LLM-Driven Library Screening for Discovery of Antibody-Based GLP-1R Agonists

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Background

LLM-based Antibody Discovery[1,2]

- Antibody-based agonists offer potential advantages over peptide agonists, including longer half-life, improved specificity, and favourable biodistribution
- Multi-pass transmembrane GPCRs remain challenging targets for antibody discovery, with relatively few FDA approvals to date. Structurally constrained extracellular epitopes can limit antibody binding and functional modulation of receptor signalling
- Recent advances in large language models (LLMs) have accelerated de novo antibody generation, increasing the potential for successful campaigns

GLP-1R as a Therapeutic Target

- Glucagon-like peptide-1 receptor (GLP-1R), a class B G protein-coupled receptor (GPCR), is a validated therapeutic target for type 2 diabetes and obesity
- Aim of isolating agonistic GLP-1R-binding antibodies capable of activating downstream cAMP signalling to promote glucose-dependent insulin secretion

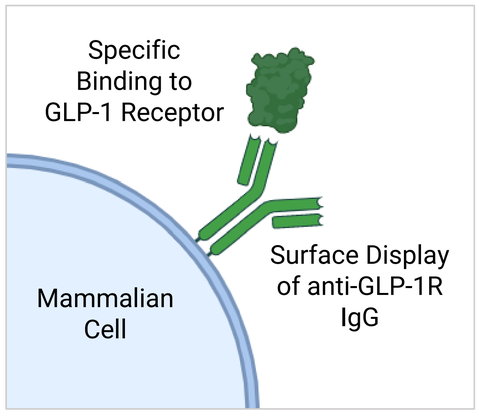

Fig 1. Mammalian cell displaying a full-length anti-GLP-1R IgG that is binding specifically to the GLP-1 Receptor

AI/ML-ab™ In-Silico Design Platform

- Traditional discovery of high affinity antibodies relies on time-consuming immunisation and manual engineering, including CDR grafting and mutation
- AI/ML-ab™ preserves native eukaryotic folding and post-translational modifications whilst facilitating selection for binding, biophysical properties, and reduced immunogenicity
- Combination of AI/ML-ab™ and OptiMAL® platforms enable screening of diverse AI/ML-designed libraries within high-throughput mammalian display

Methodology

LLM Training

- Interrogation of >2 billion human antibody sequences from large-scale databases, including OPIG Observed Antibody Space (OAS), enabling learning from naturally occurring repertoires
- Generation of 3,961 heavy-chain and 1,913 light-chain sequences, combinatorially paired to produce ~8 million unique antibody candidates predicted to bind full-length human GLP-1R
- Sequences were assessed for developability as drug candidates and prioritised according to desired sequence and germline characteristics

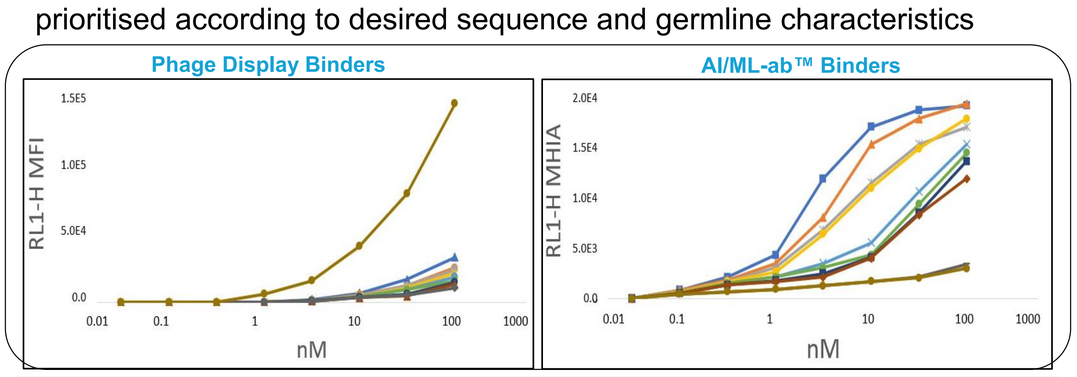

Fig 2. Comparison of Binding Profiles in Phage Display (left) and AI/ML-ab™ in-silico Libraries (right). AI/ML-ab™ binders exhibited greater concentration-dependent affinities.

Library Generation

- Library incorporated into HEK293T via lentiviral transfection and transduction with GFP as a reporter for surface display of anti-GLP-1R IgG variants
- Iterative Magnetic- and Fluorescence- Activated Cell Sorting (MACS) and (FACS) conducted to enrich GLP-1R-binding antibodies, incorporating positive and negative selection with recombinant GLP-1R protein
- Enriched hits were purified and re-expressed as recombinant H + L pairs for biophysical and functional characterisation, assessed against the theoretically-ranked top 96 sequences

Results

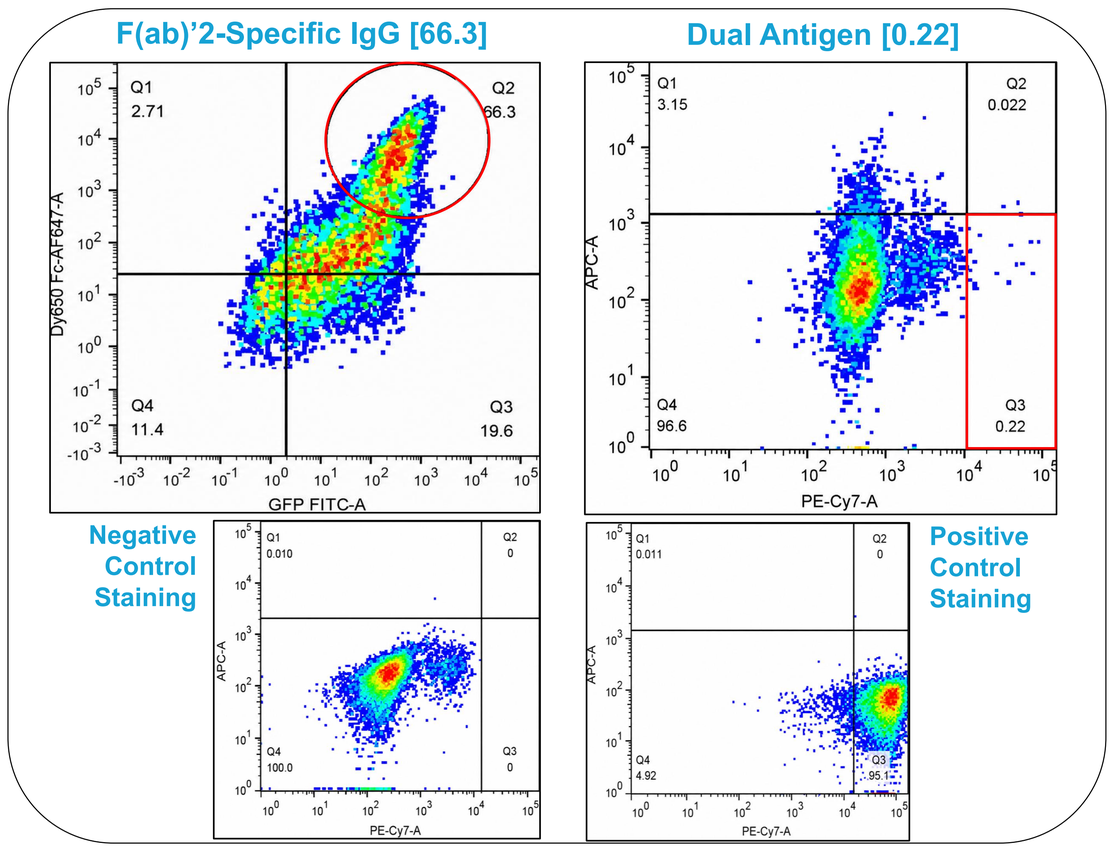

Fig 3. Selection of GLP-1R-specific Binders via FACS. Following iterative antigen-specific enrichment via Magnetic-Activated Cell Sorting (MACS), an enriched population of **GFP positive cells** was detected by flow cytometry. An initial bulk sort to isolate full-length IgG variants (via anti-hu F(ab)'2 IgG Display) was conducted, yielding **F(ab)'2-Specific IgG [Q2, 66.3%]**. Post-recovery, the bulk-sorted population underwent **dual-antigen staining**, using GLP-1R recombinant protein for positive selection **(PE-Cy-7-A)** and a negative antigen control **(APC-A)**. **GLP-1R-specific binders** were isolated whilst excluding non-specific antigen binding **[Q3, 0.22%]**. Selected events were single-cell sorted (SCS) into 96W plates, expanded, and validated for antigen binding by flow cytometry (FC).

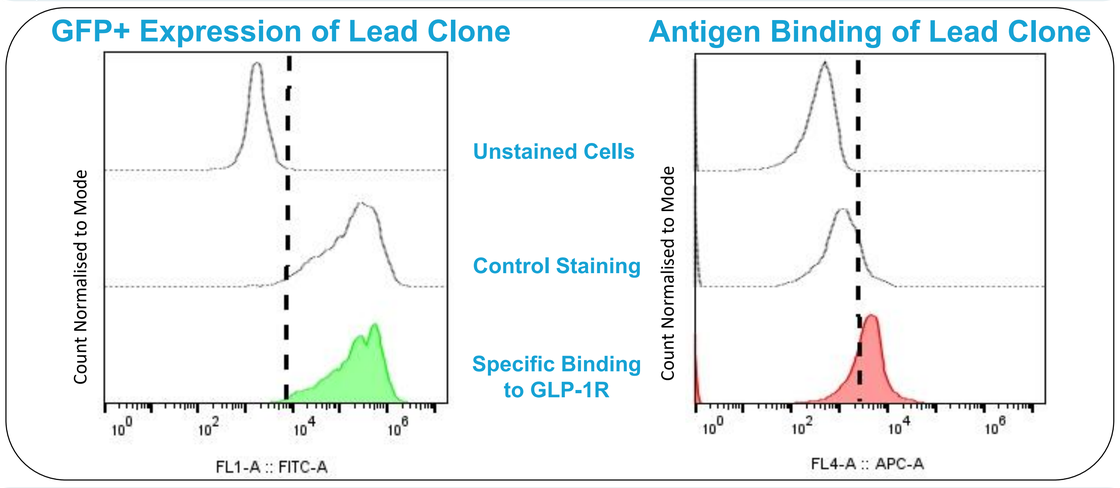

Fig 4. Flow Cytometry Validation of Identified GLP-1R Antibody Hits. Individual clones brought forward from Single Cell Sorting (SCS) were assessed by flow cytometry (FC) for GFP expression and antigen binding to anti-GLP-1R. **GFP+** fluorescence indicated stable presence of the antibody library, whilst **APC+** fluorescence confirmed surface display of antibodies capable of binding to the target recombinant protein (GLP-1R). Identified hits demonstrated **dual GFP+/APC+ signal**. Unstained HEK293T cells and a non-targeting antibody stain control showed no detectable APC signal, supporting **GLP-1R specificity**. Rescreened clones were subsequently cryopreserved and re-expressed for downstream characterisation and NGS analysis.

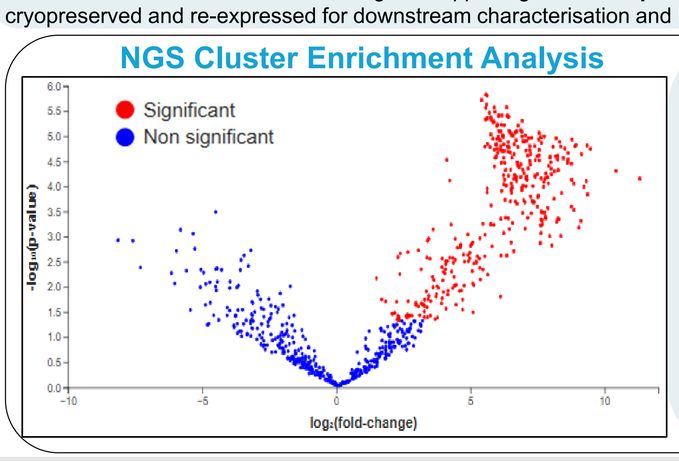

Fig 5. Cluster Analysis of Enriched IgG Sequences. Antibody sequence clusters identified following iterative MACS rounds were compared with the unprocessed library. **Red dots (n=435)** represent **statistically significant positive fold-change**, whilst **blue (n=341)** remain **unenriched or depleted**. Overall enrichment was observed; however, insufficient specificity for the desired GLP-1R binding phenotype remained. Five out of 96 top-ranked hits were contained within the enriched dataset, though only one demonstrated on-target binding.

Conclusions & Future Work

- Combining LLM-driven de novo antibody generation with high-throughput mammalian display is a promising approach to overcome key obstacles associated with discovery against challenging targets
- GLP-1R-binding antibody sequences were successfully recovered from the de novo library, validating the existing methodology and highlighting the potential of AI/ML technologies to shape the future of antibody discovery
- Optimisation of enrichment and selection stringency is necessary to increase target-specific enrichment for GLP-1R. Further characterisation studies will aim to ultimately isolate functional GLP-1R agonists

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References (1) Kim J, McFee M, Fang Q, *et al*. Computational and artificial intelligence-based methods for antibody development. *Trends Pharmacol Sci.* 2023;44(3):175-189. (2) Bai G, Sun C, Guo Z, *et al.* Accelerating antibody discovery and design with artificial intelligence: recent advances and prospects. *Semin Cancer Biol.* 2023;95:13-24.

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