from the pancreatic beta cells. To confirm TB01-3's antagonistic activity *in vivo*, C57BL/6NHsd mice were pretreated with TB01-3 or the peptide antagonist exendin 9-39 (also known as avexitide) and then challenged with insulin. Pretreatment with TB01-3 significantly elevated blood glucose levels in mice following insulin challenge (**Figure 5**). TB01-3 was effective at both dosing schedules, whereas exendin 9-39 was only effective when administered at both 19 and 2 hrs before insulin challenge (**Figure 5A**). Exendin 9-39's lack of effect when administered only 6 hrs before insulin challenge (**Figure 5B**) could reflect the peptide's short half-life of 3.5 to 4 hours[4].

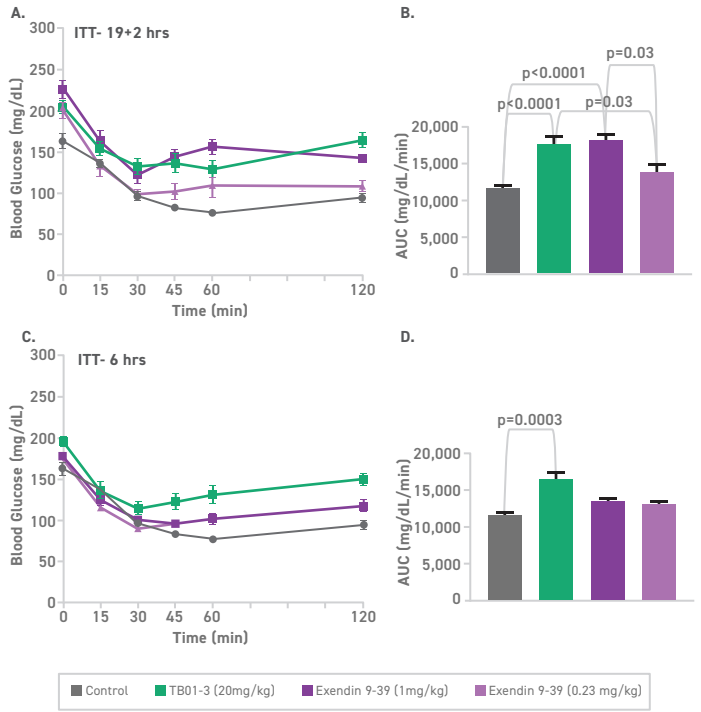

Figure 5: *In vivo* characterization of TB01-3 using an insulin tolerance test. Two dosing schedules were used: (**A**) 19 and 2 hrs before insulin challenge, and (**B**) 6 hrs before insulin challenge.

DISCUSSION

In 2019, the discovery pipeline for GPCR-targeting antibodies spanned over 170 active programs targeting 76 GPCRs, up from the 15 programs targeting 10 different GPCRs that existed in 2010[7]. This dramatic increase in interest among antibody developers presumably arose from a greater understanding of GPCR biology afforded by crystal structures as well as the advances in high-throughput antibody discovery technologies. The clinical success of these efforts was realized in 2018 when the FDA approved two GPCR-targeting antibodies for clinical use. Opportunity abounds in the GPCR antibody discovery space, with hundreds of GPCR targets still untapped by current discovery campaigns.

This application note outlines the discovery of antagonistic anti-GLP-1R antibodies from the GPCR 2.0 scFv Library, a fully synthetic human antibody library conceived and constructed by Twist Biopharma. The library's design explicitly incorporates structural information derived from known GPCR crystal structures in-complex with a wide diversity of ligands. By combining a development-focused design with precise construction enabled by large-scale gene synthesis, the GPCR 2.0 scFv Library virtually eliminates unnatural and nonfunctional antibodies from the discovery process, saving developers time and money. Antibody discovery pipelines can access this library by licensing it directly from Twist Biopharma or through a partnership with Twist Biopharma, who will take on the complete discovery process.

Pairing this rich GPCR-focused library with a cell-based biopanning strategy led to the discovery of 13 high-affinity and selective anti-GLP-1R antibodies with highly developable therapeutic properties. Interestingly, although the majority of these hits possessed GLP-1 or GLP-2 motifs in their HCDR3 loop, a minority (3/12) utilized unknown motifs to bind GLP-1R[7]. Thus, the GPCR 2.0 scFv Library is sufficiently diverse to enable the identification of motifs beyond those present in GPCR and ligand co-structures that informed its design.

The discovery of antibodies against GLP-1R was spurred by the poor pharmacokinetic properties of existing peptide antagonists, such as exendin 9-39. In this study, antibody antagonist TB01-3 outperforms exendin 9-39 in an insulin tolerance test while demonstrating an exceptional half-life of ~7 days *in vivo* in rats. Twist Bioscience's DNA synthesis capabilities allowed for the design and synthesis of a library that was both rich with functionality, and representative of the ultra-long loop regions required to interact with the deeply buried transmembrane binding regions of GLP-1R. Such a library maximized the opportunity to identify functional antibodies with desirable binding properties. We believe TB01-3 is the first fully human anti-GLP-1R antagonist antibody that is ready for therapeutic development.

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