Alloy Therapeutics

Harnessing the potential of human lambda light chains in antibody discovery campaigns

Antibody discovery technologies have advanced considerably since the development of hybridoma technology first enabled the production of monoclonal antibodies. More than one hundred therapeutic antibodies are already approved for clinical use, with new molecules being approved each year. However, despite the widespread development of novel discovery methodologies, from display technologies to single B cell sorting and sequencing, the clinical antibody armamentarium remains biased towards the use of kappa light chains. This light chain usage pattern is distinct from the observed function of the human immune system. The kappa/lambda ratio of circulating human antibodies is reported to be between approximately 1.5 and 2. The over-representation of kappa light chains in therapeutic antibodies—the result of the shortcomings of some existing discovery platforms—leaves a significant opportunity for probing novel epitopic diversity and functional activity using human lambda light chains. Indeed, it has been reported that the CDRs in kappa and lambda light chains have different physicochemical and structural properties (Townsend et al., 2016, Frontiers in Immunology). The Alloy ATX-Gx™ platform contains an *in vivo* solution for the discovery of human lambda light chain therapeutic antibodies, the ATX-GL mouse.

Why do kappa light chain antibodies predominate in the clinic?

The initial wave of therapeutic antibodies were generated using hybridoma technology. In these early discovery programs, hybridomas were generated from experimental animals (such as mice and rats) that were immunized with target antigens. As these antibodies were of rodent origin, they were often extremely immunogenic, leading to limited efficacy and poor pharmacokinetics as the patient's immune system developed anti-drug antibodies (ADAs). In the second wave of therapeutic antibodies, chimerization and humanization approaches allowed for the transferral of antigen-binding regions (such as CDRs) from the mouse/rat monoclonal antibody into a human antibody backbone. This approach significantly improved the performance of these therapeutic antibodies, however the chimerization/humanization process often led to significant challenges for development and manufacturing. Additionally, as these antibodies were not fully humanized, ADAs remained a challenge for their clinical use.

With the development of fully human antibody discovery technologies, including transgenic animals and human antibody library preparation for display technologies, the field now had the potential to generate therapeutic antibodies with significantly improved efficacy, pharmokinetics, and safety. However, as these technologies were built on the successes of the early discovery approaches, they often relied on the antibody genes utilized in previous technologies, namely human kappa light chains. Kappa light chains were often the choice used during chimerization/humanization of wild-type rodent-derived antibodies, for example, and many early display libraries were built using human kappa light chains. Kappa light chain-

containing human antibodies have been widely successful—the top 10 best selling clinical antibodies in 2018 were all kappa light chain antibodies, for example.

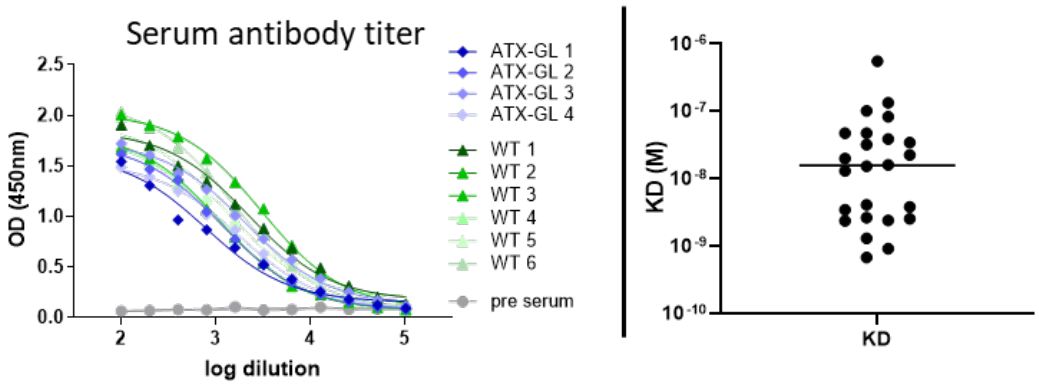
Antibody	Trade Name	Discovery method	Target	Indication
avelumab	Bavencio	Phage display	PD-L1	Merkel cell, urothelial, renal cell carcinomas
belimumab	Benlysta	Phage display	BAFF	Systemic lupus erythematosus
emapalumab	Gamifant	Phage display	IFNγ	Hemophagocytic lymphohistiocytosis
erenumab	Aimovig	Transgenic mouse	CGRP-R	Migraine
evolocumab	Repatha	Transgenic mouse	PCSK9	Hypercholesterolemia
guselkumab	Tremfya	Phage display	IL-23p19	Psoriasis

A selection of lambda light chain antibodies currently approved for clinical use.

However, as indicated in the table, lambda light chain antibodies have also found success in the clinic. The use of lambda light chain discovery technologies can potentially open up your discovery program to novel binders that may contain specific epitopic diversity and unique functional activities not captured in a kappa light chain discovery platform.

The ATX-GL platform for lambda light chain antibody discovery

The ATX-GL mouse enables *in vivo* discovery of human antibodies with lambda light chains. In designing the ATX-GL mouse, our in-house genetically engineered organism (GEO) core facility prioritized proper human gene expression by B cells, leading to a diverse representation of lambda germlines from a given *in vivo* discovery campaign. As with the other models available in the ATX-Gx™ platform, the ATX-GL mouse exhibits robust immune responses and shows levels of seroconversion that are similar to wild-type mice of the same genetic background (C57BL/6). In addition, ATX-GL mice can generate lambda light chain antibodies with strong binding to your target antigen of choice. When paired with any of the various kappa light chain expressing animals available on the ATX-Gx™ platform, you have the potential to recover both human kappa and lambda light chain antibodies from a single immunization campaign, greatly increasing your antibody diversity, epitope coverage and potential functional activity.



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ATX-GL mice generate high titers of lambda light chain antibodies with strong binding kinetics.

ATX-Gx™ Platform

The ATX-GL mouse is currently available as part of the ATX-Gx™ mouse platform, a diverse suite of transgenic animals for human antibody discovery. These include lines with diverse MHC haplotypes to maximize epitopic diversity, and those with restricted Gamma heavy chain repertoires for discovery of unique clonotypes that may be underrepresented due to expression of immunodominant germlines. The ATX-Gx™ platform allows for multiple approaches to be undertaken simultaneously in a single immunization campaign, maximizing the potential for recovery of unique antibodies with target specificity.

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 MIX		Complete functional human Gamma heavy chain and Kappa light chain on a 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	Q4-21	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	Q4-21	Genetically engineered to produce a diverse Ig response to high homology targets.

Alloy Therapeutics is a biotechnology ecosystem company empowering the global scientific community to make better medicines together. Through a community of partners, Alloy democratizes access to tools, technologies, services, and company creation capabilities that are foundational for discovering and developing therapeutic biologics. The company facilitates affordable, non-exclusive access to the entire drug discovery community from academic scientists, small and medium biotech, to the largest biopharma. Alloy's lead offering, the ATX-Gx™ platform, is a human therapeutic antibody discovery platform consisting of a growing suite of proprietary transgenic mice strains. Founded in 2017 and privately funded by visionary investors, Alloy is headquartered in Boston, MA with European labs in Cambridge, UK. As a reflection of Alloy's relentless commitment to the scientific community, Alloy reinvests 100% of its revenue in innovation and access to innovation.