AlivaMab DISCOVERY SERVICES

From Novel Assay Development to Quality Therapeutic Leads on a Rapid Timeline

A Case Study on Delivering Picomolar Affinity Drug Candidates in Three Months with AlivaMab Discovery Services

Abstract

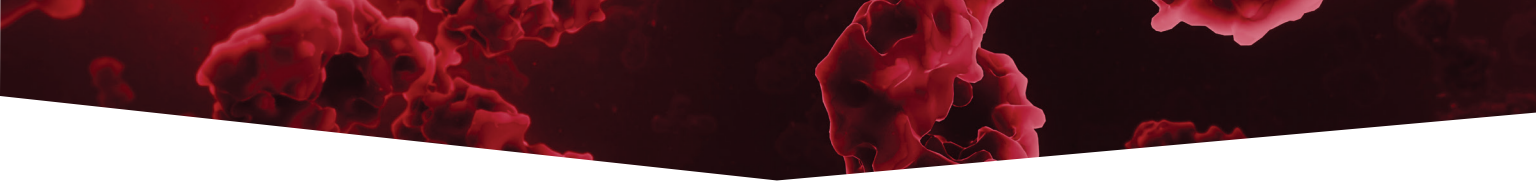
The explosion of interest and innovation in antibody engineering throughout the last three decades has led to many advances in antibody discovery platforms. AlivaMab Discovery Services (ADS) uses a transgenic mouse platform called AlivaMab® Mouse (Ablexis, LLC) and immunization strategies, called AlivaMab Mouse Prime Diversity (AMMPD), to rapidly identify and characterize human monoclonal antibodies (mAbs) that meet or exceed a client's therapeutic product profile. For one such client, ADS delivered a panel of 24 high potency, high affinity human therapeutic antibody leads in less than three months. This case study details the planning process, immunization strategies, hybridoma technique, and screening methods ADS utilized to ensure the client received results that met their needs.

By partnering with AlivaMab Discovery Services to identify therapeutic leads, a client was able to:

- Advance their drug discovery pipeline in the absence of laboratory facilities
- Obtain large panels of functionally characterized antibodies able to neutralize ligand activity to its two different G-protein-coupled-receptors (GPCRs)
- Receive a panel of high potency human antibodies with affinities in the picomolar range for further development

Challenge: *De Novo* Development of a Discovery Research Plan

The last three decades have included exponential innovation in antibody engineering, which has facilitated a seemingly limitless interest in discovering and developing therapeutic mAbs to address a wide range of diseases and match increasing demand.[1] Advances in discovery platforms have also shifted more of the responsibility of complete mAb discovery programs from individual companies or labs to contract research organizations (CROs), where consolidated expertise accelerates identification and characterization of therapeutic candidates. Leaner, more virtual companies can rely on CROs for all aspects of discovery, such as *de novo* assay development, while focusing internal resources on more basic research and downstream strategies en route to clinical testing.



As technologies continue to advance, the drug discovery landscape has become more competitive and is moving at an ever-increasing pace. The quicker a company can move through drug discovery and development with the highest quality candidate antibodies, the better their chances are of being first-to-market. Reducing timelines while selecting the most sensitive and diverse panel of leads is key to maximizing success throughout all stages of drug development. However, reducing timelines is often challenging given that several common discovery approaches require affinity maturation to achieve antigen binding affinities of 1 nanomolar (nM) or less, which is considered the upper allowable limit for therapeutic drug discovery.[2]

The innovative AlivaMab Mouse technology, coupled with ADS' proprietary immunization strategies and enhanced hybridoma techniques can eliminate the need for affinity maturation and developability optimization by improving recovery of a more diverse pool of IgG-secreting B cells that produce naturally high affinity human antibodies with inherent qualities required for developability.[3] Choosing a drug discovery partner that can deliver a panel of unique human mAbs with picomolar (pM) affinities in a short amount of time is extremely valuable for building and successfully executing a robust drug development plan.

AlivaMab Mouse Platform and Immunization Approach Accelerates mAbs Discovery

While efficient development timelines for remote and virtual drug companies can be a challenge even to experienced CROs, it is in this scenario that ADS has been able to provide high value to clients. Many transgenic mouse mAb discovery platforms take six to eight months from planning to delivery of drug candidates. However, with the AMMPD immunization strategies in AlivaMab Mice, enrichment for IgG-secreting B cells, efficient hybridoma generation, and scalable screening methods, ADS can deliver high potency, high affinity mAb leads in three months or less.

One virtual pharmaceutical company approached ADS with the need for a panel of mAb leads that neutralized a ligand that binds two different GPCRs.[4,5] This client did not have laboratory facilities and, therefore, needed to rely on ADS for sourcing of all material, *de novo* functional assay development, and expertise for designing and executing a discovery plan that would result in rapid leads that fit the therapeutic product profile. This case study examines the approach and results the ADS team achieved for this client.

Early Functional Assay Development Sets Up Screening and Discovery Success

Screening thousands of antibodies produced by the genetically engineered AlivaMab Mouse requires functional assays that will easily detect neutralizing mAbs. As many therapeutic candidates target novel proteins with specific functionalities, off-the-shelf assays are often unavailable. In addition, many pharmaceutical companies lack the capability or lab space to generate and validate their own tests. In these situations, the ADS team will collaborate with clients to develop *de novo* assays that meet or exceed screening requirements. In this particular case, the client wanted a lead candidate able to block the same ligand from binding two known GPCRs. ADS researched the target biology to understand the complexities of the system and designed a FACS-based assay that determines inhibition of ligand binding to each of its receptors. The team engineered

cell lines with each of the GPCRs and was able to test if antibody candidates could block receptor binding (**Figure 1**). Developing this functional assay early in the ADS process ensured the team had all the tools necessary to rapidly and efficiently launch a discovery campaign following immunization and antibody recovery.

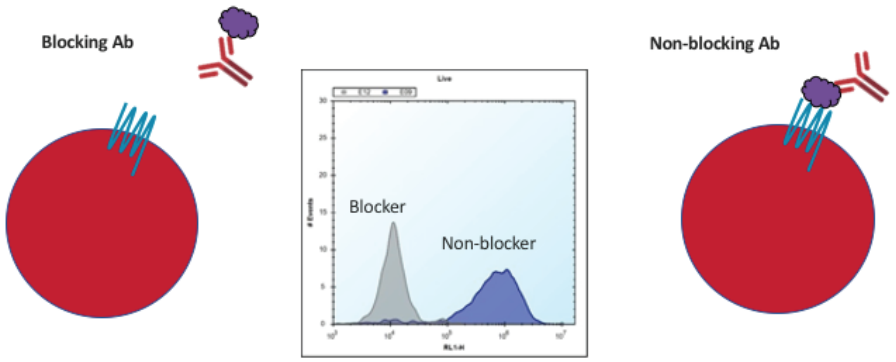


Figure 1: Functional FACS-assay that distinguishes ligand blocking and non-blocking Ab to two GPCRs.

AMMPD Immunization Strategies Lead to a Fast and Potent Start

Immunization strategies, such as optimal selection of adjuvants, routes of administration, frequency, and dosage, are all dependent on individual antigens, but ADS has the expertise and experience necessary to select the best immunization strategy on a project by project basis.[6] When targeting the ligand, ADS used two different AMMPD immunization strategies that generate high titers in two weeks and four weeks (AMMPD2 and AMMPD4), respectively. Results showed that, as early as day 10, AMMPD2 achieved a high endpoint titer of more than 1/100,000 dilution, while on day 24 AMMPD4 achieved an endpoint of greater than 1/1,000,000 dilution (**Figure 2**). These results demonstrate that in a very short period of time, both AMMPD immunization strategies produce high serum concentrations of target-specific antibodies.

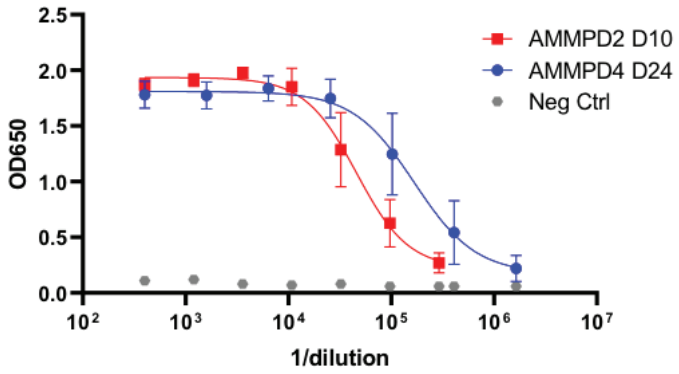


Figure 2: Serum Antibody Titers for Two AMMPD Immunization Strategies

Maintaining Hit Panel Quality Through Efficient Recovery and Screening

High serum titers are a good indication of immunization success, but efficient recovery of those antibodies is crucial to mitigating loss of high-quality therapeutic candidates. ADS' recovery system is designed to harvest and enrich for IgG-secreting B cells, which are then fused to an immortalized myeloma cell line using a proprietary electrofusion process (**Figure 3**). The hybridomas are then plated at low density on 384-well plates, while any remaining material is cryopreserved. Cryopreservation provides risk-mitigation and the opportunity to rapidly rescreen for additional hits. Importantly, plating is scalable depending upon "how deep" the recovery of the immune repertoire needs to be. Using approximately 10% of the starting material, 30 plates, and an enzyme-linked immunosorbent assay (ELISA) for detecting antigen-specific IgG antibodies, ADS was able to confirm 1,573 hits for the ligand target antigen.

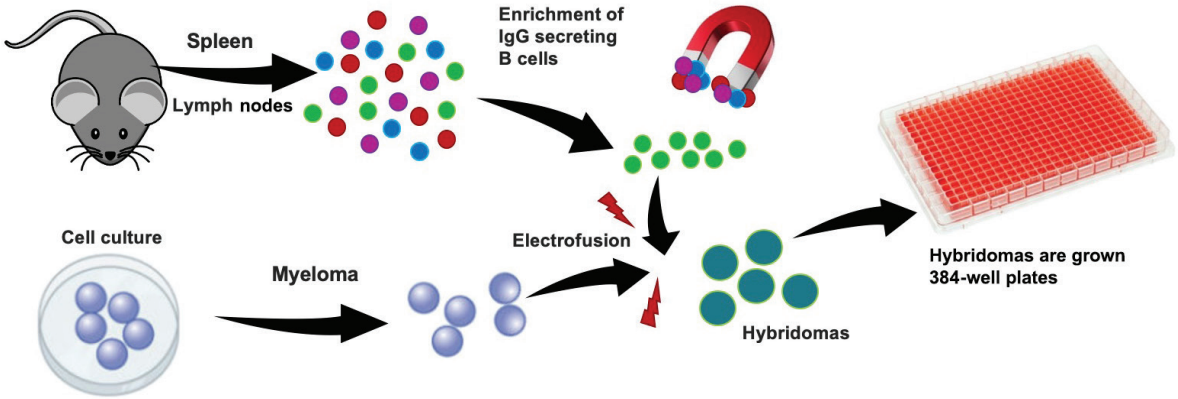
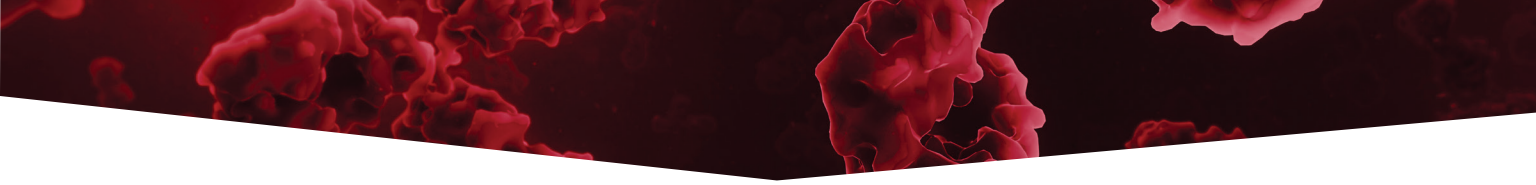


Figure 3: AlivaMab Discovery Service's Process for Hybridoma Generation

The hybridoma process is designed to quickly transfer from hit identification to screening in reliable and predictive functional assays. The hits were screened for their ability to block ligand binding to both GPCRs utilizing the functional assay developed by ADS (**Figure 4**). Selecting for antibodies that showed the highest level of binding inhibition for both GPCRs, 384 antibodies were then run through an assay to measure relative binding kinetics using the ForteBio Octet platform. The combination of these screens resulted in 200 antibodies with the desired functional activity and the highest relative affinities. Together with the client, 57 of these antibodies were selected for sequencing and further diversity characterization. The sequencing revealed a highly diverse set of combinatorial and somatic rearrangements produced by AlivaMab Mouse and AMMPD immunizations, with 95% having unique rearrangements in heavy chain CDR3 and no identical antibodies (**Figure 5**). It took approximately four weeks from identification of the initial 1,573 hits to the selection of 57 preliminary candidates.



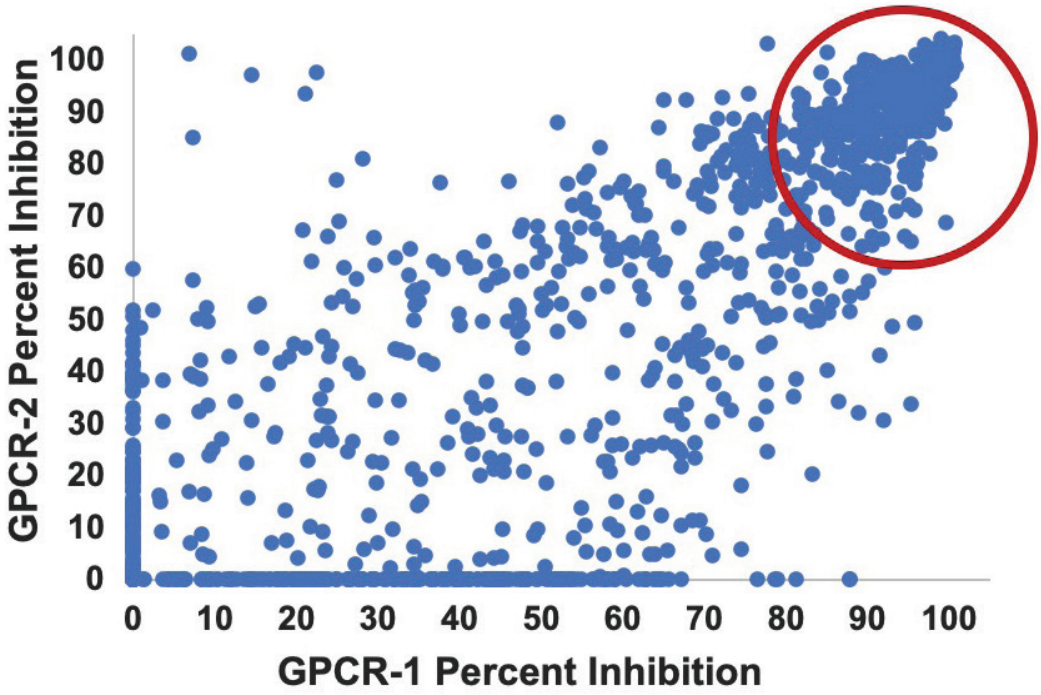


Figure 4: High-throughput Functional Inhibition Assays Identify Dual-GPCR Blocking Abs

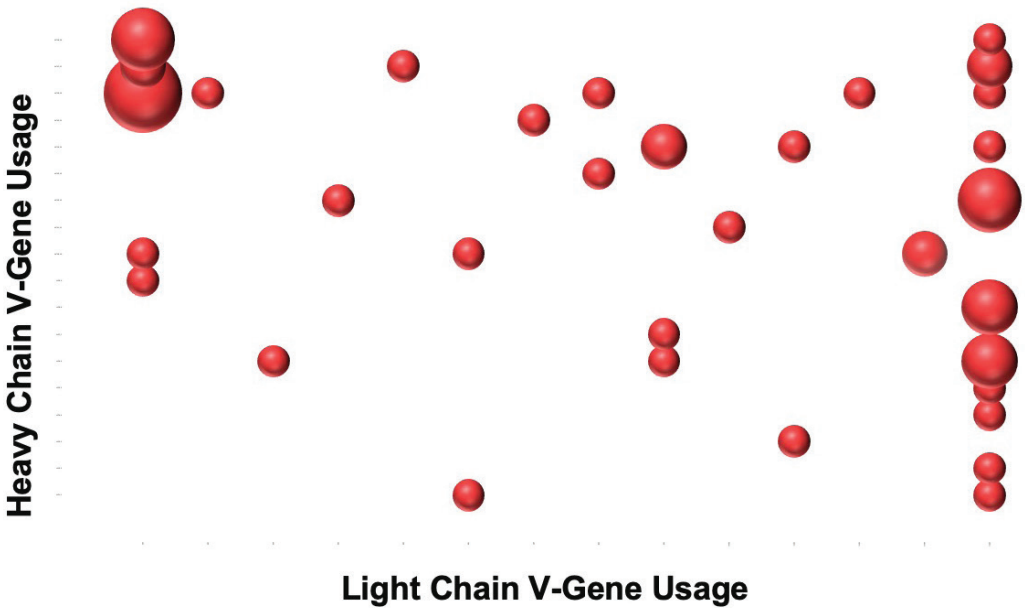


Figure 5: Antibody Diversity as Determined by Sequence Analysis

Finishing with Therapeutic Quality Leads

Having a diverse, potent, and high affinity panel of lead therapeutic candidates enables seamless downstream research and provides development alternatives ("back-ups") should there be any setbacks with any individual lead. By examining sequence data, ADS and the client were able to select 24 potential leads. These were purified and reexamined for functionality and affinity binding to both the human and cynomolgus monkey ligands. Verifying cross-reactivity in the cynomolgus monkey is important for pre-clinical development of the drug candidate for non-human primate toxicology studies required for regulatory agencies.

Functional assays demonstrated that multiple unique mAbs have IC_{50} values at the limit of detection for both receptors, indicating strong inhibition of ligand-receptor binding (**Figure 6**). Using the Octet platform to measure antibody affinity, Table 1 shows that 23 out of 24 leads have a pM K_D or below. These K_Ds match or exceed the values reported for existing FDA-approved mAb therapeutics.[3] Incredibly, seven of the antibodies have k_{off} (k_{dis}) rates that are too slow to measure on the Octet, suggesting that these antibodies have very low picomolar, or even femtomolar, affinities. The final panel included antibodies from both AMMPD immunization strategies, demonstrating that AlivaMab Mice can generate potent and high-affinity (very low pM) IgG antibodies within two weeks. This rapid production timeline was a major benefit for this virtual client.

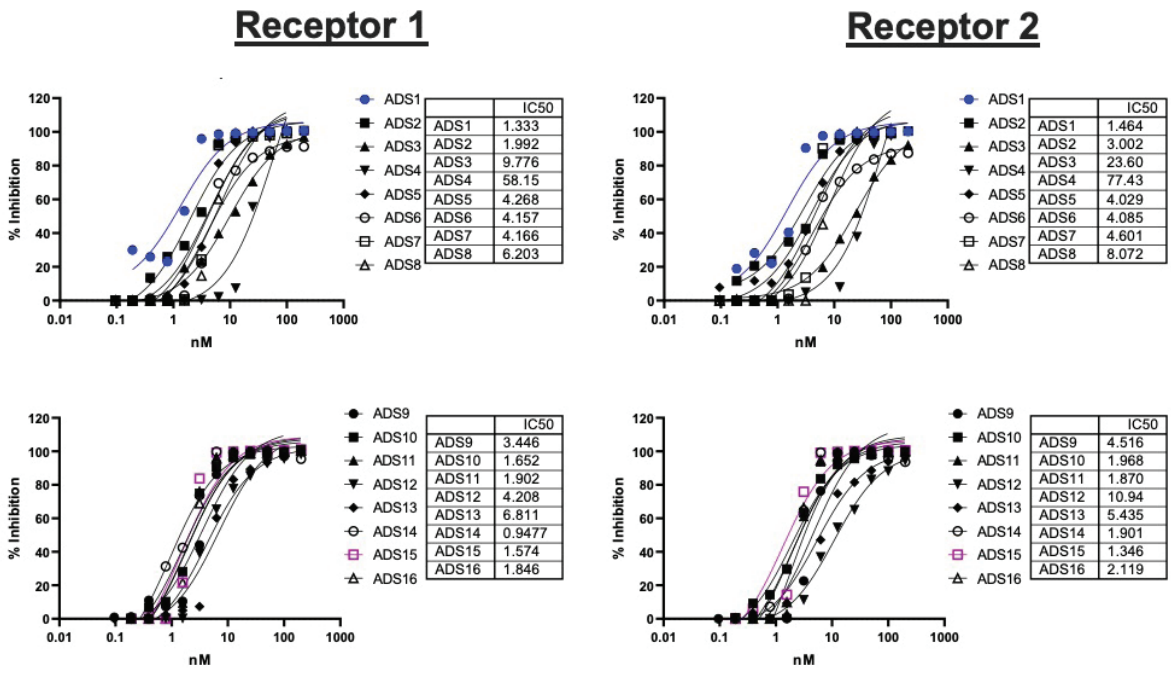
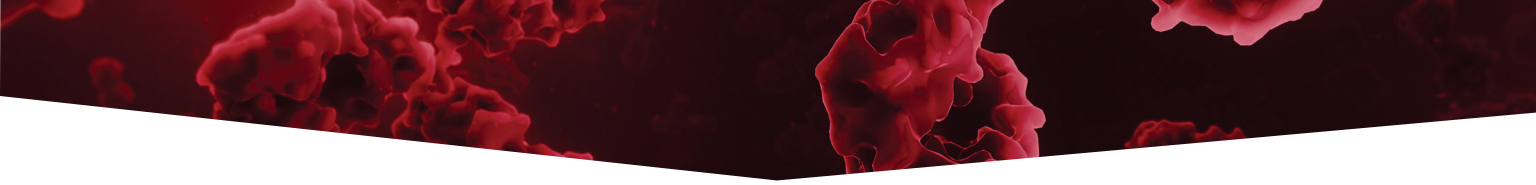


Figure 6: Functional Assays for Ligand to GPCR Receptors Detect pM Affinities and Therapeutic Quality K_Ds



	Human Ligand		Cyno Ligand	
mAb	KD (M)	kdis(1/s)	KD (M)	kdis(1/s)
1	1.0E-09	1.6E-03	1.5E-09	3.2E-03
2	6.1E-10	3.2E-03	4.6E-10	4.0E-03
3	5.8E-10	6.3E-04	2.6E-10	4.6E-04
4	4.9E-10	2.3E-03	3.0E-10	2.2E-03
5	4.6E-10	5.8E-04	1.7E-10	4.6E-04
6	4.0E-10	1.3E-03	2.5E-10	1.4E-03
7	3.6E-10	8.6E-04	2.4E-10	6.8E-04
8	2.9E-10	5.8E-04	1.1E-10	5.3E-04
9	2.3E-10	2.1E-04	1.2E-10	2.2E-04
10	2.2E-10	3.2E-04	8.8E-11	2.4E-04
11	2.1E-10	4.2E-04	1.1E-10	3.6E-04
12	2.0E-10	3.4E-04	7.8E-11	1.6E-04
13	1.8E-10	3.1E-04	1.0E-10	3.1E-04
14	1.4E-10	4.2E-04	9.3E-11	4.2E-04
15	1.1E-10	4.1E-04	3.7E-11	2.3E-04
16	5.6E-11	9.1E-05	1.1E-10	3.1E-04
17	3.5E-11	6.9E-05	2.5E-11	9.4E-05
18	<1.0E-12	<1.0E-07	1.4E-11	6.0E-05
19	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07
20	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07
21	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07
22	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07
23	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07
24	<1.0E-12	<1.0E-07	<1.0E-12	<1.0E-07

Table 1: Affinity Quantification of Selected 24 Lead Candidate mAbs

Bringing Antibody Discovery Together for Client Satisfaction

Working in partnership with the client, ADS was able to deliver a panel of 24 high potency, high affinity, unique-sequence therapeutic leads in less than three months without any starting materials provided (**Figure 7**). The timeline achieved with the AlivaMab Mouse and the AMMPD immunization strategies is several months shorter than typical hybridoma or *in vitro* display strategies and comparable to those seen with single-cell antibody discovery platforms. However, unlike microfluidic single-cell platforms, the ADS workflow includes all IgG secreting cell types, not just the plasma cells, which can lead to more diverse hit panels that include very rare antibodies. Overall, ADS' technologies, strategies, and approach were able to provide the client with a unique panel of lead candidates that set them up for continued success along the path to the clinic.

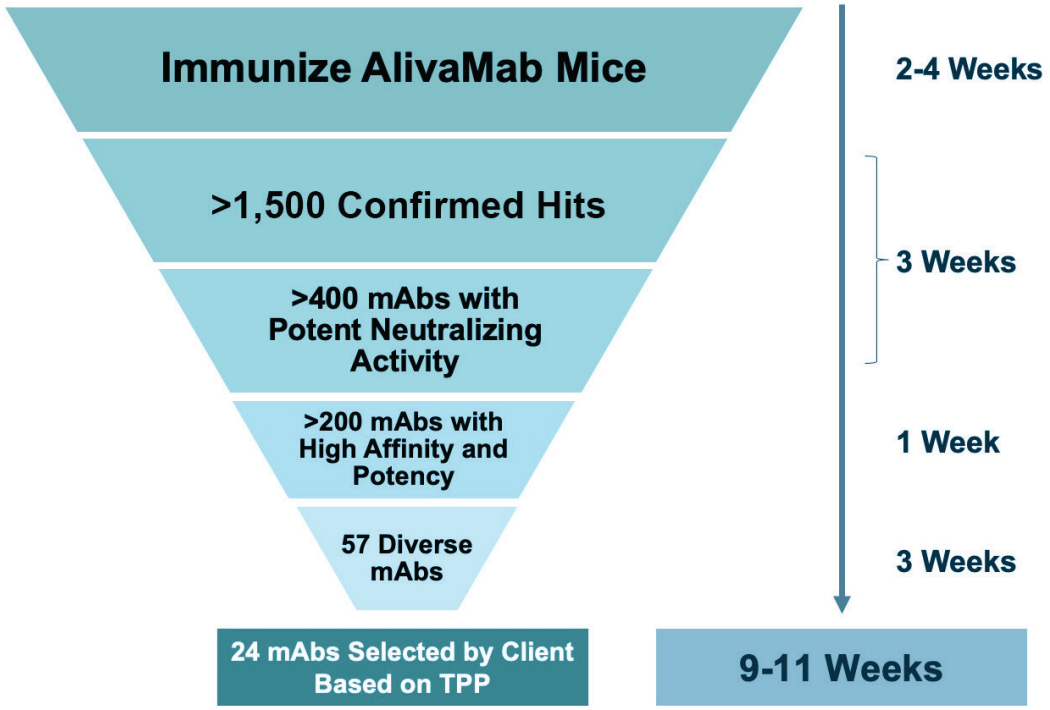


Figure 7: AlivaMab Discovery Services Timeline From Mouse Immunization to Selection of 24 mAbs.

References

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