Accelerate antibody discovery with Beacon® single cell technology and humanized mice

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Abstract

Despite demonstrated efficiency in antibody generation, classical immunization strategies and subsequent hybridoma generation often face strong limitations when it comes to complex targets like GPCRs or tetraspanins. Using WT, KO or Alloy therapeutics ATX-Gx™ humanized transgenic mice, we have developed innovative approaches combining mRNA immunization and Bruker Beacon® single cell screening platform to provide unique opportunities to dramatically speed up antibody discovery against such challenging targets.

Workflow

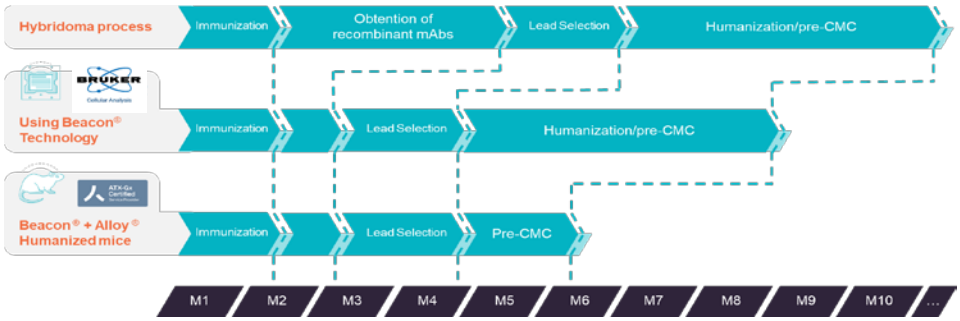


Figure 1:
Comparison of antibody production workflows between hybridoma technology and Bruker Beacon®-based single B-cell screening with WT or humanized Alloy® mice.

- Compared to the widely used hybridoma approach, larger collections of B cells are screened at higher throughput using the single cell approach.
- Compared with the approach used with WT mice, the use of Alloy® humanized mice considerably reduces antibody development time.

mRNA Immunization

Current challenges in immunization:

- **Possible issues in recombinant protein production**
- **Poor immunogenicity / cross-reactivity**

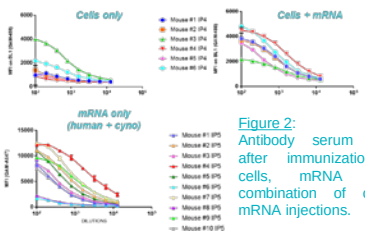


Figure 2:
Antibody serum titration after immunization with cells, mRNA or a combination of cells & mRNA injections.

- Immunization with mRNA alone or in combination with cells helps improve immune response levels.
- mRNA from different species can be used to increase cross-reactivity.

"On-chip" Functional Assays

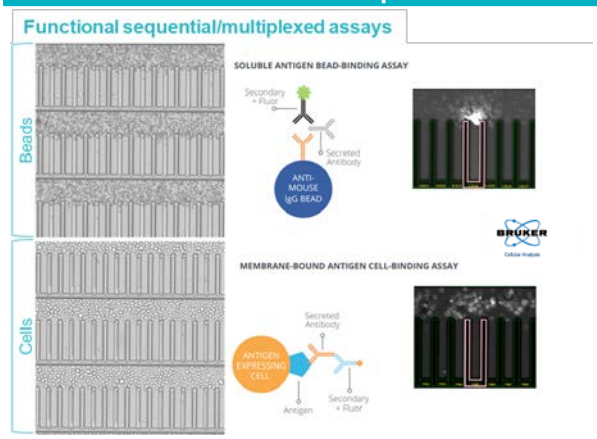


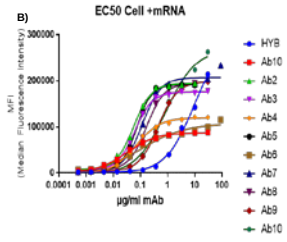
Figure 3:
"On-chip" functional assays examples.
Screening can be performed on beads (upper panel ; IgG specific, peptide- or protein-coated beads) or on target expressing-cells (lower panel).

- Sequential or multiplexed functional assays can be performed to refine candidate selection prior to hit export, antibody sequencing, production and further "off-chip" validation.
- Validated B cells are individually exported to recover corresponding antibody sequencing for further production and characterization.

GPCR Campaign Example

A

Mice Immunization (nb of mice)	Nb of campaigns	Screened colonies/clones	Positive clones
Cells only (>10)	4 (hybridoma, historical data)	> 5,000	0
mRNA only (6)	1 (hybridoma)	2,963	0
Cells + mRNA (6)	1 (hybridoma)	2,266	1
mRNA only or cells + mRNA *	**1 (Beacon®)**	**> 35,000**	**26 unique mAbs**



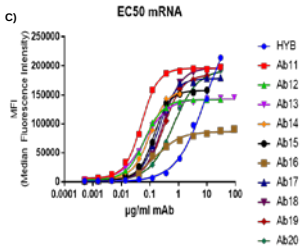


Figure 4:
Table A recapitulates data from all campaigns performed on the targeted GPCR (* remaining mice from hybridoma campaign). Graphs B and C illustrate comparative EC_{50} on human-target expressing cells for antibodies generated from different immunization strategies.

- Antibody discovery was strikingly improved using the combination of mRNA immunization and single B cell screening. No difference in affinity could be observed between clones resulting from mixed immunization or mRNA only.

Comparison of Alloy® and WT mice on a model antigen

	Alloy® MICE	WT MICE
Number of mice screened	2	2
Number of cells (millions of cells)	330	360
Sorted B cells	1,387,000	780,000
Secretory B cells	4.4 %	5 %
Specific anti-target B cells	1.2 %	1.2 %
Full antibody sequence recovery	70 %	60 %

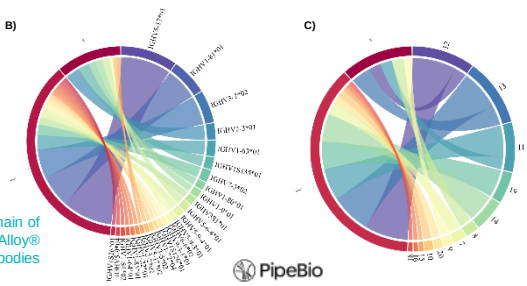


Figure 5:
Mice were immunized with recombinant protein from an extracellular domain of a membrane antigen. Table A recapitulates data from campaigns using Alloy® or WT mice. Graphs B and C illustrate the comparative diversity of antibodies obtained on VH germlines and CDRH3 length for both types of mouse.

- Data obtained with both strains of mice are very similar, except for a higher number of sorted B cells for Alloy® mice.
- VH germline diversity, CDRH3 size diversity and lengths are higher in Alloy® mice than in WT mice, suggesting more diverse paratopes in Alloy® mice.
- No common CDRH3 was found, indicating that the two strategies enable the discovery of antibodies with different types of paratopes.

Conclusion

Using innovative approaches like RNA immunization and single B cell screening, MImAbs has developed the know-how to tackle the challenge of antibody generation against difficult targets like GPRCs, ion channels or other complex proteins with multiple transmembrane domains. Combined with multiple functional assays upon candidate selection and possible use of ATX-Gx™ humanized mice, time to therapeutic candidate antibody delivery can now be significantly shortened.

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