Accelerating antibody discovery for difficult targets using mRNA immunization and BEACON® single cell technology

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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. 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

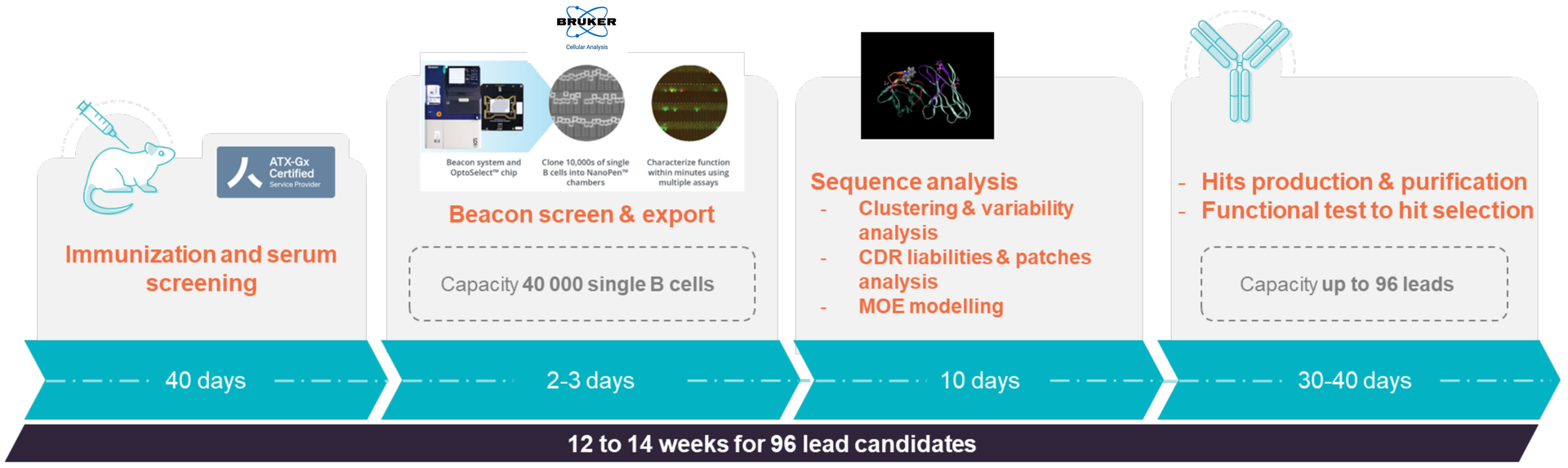


Figure 1: Representative workflow of an antibody generation campaign using Bruker Beacon®-based single B cell screening.

- Compared to the widely used hybridoma approach, larger collections of B cells are screened at higher throughput using the single cell approach.

mRNA Immunization

Current challenges in immunization:

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

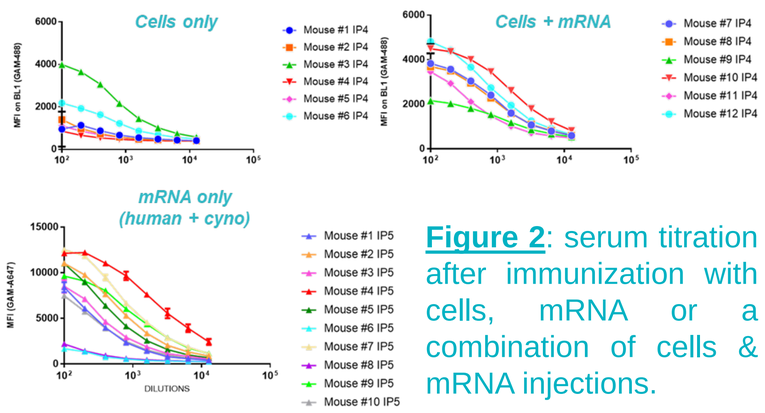


Figure 2: serum titration after immunization with cells, mRNA or a combination of cells & mRNA injections.

- 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

Functional sequential/multiplexed assays

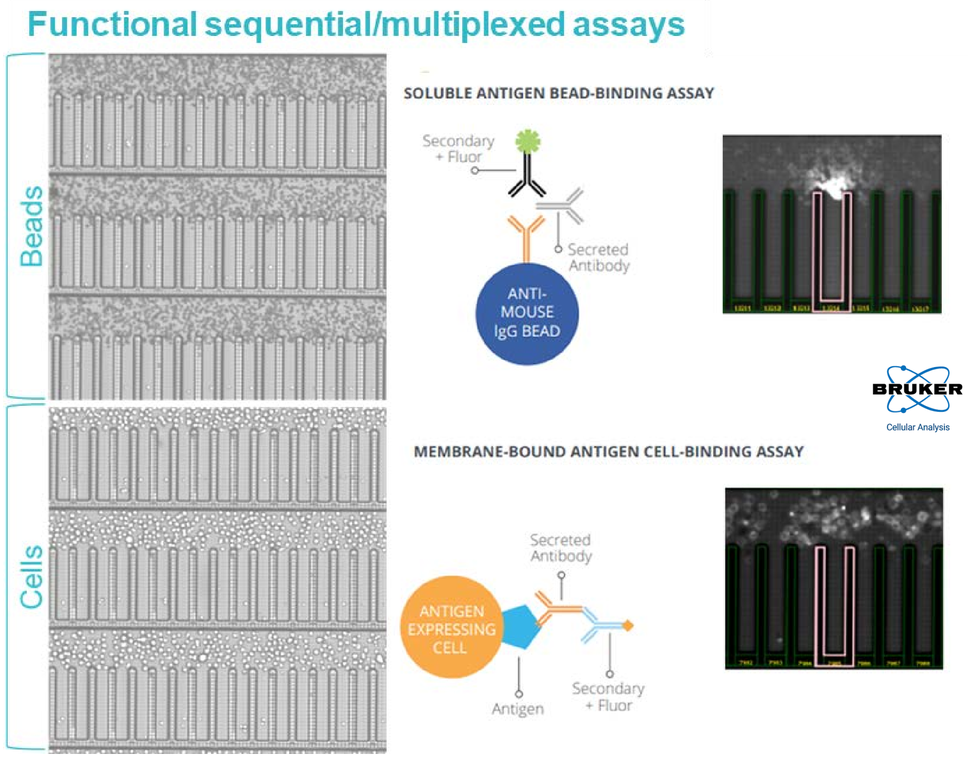


Figure 3: "On-chip" functional assays examples.
Screening can be performed on beads (upper panel ; IgG specific, peptide- or protein-coated beads) or on target expressing-cells (lower panel).

- 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**

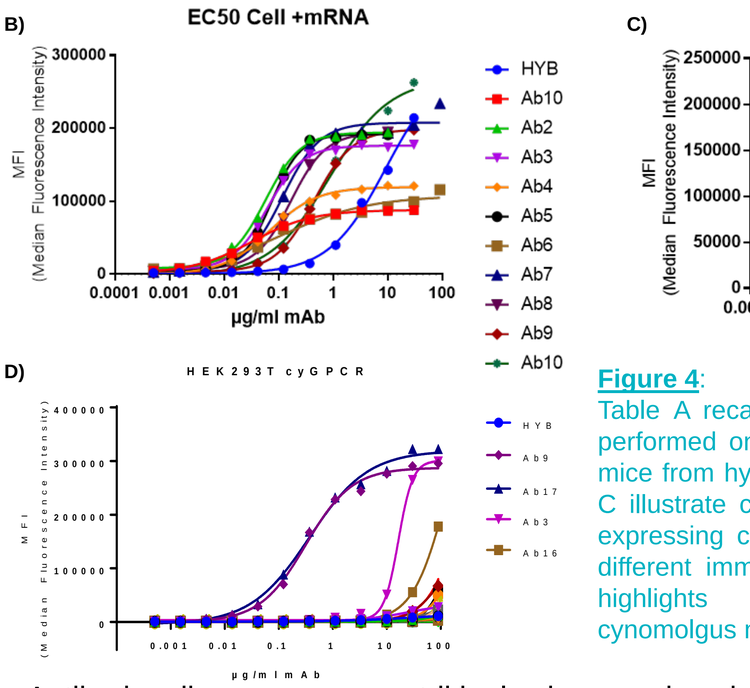
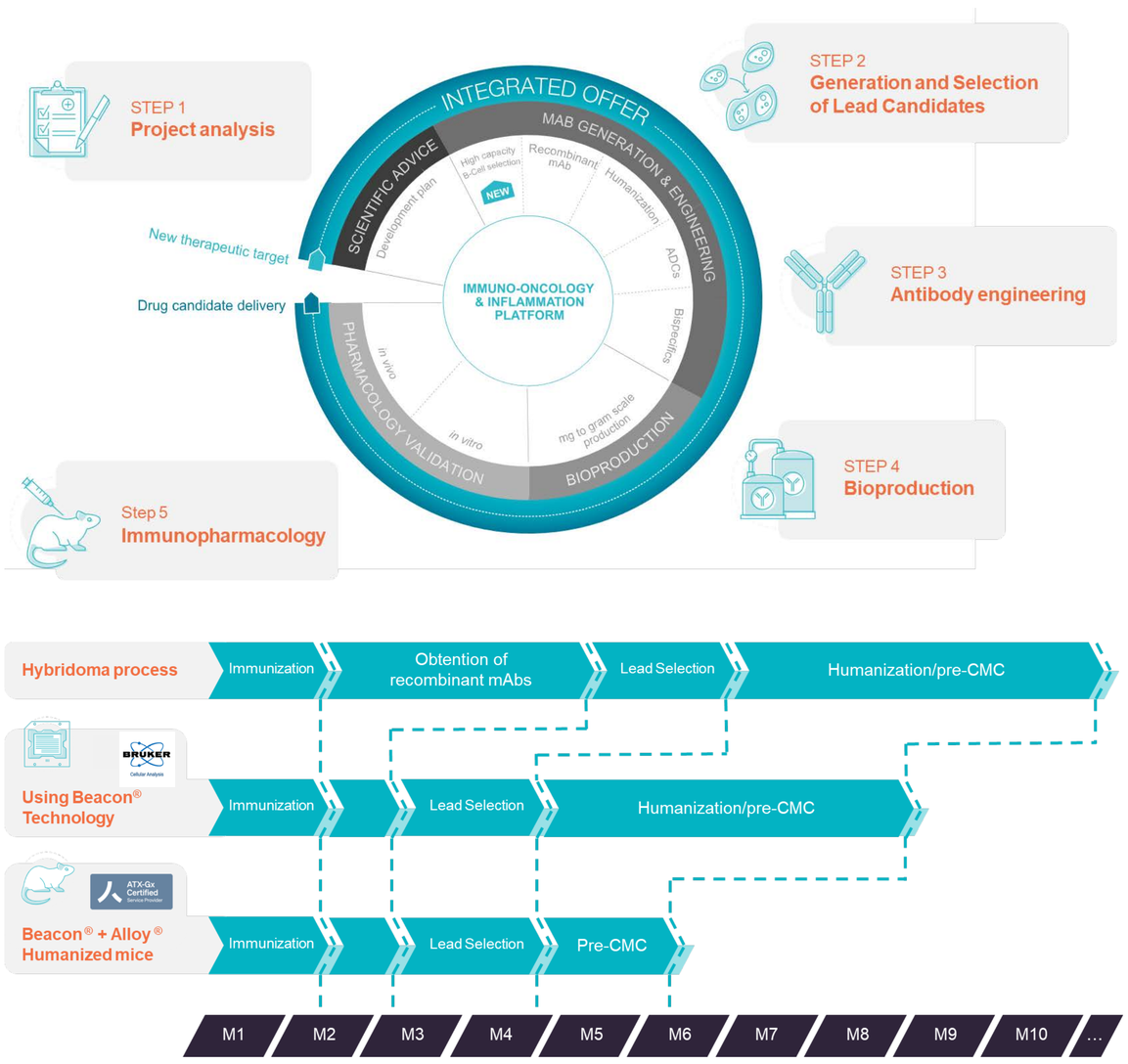


Figure 4:
Table A recapitulates data from all campaigns performed on the targeted GPCR (* remaining mice from hybridoma campaign). Graphs B and C illustrate comparative EC_{50} on human-target expressing cells for antibodies generated from different immunization strategies and graph D highlights cross-reactive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

- Antibody discovery was strikingly improved using the combination of rnRNA immunization and single B cell screening. No difference in affinity could be observed between clones resulting from mixed immunization or mRNA only and 1 cross-reactive clones was obtained from each group.

Therapeutic mAb Candidate Roadmap



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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