Optimization of a Glucagon-Like Peptide 1 Receptor Antagonist Antibody for the Treatment of Hyperinsulinism

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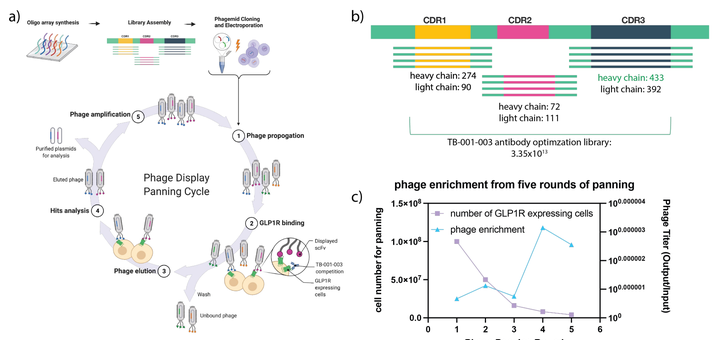
ABSTRACT

Hyperinsulinism is commonly caused by genetic mutations and as a consequence of hypobaric surgery. Hyperinsulinism can lead to hypoglycemia, psychiatric disorders and insulin resistance, a cause of type two diabetes. An effective avenue for therapeutic treatment of hyperinsulinism is antagonism of the Glucagon-Like Peptide 1 Receptor (GLP1R). Previously, we identified a highly potent antagonist antibody, called TB-001-003, which was from our synthetic antibody libraries designed to target G protein-coupled receptors (GPCRs). Here, we designed a library to optimize the activity of TB-001-003 against GLP1R and performed phage-display on cells overexpressing GLP1R. We found many optimized antagonists, including partial agonists. One antagonist, called TB-222-023, is a β-arrestin biased inverse agonist. *In vivo*, TB-222-023 effectively decreased insulin secretion in mouse pancreatic islets and increased plasma glucose in a mouse model of hyperinsulinism. We show that targeting GLP1R with an antibody antagonist is an effective strategy for treatment of hyperinsulinism.

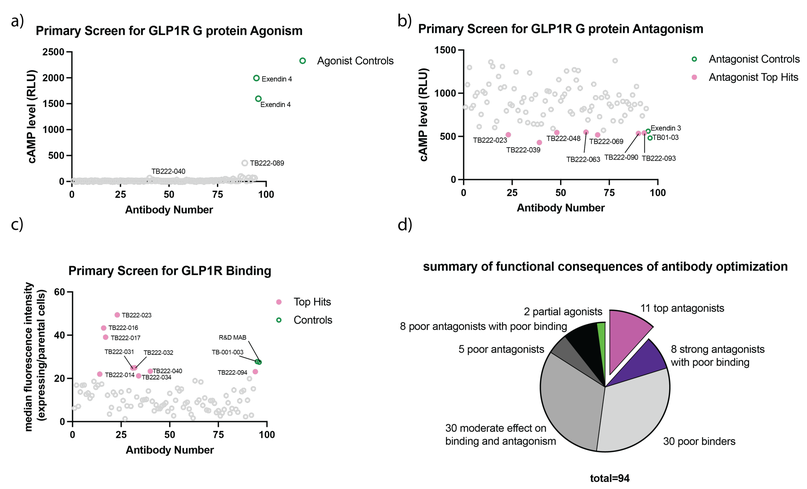
TWIST ANTIBODY OPTIMIZATION OF A GLP1R ANTAGONIST (TB-001-003)



Strategy for antibody optimization of TB-001-003

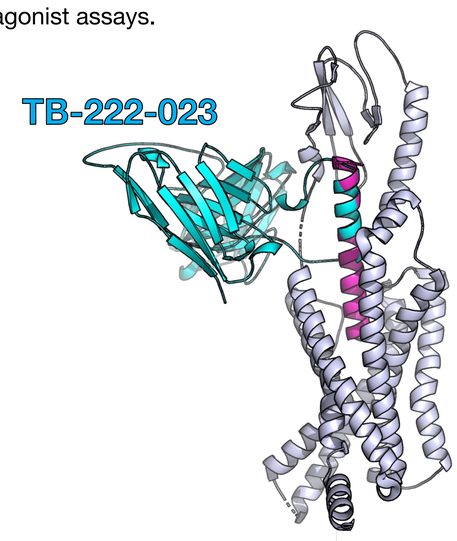
We previously identified TB-001-003 as a potent GLP1R antagonist antibody (Qiang, et al. MABS, 2021). **a)** phage panning on cells overexpressing GLP1R. **b)** Library design using Twist Antibody Optimization (TAO) algorithm. Briefly, the CDR3 from TB-001-003 was mutagenized with 433 different clones. All other CDRs were derived from naturally occurring human sequences and randomly assembled to achieve a library of $3.35x10^{13}$. **c)** phage enrichment increased through successive rounds of panning through more stringent washing and less available antigen.

DISCOVERY OF ENHANCED ANTAGONISTS AND PARTIAL AGONISTS



Parallel screening of binding, agonism and antagonism

Top 94 phage outputs were reformatted to full length IgG and screened for **a)** agonism, **b)** antagonism, and **c)** GLP1R binding. **d)** summary of results, two partial agonists were discovered and eleven optimized agonists with improved binding, antagonism or both. TB-222-023 was further characterized for antagonist assays and TB-222-040 and TB-222-089 were characterized for partial agonist assays.



AlphaFold 2.0 model of TB-222-023 aligned to GLP1 bound to GLP1R (PDB: 6X18)

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TWIST BIOPHARMA

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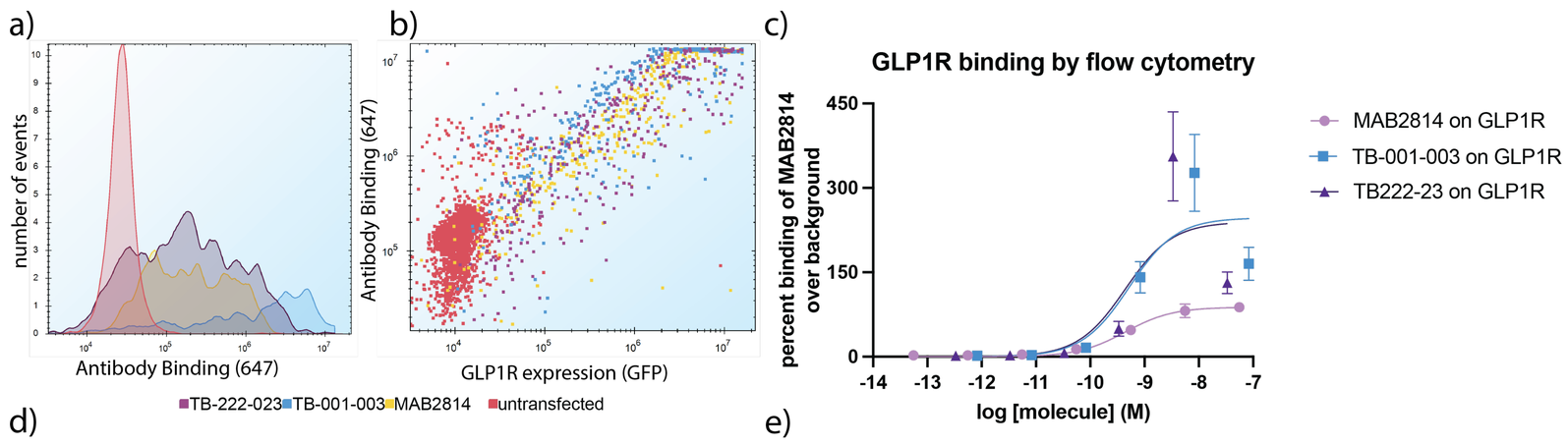
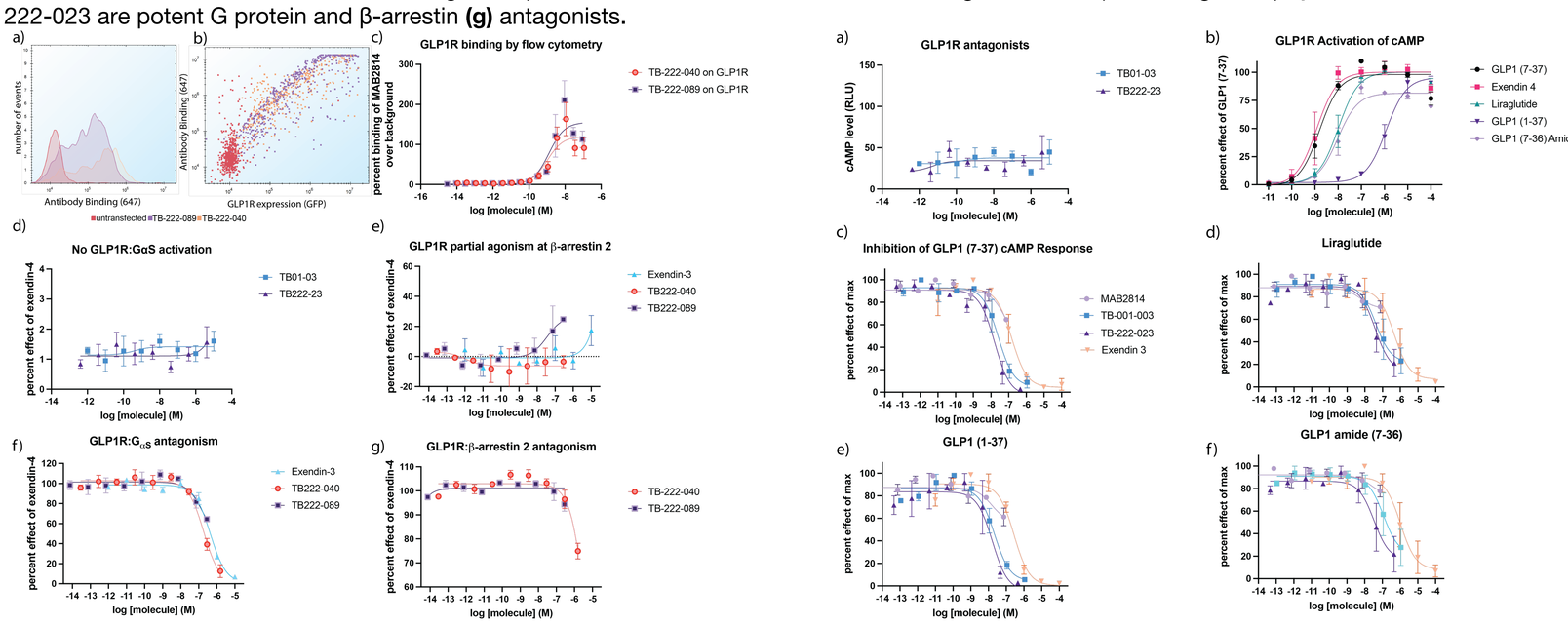
DISCOVERY OF β-ARRESTIN BIASED GLP1R ANTIBODIES



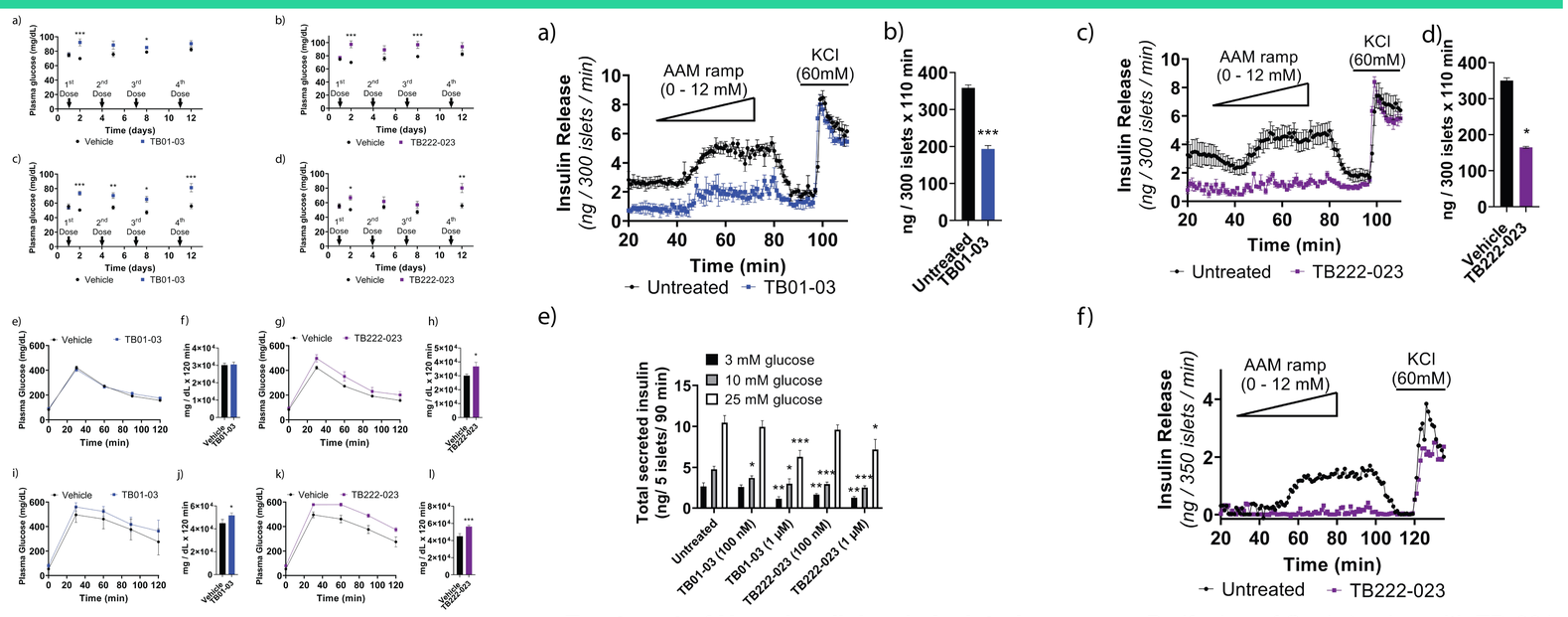
Pharmacological characterization of enhanced GLP1R antibodies

a) Histogram from binding by flow cytometery. **b)** Flow binding shows receptor dependent increase in antibody binding. **c)** TB-001-003 and TB-222-023 show enhanced binding to above MAB2814 (commercial control antibody). **d)** TB-222-040 and TB-222-089 very weakly activate cAMP. **e)** TB-001-003 and TB-222-023 reduce background β-arrestin recruitment when screened in agonist mode (inverse agonism). **f)** TB-001-003 and TB-222-023 are potent G protein and β-arrestin **(g)** antagonists.



Pharmacological characterization of novel GLP1R partial agonists **TB-001-003 and TB-222-023 block other known GLP1R agonists**

TREATMENT OF HYPERINSULINISM IN VIVO



Treatment of Hyperinsulinism in mice. Wild-type (a,b,e,g,h) and SUR1$^{-/-}$ knockout mice (c,d,i,j,k,l) are effectively treated with TB-001-003 and TB-222-023

Treatment of Hyperinsulinism in isolated pancreas. Perifusion of Sur1-/- mice with TB-001-003 (**a,b**) and TB-222-023 (**c,d**) blocked insulin release. **e)** static incubation of WT mouse islets with glucose after treatment of TB-001-003 and TB-222-023 blocks insulin release. f) perifusion of islets isolated from an infant with K_{ATP}HI treated with TB-222-023.