AlivaMab
DISCOVERY SERVICES

Breaking Immune Tolerance to Generate Highly Potent Species Cross-Reactive Antibodies

Abstract

Although several techniques exist to produce therapeutic monoclonal antibodies, those using transgenic mice carrying human immunoglobulin (Ig) genes are among the most successful at delivering drug approvals. This is because a well-executed immunization of good transgenic mouse systems harnesses the natural complexity of the immune response, with all of its diversity and checkpoints, to rapidly produce panels of antibodies with the inherent qualities needed in a drug. From these panels the drug candidates best meeting or exceeding the target product profile can be identified by screening for requisite binding affinity, specificity, and functional efficacy. In addition, the use of human antibodies derived from transgenic mice generally means they possess inherent qualities needed for manufacturing, formulation, and stability in a drug plus an innately low risk of immunogenicity or toxicity in human patients.[1] However, there are difficult occasions when a target human protein has high homology to an orthologous protein within the mouse. In those cases, the mouse immune system may not recognize the target protein as foreign, thereby limiting antibody production in the mouse against the antigen. Therefore, it is necessary to break immune tolerance to generate drug-quality antibodies against the antigen of interest. To generate panels of cross-reactive antibody leads in these cases, AlivaMab Discovery Services (ADS), offers tolerance breaking strategies for all of our protocols to suit our clients' diverse needs.

During a project that required breaking immune tolerance for an antigen of interest, AlivaMab Discovery Services delivered:

- Rapid recovery of large human antibody panels cross-reactive to mouse, monkey, and human targets at high IgG titers (10^6)
- Characterization of ~600 antibodies to select diverse candidates
- Analysis of structural binding mechanisms related to cross-reactivity
- Functional assays that analyze panels to identify triple-cross-reactive antibody leads with therapeutic potential

The Challenge: *Breaking Immune Tolerance to Generate Cross-reactive Antibody Drug Candidates*

While mouse immunization is a common approach for antibody production, therapeutic antibody drug discovery for human diseases such as cancer, infection, metabolic malfunction, and autoimmunity must sometimes target a protein with significant or partial homology between human and mouse versions.[2] As a result, it can be difficult to generate antibodies against the human protein due to immune tolerance and the consequent low immunogenicity in mice. This means that drug discovery efforts must find a way to break immune tolerance towards these homologous proteins in order to generate antibodies capable of drugging the human target.[3]

That said, high homology across multiple model-organism species can also be beneficial to drug developers, once immune tolerance is broken. Antibodies that are cross-reactive to species orthologs of the target can be useful for first assessing antibody efficacy in mouse disease models and almost always are required for toxicology testing in non-human primates (cynomolgus monkeys) before advancing to clinical testing in humans.[4] This means that the antibody can be tested unaltered throughout the entire drug development process.

Phage and other in vitro display technologies are proposed as platforms for producing cross-reactive antibodies. However, the unnatural selection of in vitro display technologies is divorced from important checkpoints of the immune system and is prone to producing promiscuous binding antibody candidates that can manifest as dangerous off-target effects in human clinical trials as well as developability issues in manufacturing.[5]

Ninety percent of approved antibody drugs in the United States were generated in rodents, almost all of those from mice. Even Humira, the first approved drug from an in vitro phage display platform, was engineered using a mouse antibody against TNF-α as template. In techniques using normal mice, the antibodies must be engineered subsequently to be chimeric or humanized after being isolated to reduce the chance of adverse events in human clinical trials. During these processes, an antibody candidate is engineered to carry sequences of both mouse and human antibodies.[1,6,7] However, re-engineering an antibody to make it more human takes a significant amount of resources, which limits throughput and often forces developers to only take limited numbers of candidates through the process. Furthermore, the process can sometimes render the selected antibody less soluble, introduce alterations in binding affinity and specificity, and induce changes in protein expression.[1] Because these issues may not be apparent until later in the development process, they can lead to dramatic setbacks. In fact, some antibodies cannot be humanized and still retain all the requisite drug-like properties. Especially when users are seeking cross-reactive antibodies, the lack of antibody options for humanization can be a detriment.[1] Some mouse model immune systems have now been genetically engineered to carry human Ig genes so as to directly produce human antibodies.[1] However, like normal mice, these mice still carry immune tolerance mechanisms inherent to the immune system.

As a result, there is a standing need for immunization strategies able to break immune tolerance in mouse systems carrying human Ig genes in such a manner that a very large number of diverse hits are generated and recovered to better find cross-reactive drug candidates.

The Approach: High-Throughput Screening After Breaking Immune Tolerance in the AlivaMab® Mouse

To meet this drug discovery need, AlivaMab Discovery Services has optimized the use of the human Ig gene-bearing AlivaMab® Mouse of Ablexis, LLC with our proprietary immunization strategies.[1] While other genetically engineered mouse systems may still struggle to break tolerance, the AlivaMab Mouse was engineered to produce excellent antibody diversity, even in cases of highly homologous targets.[1] The AlivaMab Mouse quickly produces hundreds to thousands of hit antibodies, which are further screened to uncover cross-reactive antibodies with functional characteristics that meet the therapeutic target product profile.

In a matter of weeks, through ADS's enhanced hybridoma process, the team can recover a large pool of monoclonal antibody candidates and determine which are sufficiently high affinity, high potency, and cross-reactive.

To assess and demonstrate ADS's tolerance breaking technologies, target proteins with high homology between mouse, cynomolgus monkey, and human orthologs were used to generate antibody producing hybridomas. ADS subsequently put candidate antibodies through functional assays designed to efficiently screen leads and select the most desirable based on specificity to the target of interest, affinity, cross-reactivity, and epitope binding diversity.

Rapidly Achieving Cross-Reactive Immunity

Using its proprietary immunization technologies, the AlivaMab Discovery Services team immunized AlivaMab Mice against two proteins, referred to as Target 1 and Target 2, each with different percentages of amino acid sequence conservation with their cynomolgus monkey and mouse orthologs, and recovered triple-cross-reactive antibodies against mouse, cynomolgus monkey, and human versions of each target. Targets 1 and 2 have significant homology across all the three species with mouse to human homology at ~85% and ~65%, respectively. Because it can be challenging to recover cross-reactive antibodies for any protein that carries less than 90% homology,[1] the use of proprietary methods that break immune tolerance is key; this enables antibody production against conserved protein segments and not just epitopes unique to the human protein.

Within weeks of immunization, ELISA revealed the achievement of robust cross-species recognition. The resulting serum titers were quite potent against all homologs, which points to success with respect to overcoming tolerance **(Figure 1).**

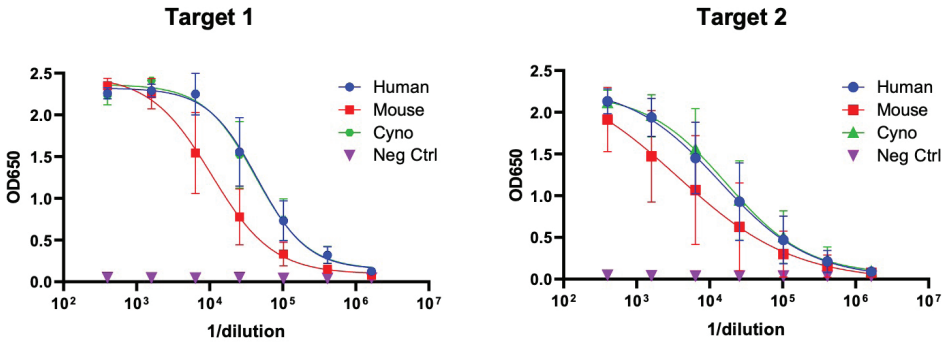

Figure 1: Immunization of the AlivaMab Mouse yielded high serum titers cross-reactive for mouse, cynomolgus monkey, and human for versions of both Targets 1 and 2.

Efficiently Evaluating Individual Antibody Candidates for Cross Reactivity

A cornerstone of ADS's approach is generating and working with large panels of antibodies, which enables recovery of a larger selection of unique antibodies with the desired attributes. In this case, an ELISA enabled screening of almost 600 antibodies against the human, cynomolgus, and mouse orthologs of Target 1 and Target 2 **(Figure 2)**. Candidates were then binned based on the extent they bound each versions of the target.

This experiment illustrates the breadth of antibody diversity recovered from the AlivaMab Mouse. Some antibodies were more reactive to one version of a target than the others (Ex: **Figure 2**, right), whereas some antibodies bound to all three versions (**Figure 2**, left). In addition, variation of binding profiles to the three versions indicated that the antibody hits were binding via different structural mechanisms. Different binding mechanisms strongly suggest that different antibodies hits recognized different epitopes on the target.[8]

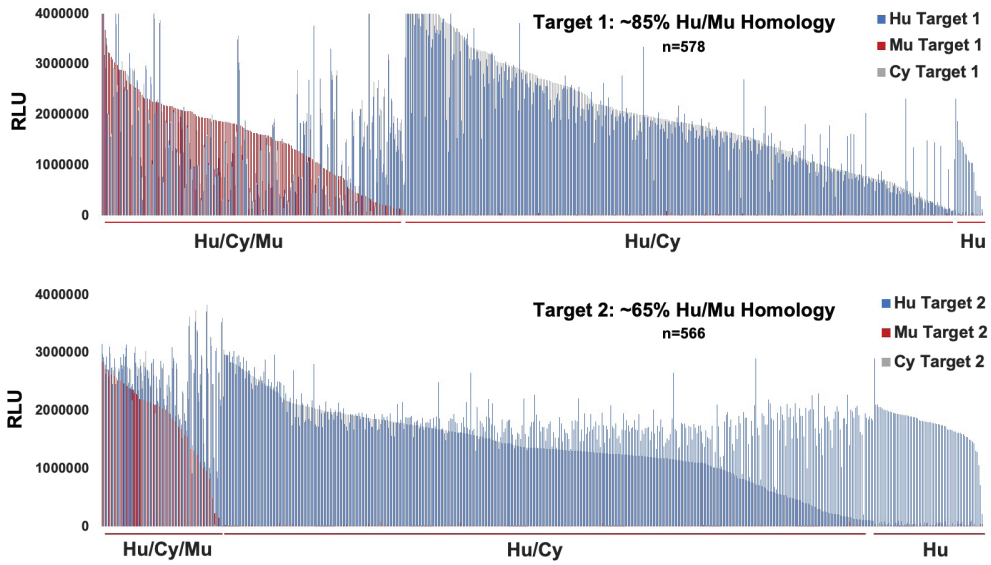

Figure 2: AlivaMab Mouse Prime Discovery immunizations result in cross-reactive antibodies for Targets 1 and 2, as determined by ELISA. Bars represent the individual antibody binding profile towards each target version, with triple cross-reactive antibodies on the left, human/monkey reactive in the center, and human only reactive on the right.

Applying Strategies that Break Immune Tolerance to Rapidly Generate Panels of Functional, Cross-Reactive Antibodies

A longstanding challenge for generating therapeutic antibodies in vivo against highly conserved targets is overcoming immune tolerance; yet, having cross-reactive antibodies between mouse and human orthologs can be useful for in vivo studies in mouse models. AlivaMab Discovery Services can conduct immunizations that break immune tolerance and produce large and diverse panels of antibodies that recognize mouse and cynomolgus orthologs of the human target. In addition, downstream functional testing of the panel can reveal functional, triple-cross-reactive leads with sufficiently high affinity and therapeutic potential.

Collectively, this case study shows that ADS's platform can enable future antibody discovery against challenging antigens and address unmet targets. Combining the power of ADS's technologies with fast turnaround, clients can quickly advance through multiple stages of antibody drug development, minimize the risk of costly setbacks, and increase the clinical relevance of their pre-clinical research.

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