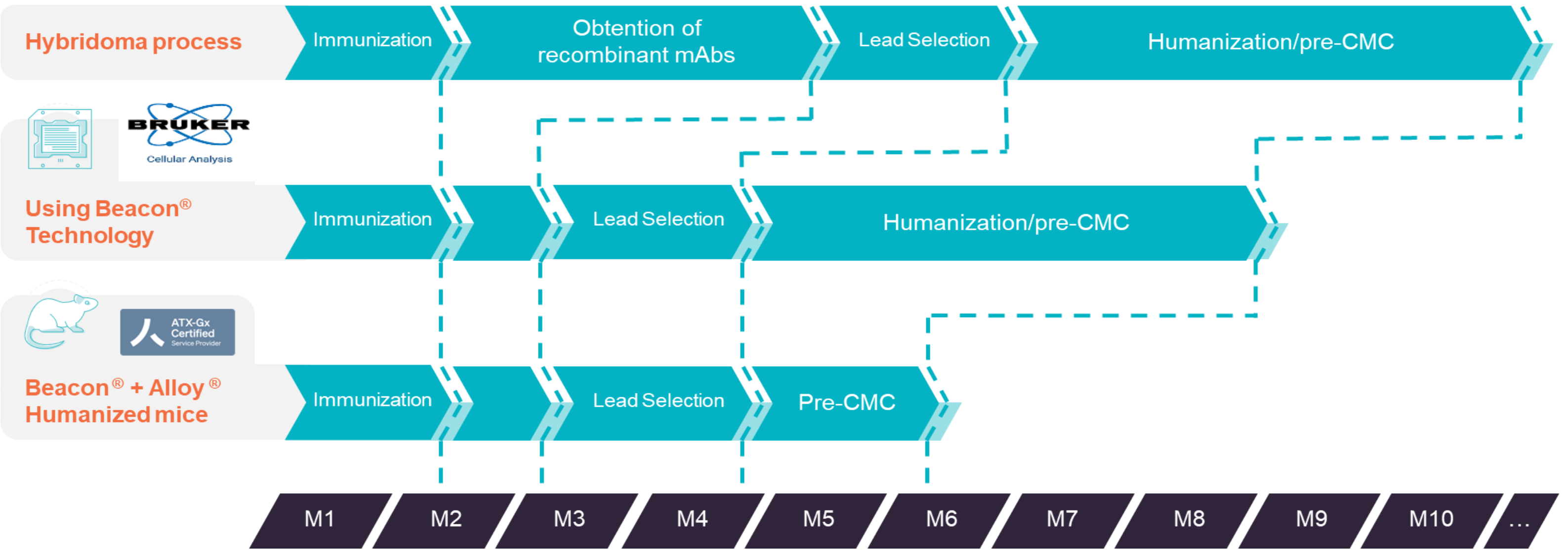
Accelerate antibody discovery with Beacon® single cell technology and humanized mice

Eric CHABROL, Manuel PELE, Marie-Claire PHELIPOT, Laura CHLADNI, Alice AYMARD, Alexandre BAGNOLINI, Laurent POUYET, François ROMAGNE

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



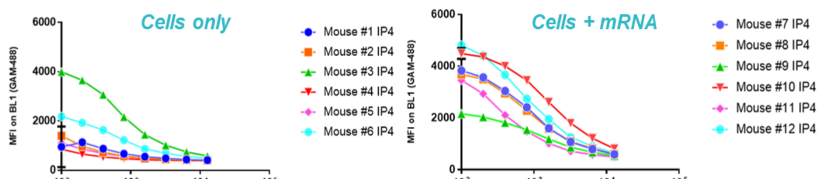
- Compared to the widely used hybridoma approach, larger collections of B cells are screened at higher throughput using the single cell approach.
- Compared with the approach used with WT mice, the use of Alloy® humanized mice considerably reduces antibody development time.

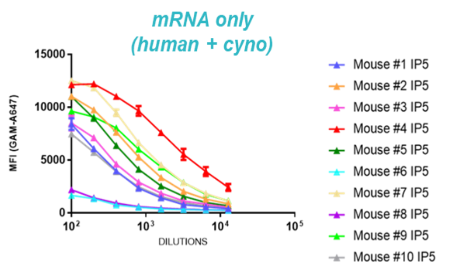
Figure 1: Comparison of antibody production workflows between hybridoma technology and Bruker Beacon®-based single B-cell screening with WT or humanized Alloy® mice.

mRNA Immunization

Current challenges in immunization:

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

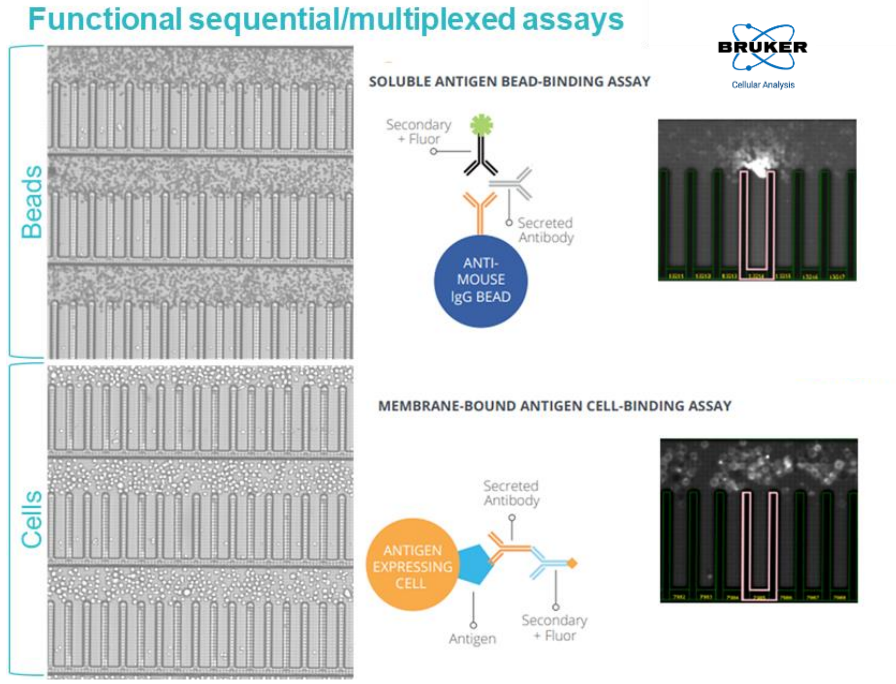




- Immunization with mRNA alone or in combination with cells helps improve immune response levels.
- mRNA from different species can be used to increase cross-reactivity.

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

"On-chip" Functional Assays



- 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.

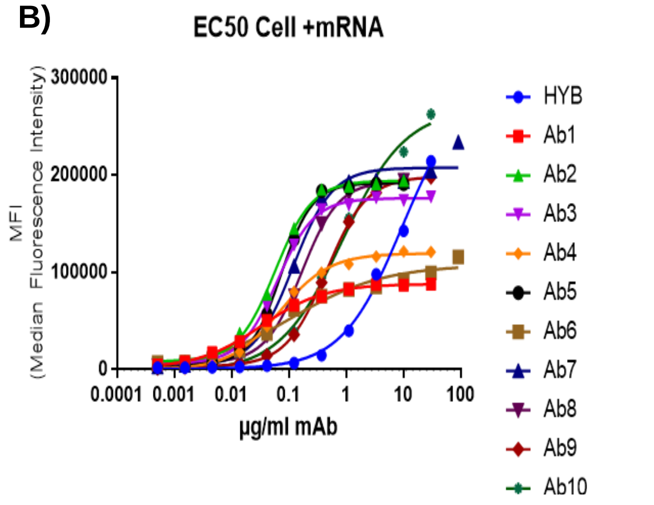
Figure 3: "On-chip" functional assays examples with screening on beads (upper panel ; IgG specific, peptide- or protein-coated beads) or on target expressing-cells (lower panel).

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**

B)



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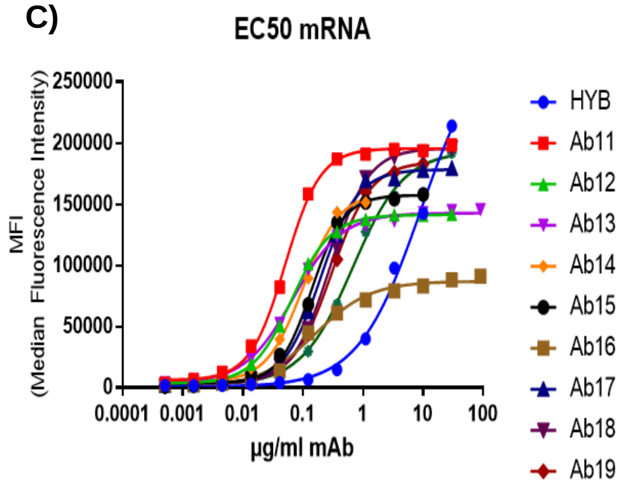


- Antibody discovery was strikingly improved using the combination of mRNA immunization and single B cell screening. No difference in affinity could be observed between clones resulting from mixed immunization or mRNA only.

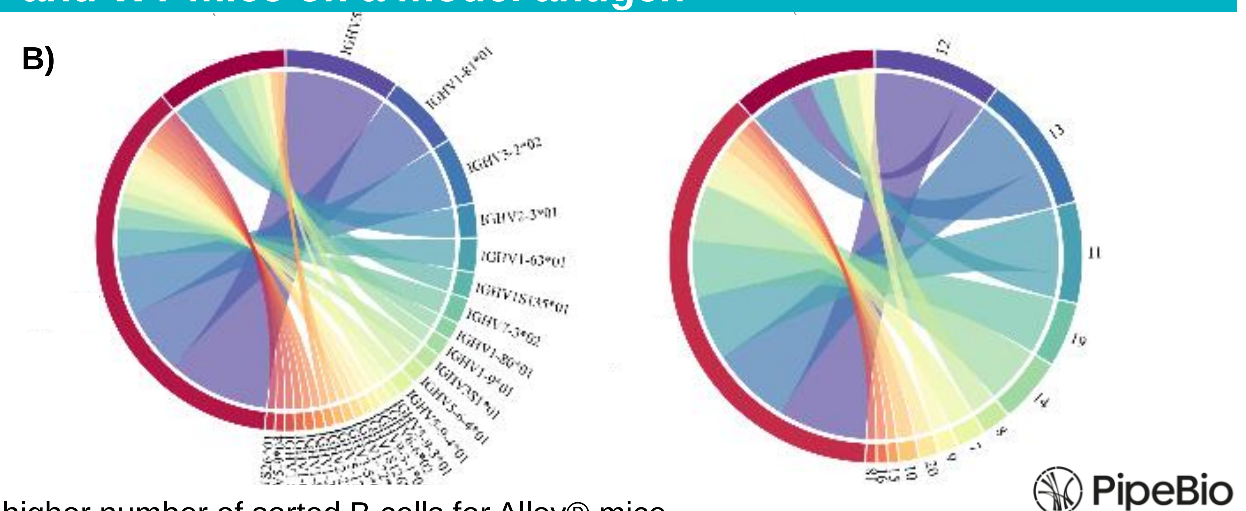
Figure 4: Table A recapitulates data from all campaigns performed on the targeted GPCR (* remaining mice from hybridoma campaign). Graphs B and C illustrate comparative EC_{50} on human-target expressing cells for antibodies generated from different immunization strategies.

Comparison of Alloy® and WT mice on a model antigen

A)

	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 %

B)



- 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® 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.

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.

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.

MImAbs, 117 Avenue de Luminy, 13009 Marseille, France | contact@mimabs.org

OUR OFFER

Beacon® package
(mAbs generation)

- Antigen or mRNA immunization
- Beacon® screen
- Hundreds of hits recovery, wave of 50-100 hits production at 100 µg scale
- Characterization/validation of hits (Affinity, EC_{50})

mAb bioproduction / engineering
(1 to 500 mg scale)

- Naked mAbs, bispecific (all common formats)
- ADC (linker payloads validated in the clinics)
- Industry standard quality controls (SDS, SEC, MS)

Pre-CMC

- Humanisation (molecular modeling and CDR grafting)
- Biophysical characterization
  - *In silico* "hotspots" sequence analysis
  - Formulation and concentration testing
  - Biochemistry, thermal and pH stability studies
  - *Ex-vivo* stability study in serum or PK ADC

In vitro pharmacology

- Direct cytotoxicity test (incl. CDX and PDX-derived *vitro* models)
- Immune modulation/functional tests (ADCC, ADCP, CDC...)
- Generation of transfectants

In vivo immuno-pharmacology

- Efficacy in syngeneic models
- Immunophenotyping
- Efficacy in xenogeneic models (CDX)

OUR TRACK RECORD

- **Transferred to clinical development:** naked mAbs CD39 (Astra-Zeneca) and CD73 (Innate Pharma)
- **Transferred to regulatory driven development:** naked mAb Siglec (Innate Pharma), Bispecific NK cell engager (Sanofi)
- **Close to regulatory driven development:** 3 ADCs : MICA, NKp46 (Innate Pharma, and undisclosed)
- **More than 100 immunizations programs** (tools and drug candidates)
- **~20 target validation programs:** 16 naked, 3 ADCs, 1 bispecific
- **13 publications in peer reviewed journals:** Cell, Cell report, Immunity…
- **1 new tool for bispecifics:** a human CD3e KI mice model

FOCUS ON: Nectin 4 ADC
Emergence Therapeutics

- Generation, selection and humanization of candidates
- Conjugation with several linker payloads to 300 mg level for PK and efficacy in mice (in house) and pre-tox in monkey (outsourced)
- Selection of final candidate/linker payload, transfer to CDMO
- This work was the basis of ETX 87 million fundraising in 2022

Emergence Therapeutics was acquired by Eli Lilly in 2023

OUR BUSINESS MODELS

Fee-for-service collaboration or **FTE-based partnership**

Either way, wo do not retain any IP rights.

LET'S WORK TOGETHER

Tell us about your project at contact@mimabs.com

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

Manuel PELE, Marie-Claire PHELIPOT, Frédérique LEMBO, Laura CHLADNI, Alice AYMARD, Eric CHABROL, Laurent POUYET, François ROMAGNE

mAbs
ANTIBODIES FOR MEDICINE

The highway to mAb drug candidates

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.

MImAbs ANTIBODIES FOR MEDICINE

Workflow

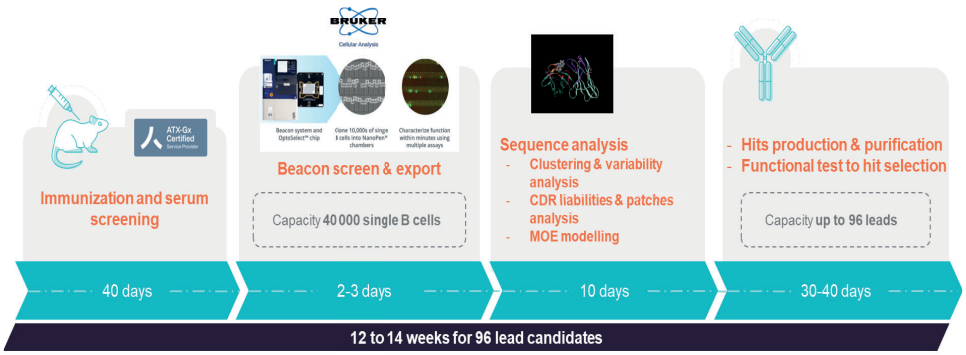


Figure 1: Representative workflow of an antibody generation campaign using Bruker Beacon®-based single B cell screening

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

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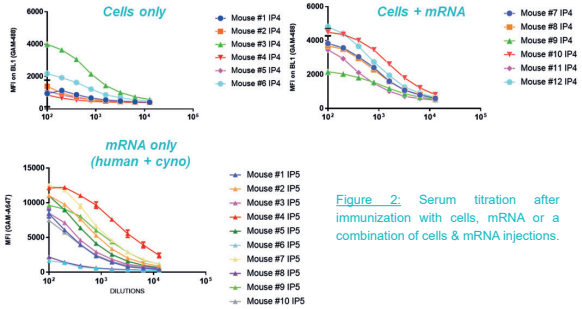

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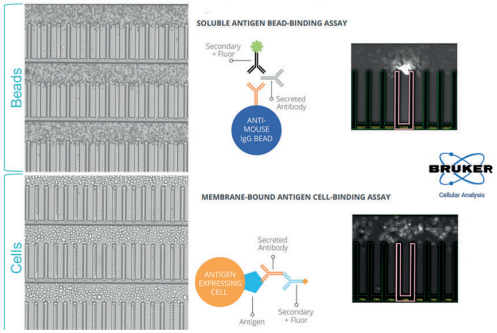
- Immunization with mRNA alone or in combination with cells helps improve immune response levels.
- mRNA from different species can be used to increase cross-reactivity.

«On-chip» Functional Assays

Functional sequential/multiplexed assays



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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.
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mRNA only or cells + mRNA *	**1 (Beacon®)**	**> 35,000**	**26 unique mAbs**

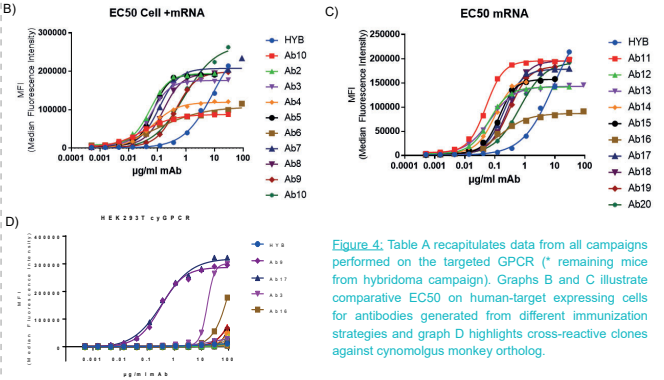
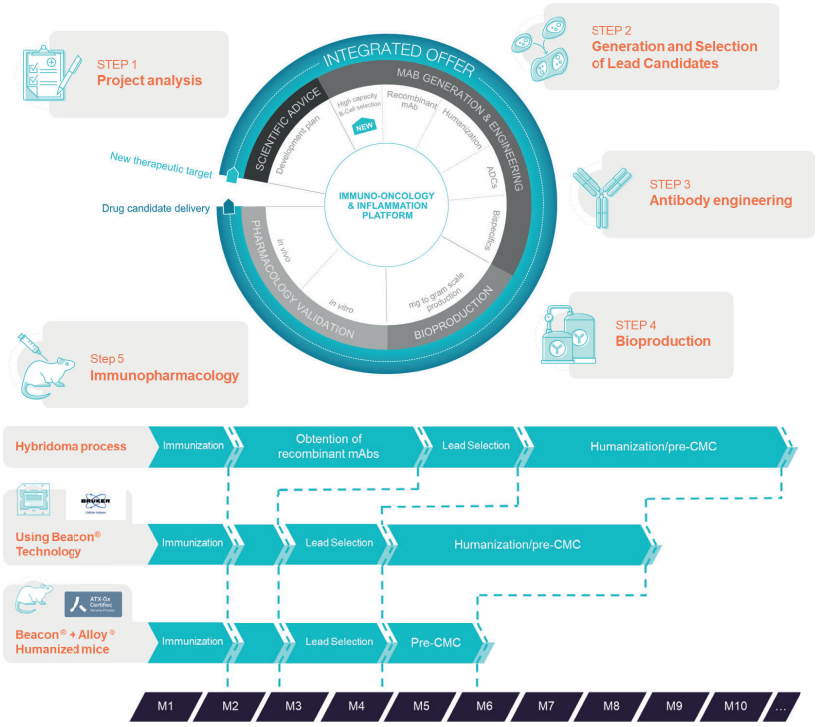


Figure 4: Table A recapitulates data from all campaigns performed on the targeted GPCR (* remaining mice from hybridoma campaign). Graphs B and C illustrate comparative EC50 on human-target expressing cells for antibodies generated from different immunization strategies and graph D highlights cross-reactive clones against cynomolgus monkey ortholog.

- Antibody discovery was strikingly improved using the combination of mRNA 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.

mAbs
ANTIBODIES
FOR MEDICINE

GET YOUR PURIFIED RECOMBINANT MAB HITS AGAINST DIFFICULT TARGETS IN LESS THAN 5 MONTHS USING BEACON® SINGLE CELL TECHNOLOGY

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 single cell screening platform to provide unique opportunities to dramatically speed up antibody discovery against such challenging targets.

Typical Beacon Workflow

Get your purified recombinant mAb hits against difficult targets in less than 5 months using Beacon® Single Cell technology

Key advantages of the technology

Increased depth of screening, and diversity for difficult antigens, without the need for recombinant soluble antigen

- 40,000 plasmocytes from either spleen or bone marrow screened per Beacon® campaign
- mRNA immunization followed with bone marrow plasmocyte selection
- Compatible with the use of human IgG expressing mice (e.g. Alloy Therapeutics)

Rapid functional on-chip screening at single cell level with recombinant antigen or transfectant expressing antigen

- A choice of selection criteria as recognition of antigen, preliminary functional (blocking) or crossreactivity properties
- Obtention of the sequences within a week of all the screened hits allowing *in silico* pre-selection to avoid antibodies with obvious liabilities
- Production of the selected hits based on binding or functional criteria and *in silico* pre-selection

Decreased turnaround time from hit identification to recombinant production and evaluation

- Recovery and production of your hits without the need for cell culture or gene synthesis
- Delivery of up to 100 hits at 100µg scale (i.e. purified antibody) within 8 weeks after initial screening.

What we require and what we deliver

What do we need?

- 1 mg of recombinant antigen, or mRNA coding for your antigen or a cell line expressing your antigen for immunization
- A cell line expressing the antigen or recombinant protein for screening

What do you get within 5 months?

- A collection of up to 100 recombinant mAbs, at 100 µg scale level, with EC_{50} characteristics

MIMABS DOES NOT RETAIN ANY IP RIGHTS AND DOES NOT CARRY-ON PROPRIETARY DISCOVERY PROGRAMS.

Track record

- We have run more than 10 successful Beacon® campaigns with standard recombinant antigen, or peptide with 50-200 high affinity mAbs recovered in each run.
- We have obtained collections of antibodies against difficult antigens where no recombinant protein antigen was available :
  - **50 antibodies against a GPCR with minimum extracellular domain**
  - **12 antibodies against an ion channel where no antibodies were available worldwide.**

Conclusion

Using innovative approaches like mRNA 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, the delivery time of therapeutic candidates can now be significantly shortened.

GPCR Campaign Example (comparison with hybridoma)

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

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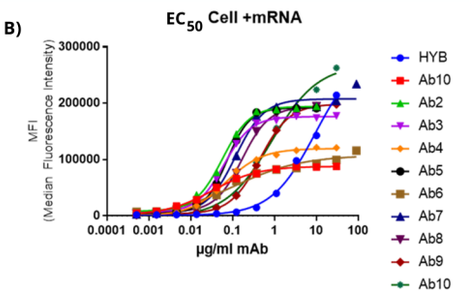

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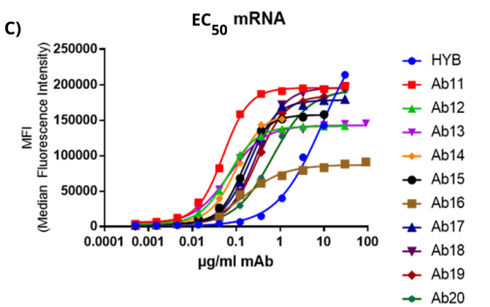

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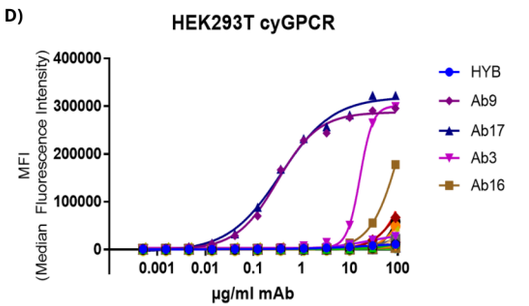

ANTIBODY DISCOVERY WAS STRIKINGLY IMPROVED USING THE COMBINATION OF mRNA IMMUNIZATION AND SINGLE B CELL SCREENING. NO DIFFERENCE IN AFFINITY COULD BE OBSERVED BETWEEN CLONES RESULTING FROM MIXED IMMUNIZATION OR mRNA ONLY AND 1 CROSS-REACTIVE CLONE WAS OBTAINED FROM EACH GROUP.

Contact

Wish to get more information about our Single B cell platform to speed up your antibody development ?

Contact our business team at contact@mimabs.com or +33 675177351
https://www.mimabs.org
MImAbs, 117 Avenue de Luminy, 13009 Marseille, France

- Scientific advice
- mAb generation & engineering
- Antibody Bioproduction
- ADC's
- Bispecific antibodies

- In vitro immunopharmacology
  - Direct Cytotoxicity Assays
  - Cell-Mediated Cytotoxicity
  - Immune Modulation Assays
  - Available Cell Lines

- In vivo immunopharmacology
  - Mouse Models for Cancer
  - PD1/CTLA4-based Combination Models
  - Mouse Models for Inflammation
  - Antibody Bioanalysis & Safety
  - Efficacy Readouts

- Mouse genetic engineering

Contact

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https://www.mimabs.org/

117 Av de Luminy 13009 Marseille, France

Testimonial

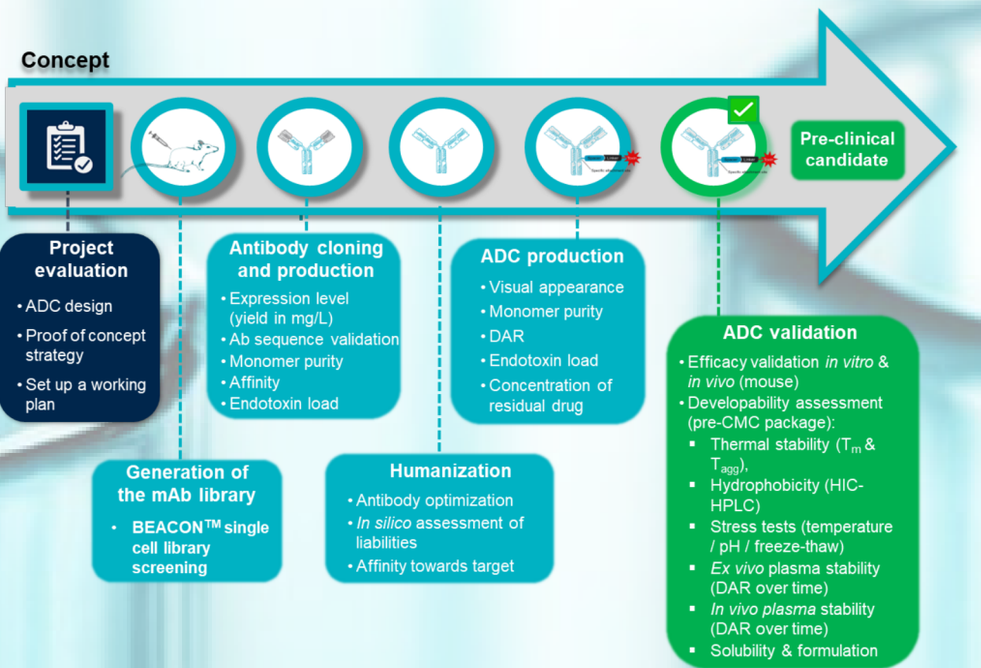
Jack Elands PhD, founder and former Chief Executive Officer of Emergence Therapeutics, a wholly owned subsidiary of Eli Lilly and Company

"Emergence Therapeutics was founded on the basis of ETx-22, a next-generation nectin-4 ADC. The collaboration with MImAbs has been absolutely essential for Emergence's success. MImAbs continues to be a valued partner and collaborator for most of our on-going R&D for ETx-22 and our other ADC projects and has generated critical data enabling our successful series A financing in 2021 as well as our recent acquisition."

ADC DEVELOPMENT: A STEP-BY-STEP PROGRAM TOWARDS SUCCESS

Developing an Antibody-Drug Conjugate (ADC) that shows clinical promise is a challenging step-by-step process that involves a combination of scientific, technical, regulatory, and strategic considerations. Our scientific team brings all its expertise to the early-stage development of your ADC candidates. Before engaging in costly GMP productions, we can maximize the chances of success by guiding you in the best suited combination of antibody/coupling technology/linker and payload for your specific target.

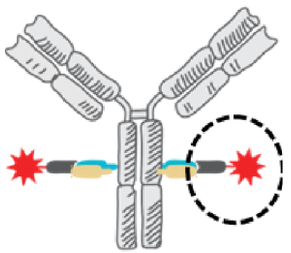
Global Project Schedule: 18 months



THE COMBINED EXPERTISE IN ADC DESIGN AND PHARMACOLOGY ASSESSMENT

After the generation and the engineering of the antibody candidates, we offer a wide array of conjugation solutions, to ensure the production of homogeneous ADCs incorporating the desired payloads. The retained ADC candidates are characterized pharmacologically using tailored proof-of-concept experiments in the most relevant in vitro and in vivo mouse models. Finally, the developability assessment of the most promising ADC candidates can also be led at MImAbs using in silico modelling tools and analytical biochemical assays.

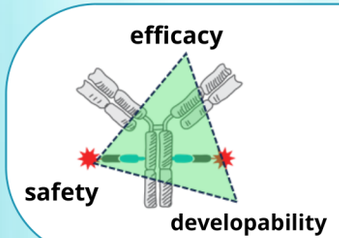
DRUG-LINKERS



Choose from a wide range of clinically validated drug-linkers (DLs).

DLs containing auristatins, maytansinoids, pyrrolobenzodiazepines (PBDs), duocarmycins or exatecans as warheads are directly available for conjugation (e.g., MC-Val-Cit-PAB-MMAE, Mal-PEG4-VC-DMEA-Seco-Duocarmycin, MC-GGFG-DX8951 or Mal-PEG8-Val-Ala-PAB-SG3200). Alternatively, custom conjugations with different structures can also be evaluated. Typically, we address random conjugations through maleimide chemistry on naked mAbs. Alternatively, the site-specific conjugation can be achieved with cys engineered Ab backbones (THIOMAB) or to other specific residues through enzymes (such as bacterial transglutaminase).

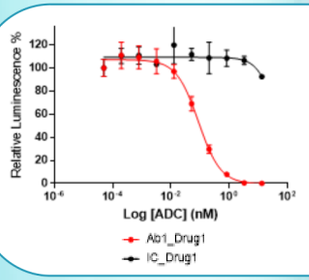
DEVELOPABILITY



Developability profile of your candidates (ADC derisking).

We propose a series of analytical tests to best characterize physico-chemically your most promising candidates. The thermal stability, hydrophobicity or plasma stability (among others) are predictive and reliable attributes of ADC developability that need to be evaluated during pre-clinical development. This pre-CMC characterization is an important tool for ranking the developability of all your candidates.

EFFICACY EVALUATION



The best cell and mouse models to validate ADC efficacy in vitro and in vivo.

Our Pharmacology Team evaluates the *in vitro* efficacy of your candidates in panel of cell lines. The i*n vivo* efficacy is evaluated using relevant syngeneic or xenogeneic mouse models or also on PDX models available from Xentech. For a comprehensive preclinical evaluation of your ADCs, pharmacological aspects can also be explored with available engineered mice (human target knock-in mice) or newly created models by JC Discovery.

FROM STANDARD TO CUSTOMIZED SERVICES FOR YOUR ANTIBODY AND ADC LEAD CANDIDATES

JANVIER GROUP
BIOSCIENCES

In a constant and fully transparent dialog, our experts will guide you through our most relevant solutions to develop your leads in the best cost- and time-effective step-by-step process. MImAbs is now part of the Janvier Group.
You may thus benefit from complementary services to further characterize your Abs & ADCs.

R&D solutions for the development of mAbs, ADCs or Bispecifics

- Recombinant mAb generation.
- Generation of mouse or rabbit mAbs libraries and selection through single cell screening Beacon® technology.
- Cloning of VH and VL and sequencing.
- Humanization.
- mAb bioproduction: from mg to gram scale. All formats: naked, mutated Abs or bispecifics.
- Generation and developability of ADCs with a choice of coupling technologies, linkers and payloads. Selection of the best option for your target.
- In vitro mAb validation using direct cytotoxicity, ADCC and ADCC-like assays.
- In vivo validation of mAbs, ADC or bispecifics in relevant syngeneic, genetically engineered or xenogeneic mouse models.

Complementary services within the Janvier Group

- Newly designed engineered mice and rat models (Knock-out or Knock-in models).
- Humanized models (human CD34 reconstituted mice).

JC DISCOVERY®

- Evaluation with a wide range of PDX (Patient-Derived Xenograft) models.

XenTech