Alloy Therapeutics®

Better medicine. Together.

# Targeting G protein-coupled receptors with DeepImmune™ Antibody Discovery

G protein-coupled receptors (GPCRs) play a central role in multiple physiological functions, from endocrine sensing to neurotransmission to chemotaxis of immune cells. GPCRs have long been an attractive target for novel antibody drug discovery campaigns in multiple disease indications. However, the unique nature of GPCRs lead to many challenges for in vivo antibody discovery. GPCRs are multi-pass transmembrane proteins that often require stabilization within a lipid bilayer when expressed in a recombinant fashion, unlike single-pass transmembrane receptors that can be expressed in soluble forms using techniques such as Fc-fusions. The large and complex nature of GPCRs also creates challenges with confirming proper protein confirmation in recombinant versions of these targets. Additionally, as only a small portion of the total GPCR may be exposed from the surface of the lipid bilayer, this limitation of available epitopes can restrict immunogenicity and limit the diversity of recovered antibody clones. Despite these challenges, GPCRs remain compelling targets for antibody drug discovery, requiring the development of more powerful tools for targeting these proteins in an antibody drug discovery campaign.

Alloy Therapeutics offers a powerful suite of mouse models to enable the discovery of unique, fully human antibodies with therapeutic potential. Alloy offers a broad range of transgenic mice as part of the ATX-Gx™ platform, with each providing a distinct approach to antibody drug discovery. Each model provides a unique immune landscape for mounting responses to target antigens, and these models can be utilized in parallel campaigns to maximize the potential output of a particular discovery campaign. Together, the ATX- portfolio of models offer (i) full human heavy chain repertoire, (ii) human kappa and human lambda chain repertoire, (iii) haplotype diversity, and (iv) limited immunodominance. These features enable the ATX-Gx™ platform to provide broad epitopic diversity to a wide range of targets, including more challenging targets like high homology proteins and multipass transmembrane receptors, while also yielding fully human antibodies that can utilize both kappa and lambda light chains.

The ATX-Gx™ platform exhibits robust immune responses that are similar to wild-type mice, allowing for the utilization of a broad range of immunization techniques, including state-of-the-art approaches for targeting GPCRs. ATX-Gx™ mice have been successfully utilized for GPCR-targeted antibody discovery, yielding target-specific serum titers and successful recovery of strong binders. Alloy's DeepImmune™ Antibody Discovery process builds on this robust *in vivo* discovery platform, coupling best-in-class immunogen production with multiple binder recovery approaches, including single B cell sequencing and immune display libraries, supported by advanced artificial intelligence and machine learning (AI/ML) approaches for lead identification and optimization. Below are two examples of how the ATX-Gx™ platform and DeepImmune™ Antibody Discovery can support discovery campaigns against G protein-coupled receptors.

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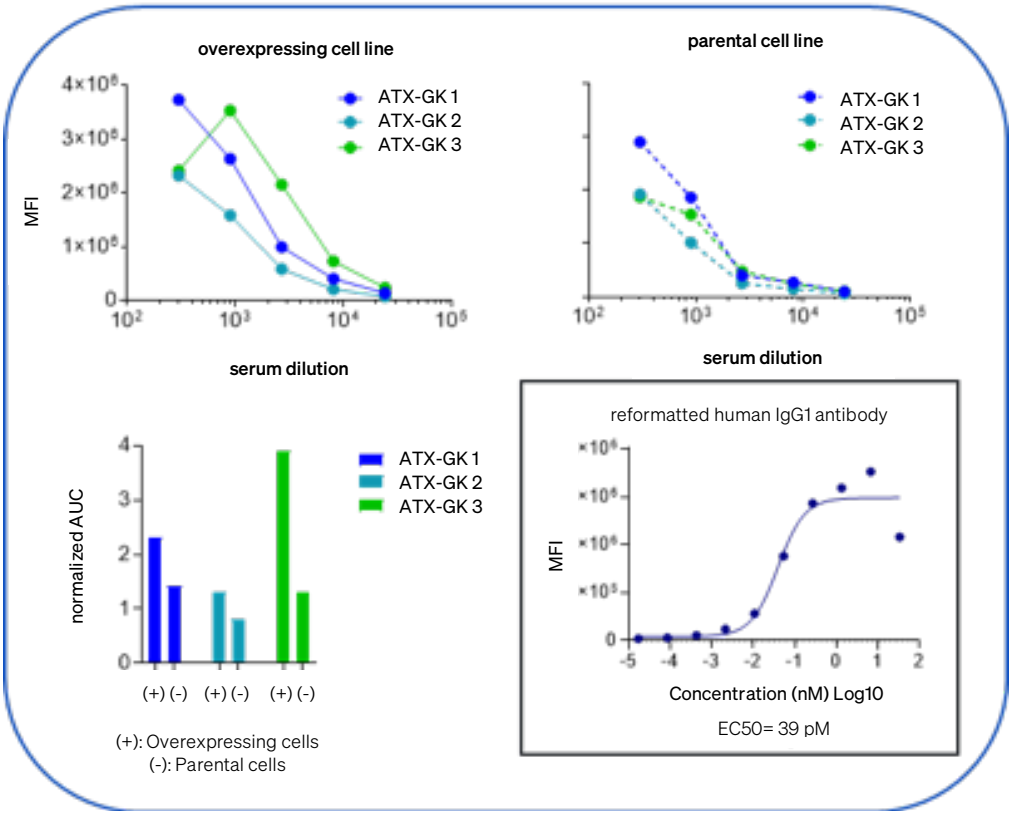
## Cell immunizations with the ATX-Gx™ platform

In this first campaign performed in-house by DeepImmune™ Antibody Discovery, the target GPCR of interest was one with high homology between human and mouse (>85%). This presented an additional challenge of overcoming the potential of central tolerance to the target, in addition to those inherent when targeting integral membrane proteins such as GPCRs. Based on these challenges, our DeepImmune™ team identified the need for cell-based immunizations to help drive the generation of antibodies specific for the native conformation of the receptor target. This would require the generation of novel overexpression cell lines for immunization, along with a well-designed immunization protocol to support strong titers to this high homology target. For the immunization protocol, Alloy developed a cell line with overexpression of the GPCR of interest. This target-expressing cell line was then utilized for a 28-day repetitive immunization at multiple sites (RIMMS) protocol with the ATX-GK strain for generating antibody responses to the GPCR of interest.

Following immunization, the ATX-GK mice showed high titers to the target GPCR of interest, as measured by flow cytometry using the overexpressing cell lines. Once target-specific titers were confirmed, splenocytes from these animals were isolated and used to generate hybridomas for the selection of monoclonal antibodies. Over 1000 individual hybridoma clones were screened, yielding 43 GPCR specific clones. Sequencing of these clones yielded over 10 different clonotypes within this collection, indicating the breadth of responses generated by the ATX-GK mice. Following reformatting to human IgG1, individual clones showed excellent target specificity with picomolar affinities to their target.



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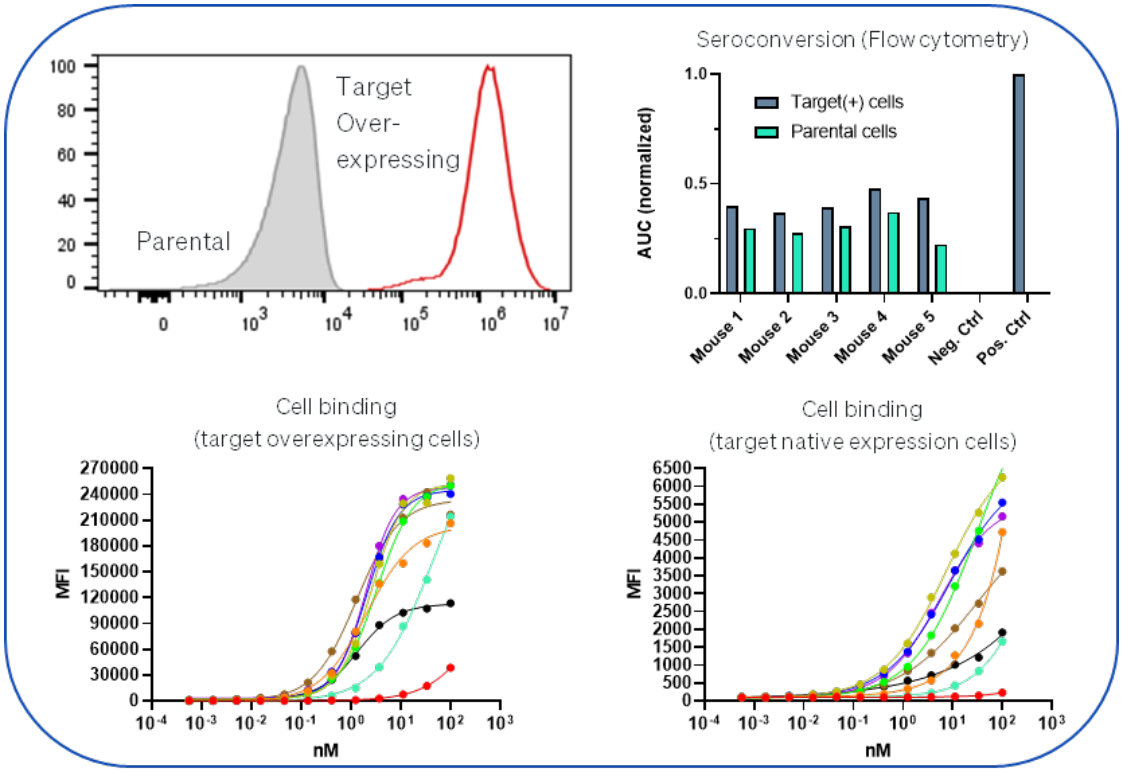
In this second campaign, also performed in-house by DeepImmune™ Antibody Discovery, the target GPCR of interest was also of significant homology between human and mouse (>80%). As with the previous example, DeepImmune™ Antibody Discovery engineered a cell line for overexpression of the target of interest and confirmed high expression via flow cytometry. This overexpressing cell line was then utilized in a RIMMS protocol with ATX-GK mice, and seroconversion levels were monitored using a flow cytometry-based cell binding assay. In response to cell immunization, all ATX-GK mice within the cohort exhibited seroconversion to target, as evidenced by increased serum binding to target overexpressing cells as compared to the parental cell line. Following confirmation of seroconversion, hybridoma fusions were performed and supernatants were screened for anti-target activity. From this hybridoma campaign, over 50 target-reactive clones were identified. Following reformatting to human IgG1, clones were tested for binding both against the target overexpressing cells, as well as cells that natively expressed the target of interest. Positive clones showed binding to the GPCR target on both cell types, confirming that these clones could engage with the GPCR target when expressed at physiological levels on target cells. From this campaign, over 30 individual clones were identified for the final lead set.




As shown in the above examples, the ATX-Gx™ mouse platform generates robust antibody responses to GPCR targets. In response to immunization with GPCR-overexpressing expressing cells, ATX-Gx™ mice exhibit *high titers* of *target-specific antibodies* when measured against cells expressing the target proteins in their native conformations. When coupled with the diverse suite of transgenic animals available within the ATX-Gx™ mouse platform, including animals with diverse MHC haplotypes and those with human kappa or lambda light chains, the ATX-Gx™ platform can enable your GPCR drug discovery program for success. Alloy's DeepImmune™ Antibody

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Discovery services group is ready to assist with your discovery campaign, leveraging our protein sciences, molecular biology, immunization, and binder recovery expertise in collaboration with our AI/ML approaches and lead optimization capabilities. Additionally, the ATX-Gx™ platform can be accessed through any of our certified CROs throughout North America, Europe, or Asia. Your team can also work with the ATX-Gx™ platform directly in your own research facilities. Whether on your own or through a CRO, Alloy provides all necessary technical support and know-how for your team to find success with the ATX-Gx™ platform when pursuing discovery campaigns against your challenging target of interest.

## The ATX-Gx™ Platform

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| **ATX-GK BL/6** | | Complete functional human Gamma heavy chain and Kappa light chain on a BL/6 background (MHC Haplotype H-2b); custom KO strains available. |
| **ATX-GK BALB/c** | | The same ATX-GK antibody diversity on a BALB/c background (MHC Haplotype H-2d). |
| **ATX-GK CROSS** | | Complete functional human Gamma heavy chain and Kappa light chain on a steady-mix BL/6 & BALB/c background (MHC Haplotypes H-2b & H-2d). |
| **ATX-pGK** | | First half of human Gamma heavy chain with full Kappa light chains on a BL/6 background to limit immunodominance. |
| **ATX-dGK** | | Second half of human Gamma heavy chain with full human Kappa light chains on a BL/6 background to limit immunodominance. |
| **ATX-GL** | | Complete functional human Gamma heavy chain and 21/30 Lambda light chain genes on a BL/6 background. |
| **ATX-HYPERIMMUNE** | Q4-22 | ATX-Gx™ strain genetically engineered to produce a diverse Ig response to high homology targets. |

### About Alloy Therapeutics

Alloy Therapeutics is a biotechnology ecosystem company empowering the global scientific community to make better medicines together. Through a community of partners across academia, biotech, and the largest biopharma, Alloy democratizes access to tools, technologies, services, and company creation capabilities that are foundational for discovering and developing therapeutic biologics across six modalities, including antibodies, TCRs, genetic medicines, peptides, cell therapy, and drug delivery. Alloy's first foundational platform, the ATX-Gx™ mice, is a suite of proprietary transgenic mice strains for human therapeutic antibody discovery. Alloy is a leader in bispecific antibody discovery and engineering services, utilizing its proprietary ATX-CLC common light chain platform integrating novel transgenic mice and phage display. Alloy's DeepImmune™ Antibody Discovery service integrates Alloy's full complement of proprietary in vivo, in vitro, and in silico discovery and optimization technologies into one comprehensive service offering for fully human monoclonal and bispecific antibody discovery. Like all of Alloy's technologies, DeepImmune™ is also available for platform transfer and can be accessed as part of Alloy's novel Innovation Subscription model. Alloy is headquartered in Boston, MA, with labs in Cambridge, UK; Basel, CH; San Francisco, CA; and Athens, GA. As a reflection of Alloy's relentless commitment to the scientific community, Alloy reinvests 100% of its revenue in innovation and access to innovation.

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