Rapid Single-Cell Discovery of Tumor-Targeting Antibodies Using the Bruker Beacon® Platform for Therapeutic Development
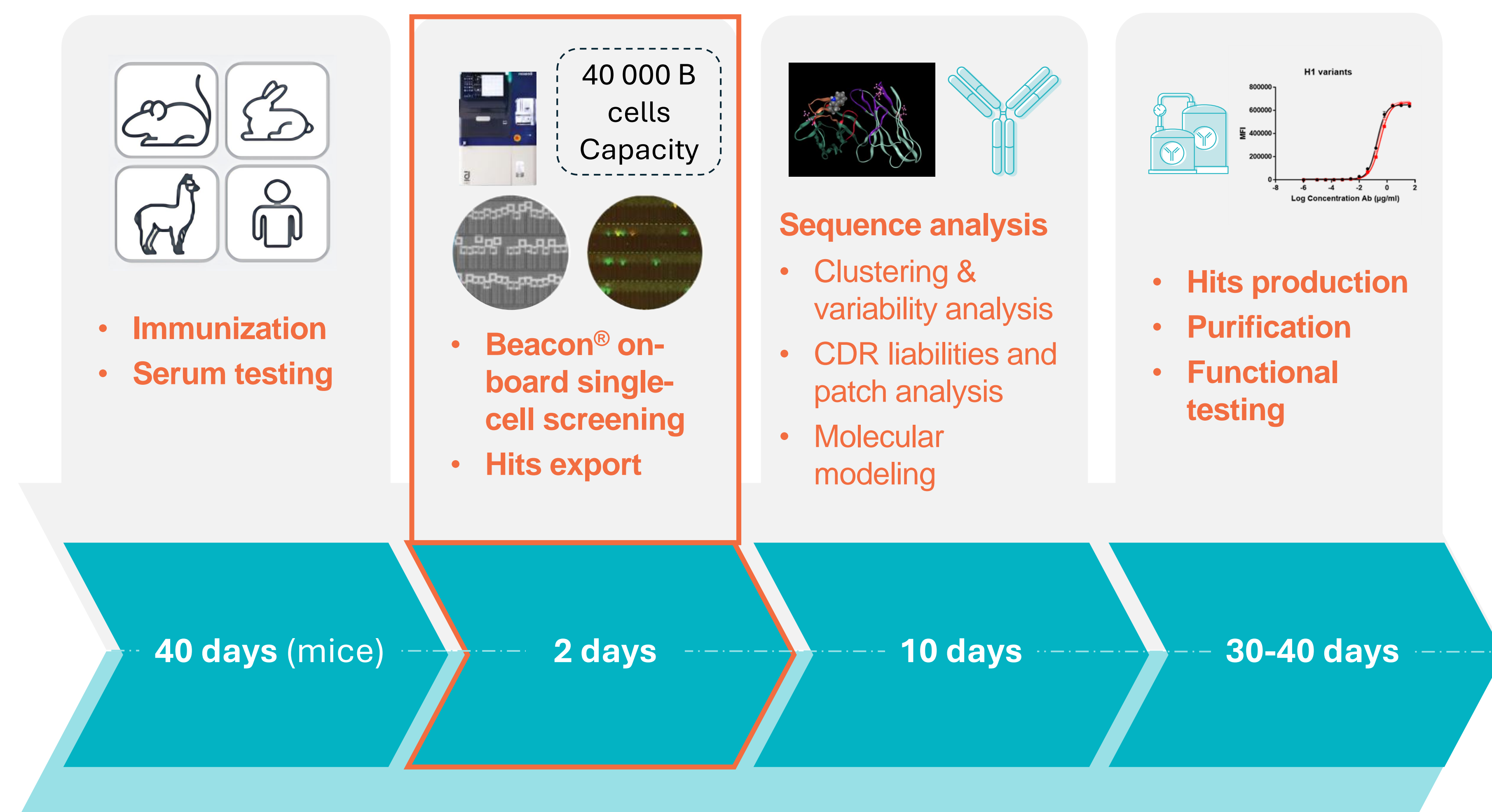
E. CHABROL, M-C. PHELIPOT, J. LICHIERE, A. AYMARD, A. BAGNOLINI, A. BROCERO, M. TASSA, M. SEILLIER, E. MORGADO, J. FIESCHI
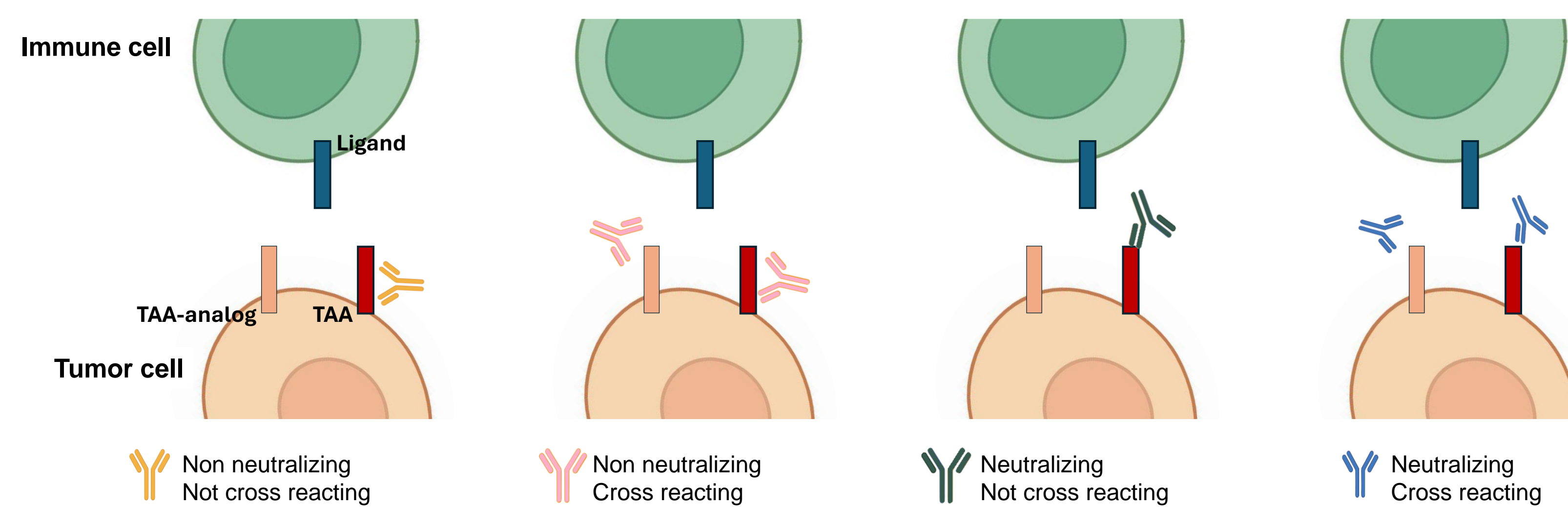
Abstract

In a single-day experiment, the Bruker Beacon® platform was employed to identify and isolate individual plasma cells secreting antibodies against a tumor-associated antigen (TAA). The platform enabled high-throughput screening of antibodies with or without neutralizing activity, and with or without cross-reactivity toward an antigen analog. This rapid, single-run workflow yielded a diverse panel of candidate antibodies. These antibodies will undergo further characterization and be used in the development of therapeutic antibodies or antibody-drug conjugates to advance cancer treatment strategies.

Fast antibody discovery & engineering workflow

- **Immunization**
- **Serum testing**

40 000 B cells Capacity

- **Beacon® on-board single-cell screening**
- **Hits export**

Sequence analysis
- Clustering & variability analysis
- CDR liabilities and patch analysis
- Molecular modeling

- **Hits production**
- **Purification**
- **Functional testing**

40 days (mice) | 2 days | 10 days | 30-40 days

Properties of antibodies with relevance in drug development

Immune cell
Ligand
TAA-analog TAA
Tumor cell

Non neutralizing Not cross reacting | Non neutralizing Cross reacting | Neutralizing Not cross reacting | Neutralizing Cross reacting
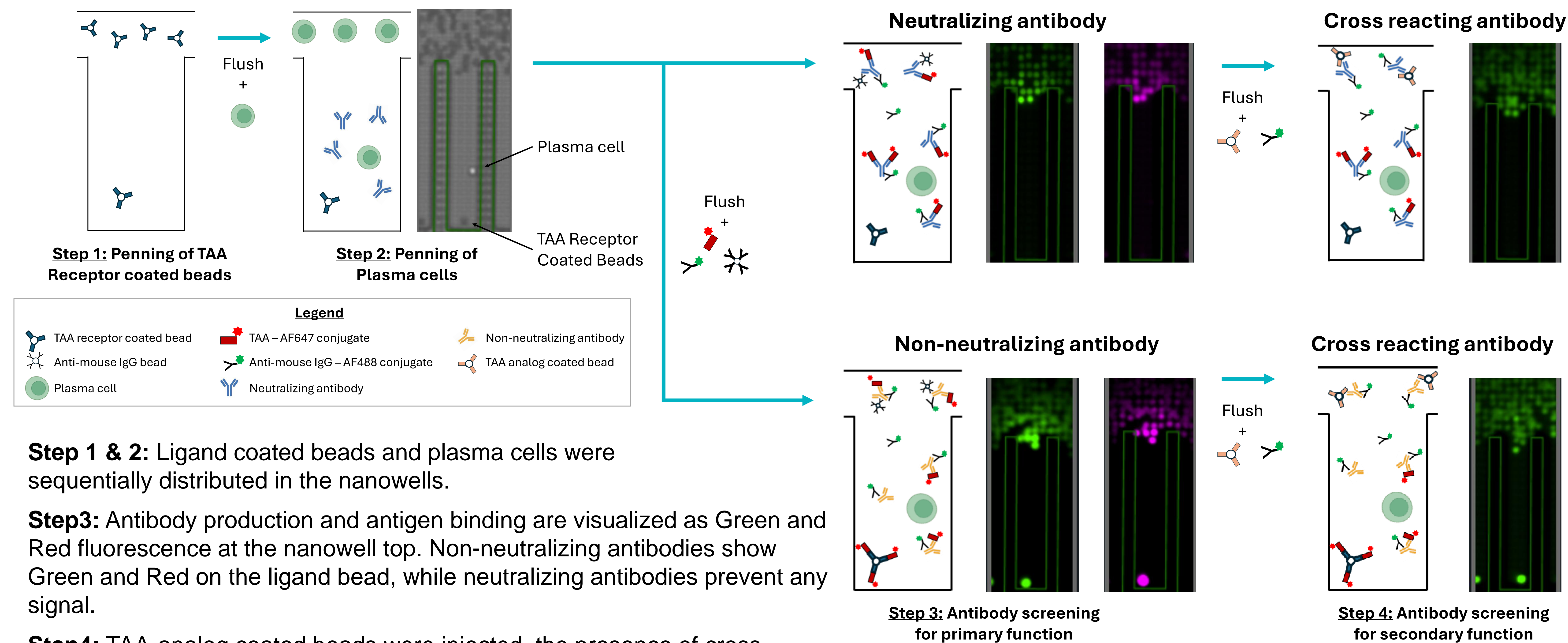
Each antibody type will be key to their application in drug discovery, diagnostics, and therapeutic development: blocking molecular interactions, use in antibody-drug conjugates (ADCs), detection and imaging, precision medicine.

Single-cell resolution screening of antibodies for multiple functional characteristics

Step 1: Penning of TAA Receptor coated beads
Step 2: Penning of Plasma cells

Plasma cell
TAA Receptor Coated Beads

Flush +

Neutralizing antibody
Flush +
Cross reacting antibody

Non-neutralizing antibody
Flush +
Cross reacting antibody

Step 3: Antibody screening for primary function
Step 4: Antibody screening for secondary function

Legend

|		
TAA receptor coated bead	TAA – AF647 conjugate	Non-neutralizing antibody
Anti-mouse IgG bead	Anti-mouse IgG – AF488 conjugate	TAA analog coated bead
Plasma cell	Neutralizing antibody	

Step 1 & 2: Ligand coated beads and plasma cells were sequentially distributed in the nanowells.

Step3: Antibody production and antigen binding are visualized as Green and Red fluorescence at the nanowell top. Non-neutralizing antibodies show Green and Red on the ligand bead, while neutralizing antibodies prevent any signal.

Step4: TAA-analog coated beads were injected, the presence of cross-reacting antibodies result in green fluorescence
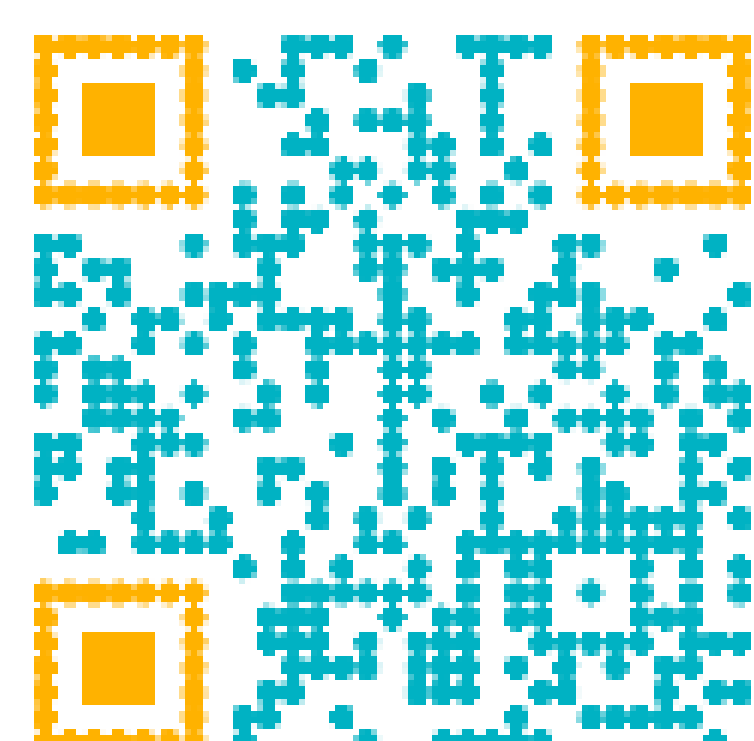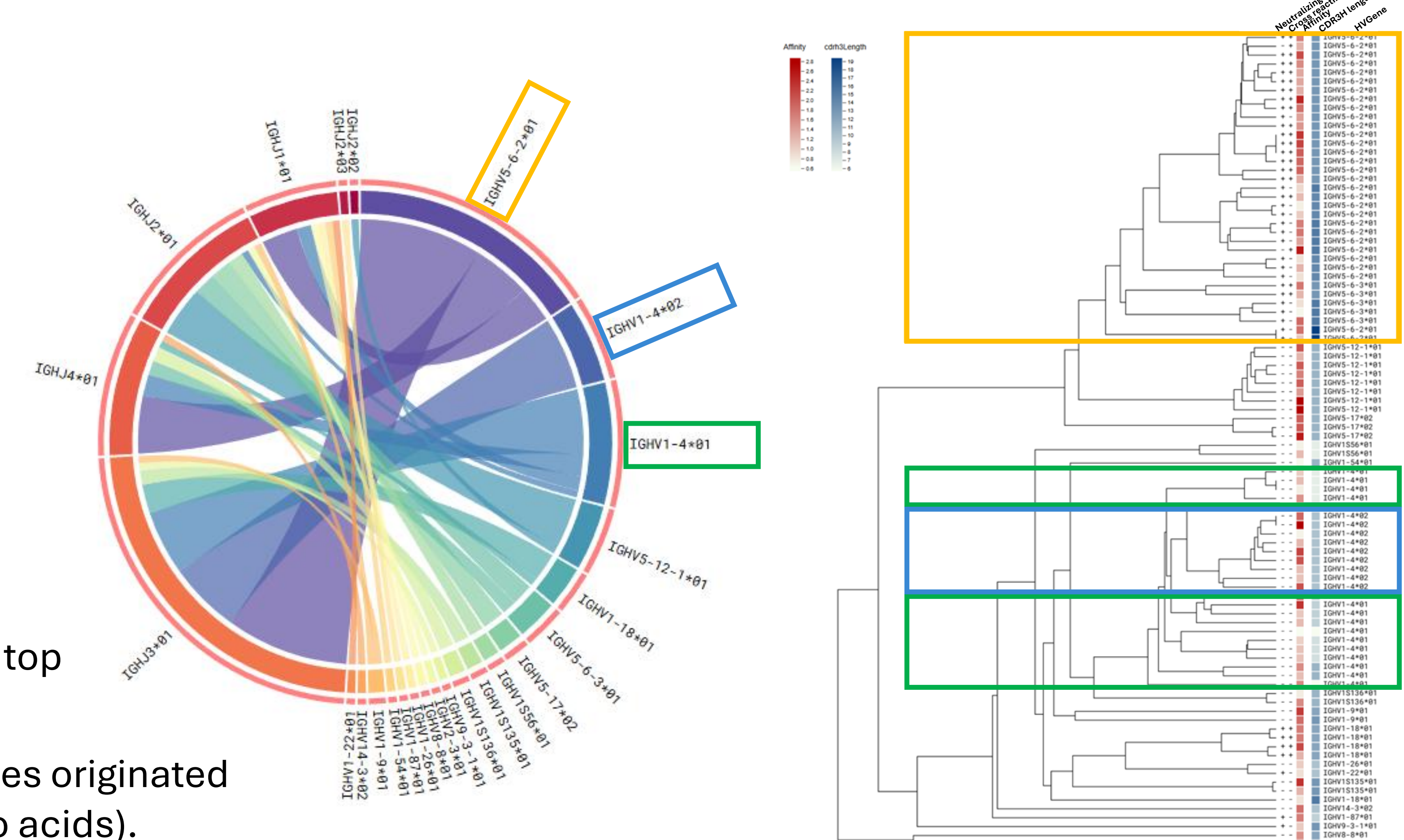
Affinity-based ranking of hits

- Non-neutralizing, Non-cross reacting
- Neutralizing, Non-Cross reacting
- Non-neutralizing, Cross reacting
- Neutralizing, Cross reacting
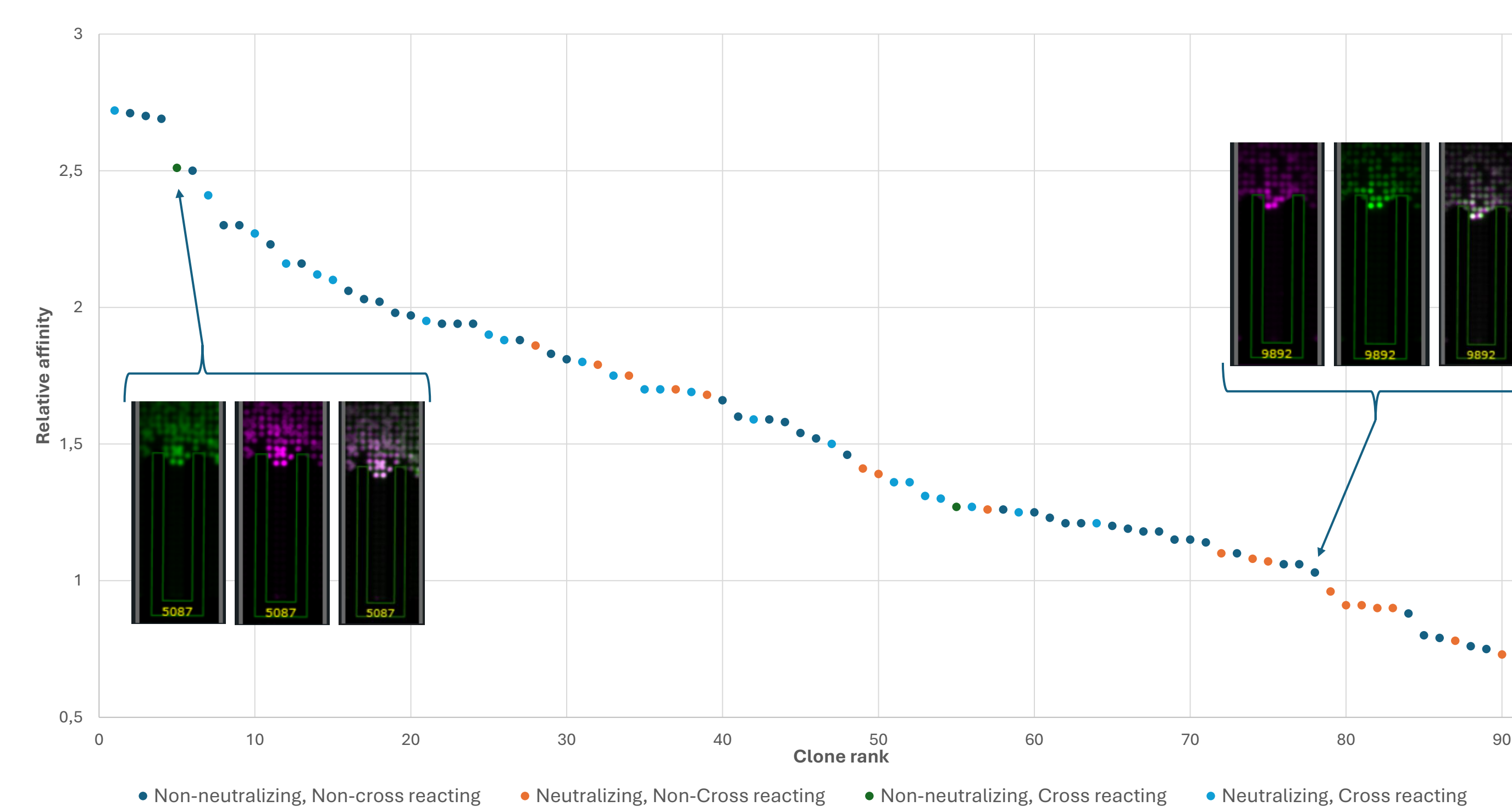
On-chip fluorescence-based affinity scoring enabled early selection of top antibody candidates in the discovery process.

Preliminary sequence analysis revealed that most neutralizing antibodies originated from a single VH germline and featured long CDR3H regions (>12 amino acids).

VH Germline clustering

Conclusion

- Utilization of the Bruker Beacon® platform enabled the identification of monoclonal antibodies based on their specificity and affinity within a single run.
- Within a day, we were able to select several dozen clones as potential therapeutic candidates exhibiting distinct mechanisms of action.
- The ability to identify and characterize hits at an early stage significantly reduces the risks associated with the development of innovative therapeutics.

MI mAbs
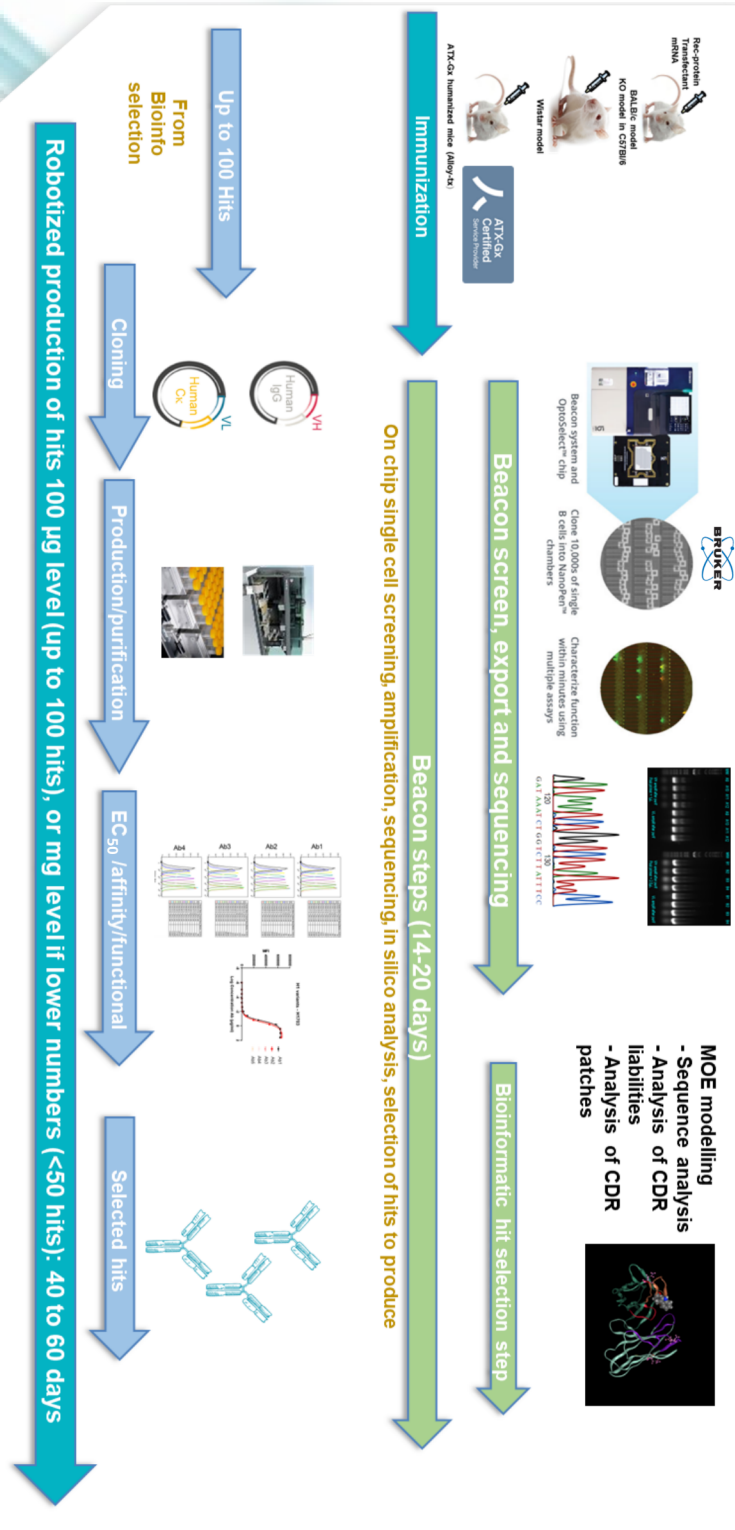### ANTIBODIES FOR MEDICINE

Typical Beacon Workflow

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.

Workflow labels

- Immunization
  - Rec-protein Transfectant mRNA
  - BALB/c model
  - KO model in C57Bl/6
  - Wistar model
  - ATX-Gx humanized mice (Alloy-Gx)
  - ATX-Gx Certified Service Provider

- Beacon screen, export and sequencing
  - Beacon system and OptoSelect™ chip
  - Clone 10,000s of single B cells into NanoPen™ chambers
  - Characterize function within minutes using multiple assays
  - BRUKER
  - Beacon steps (14-20 days)
  - On chip single cell screening, amplification, sequencing, in silico analysis, selection of hits to produce

- Bioinformatic hit selection step
  - MOE modelling
  - Sequence analysis
  - Analysis of CDR liabilities
  - Analysis of CDR patches

- From Bioinfo selection
- Up to 100 Hits
- Cloning
  - Human CL — VL
  - Human IgG — VH
- Production/purification
- EC$_{50}$/affinity/functional
  - Ab4 Ab3 Ab2 Ab1
- Selected hits
- Robotized production of hits 100 µg level (up to 100 hits), or mg level if lower numbers (<50 hits): 40 to 60 days

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**
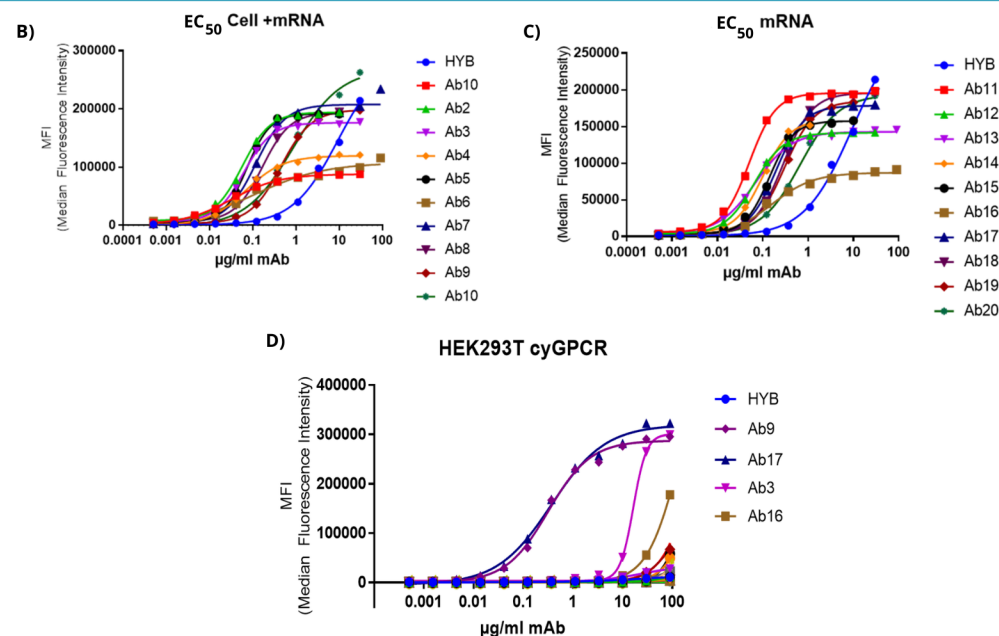 • **12 antibodies against an ion channel where no antibodies were available worldwide.**

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.

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) EC_{50} Cell +mRNA

C) EC_{50} mRNA

D) HEK293T cyGPCR

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

![MImAbs – ANTIBODIES FOR MEDICINE logo]

- **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

Wish to get more information about our solutions to develop your ADC candidate surely and efficiently?

Contact our business team at contact@mimabs.com or +33 675177351

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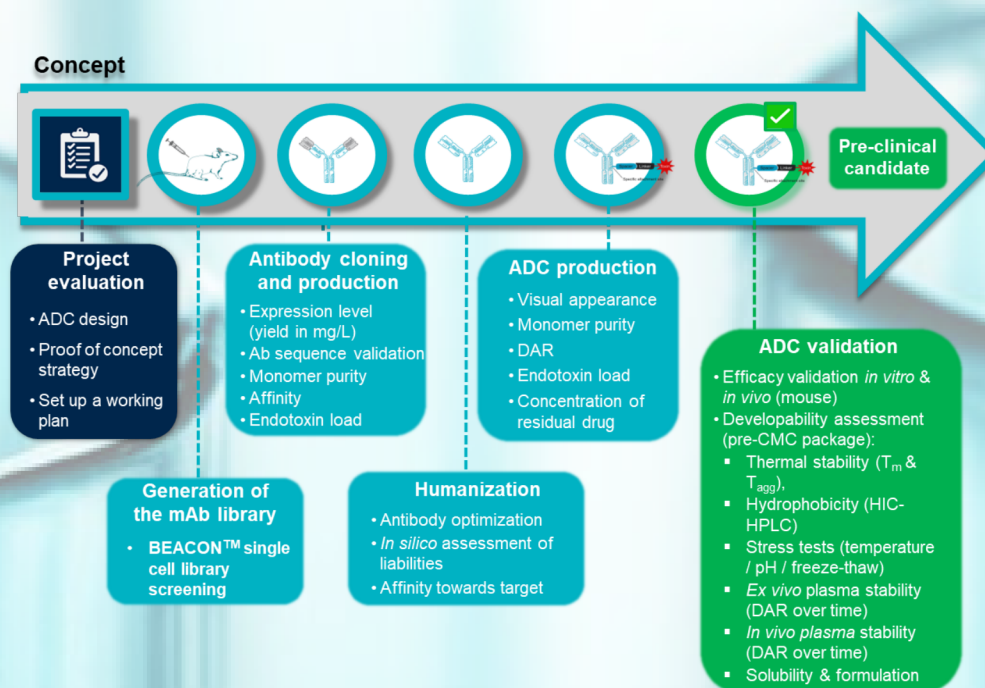
"Emergence Therapeutics was founded on the basis of ETx-22, a next-generation nectin-4 ADC. The collaboration with MImAbs has been absolutely essential for Emergence's success. MImAbs continues to be a valued partner and collaborator for most of our on-going R&D for ETx-22 and our other ADC projects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

![MImAbs – ANTIBODIES FOR MEDICINE logo]

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

Concept

Pre-clinical candidate

Project evaluation
- ADC design
- Proof of concept strategy
- Set up a working plan

Antibody cloning and production
- Expression level (yield in mg/L)
- Ab sequence validation
- Monomer purity
- Affinity
- Endotoxin load

ADC production
- Visual appearance
- Monomer purity
- DAR
- Endotoxin load
- Concentration of residual drug

Generation of the mAb library
- BEACON™ single cell library screening

Humanization
- Antibody optimization
- *In silico* assessment of liabilities
- Affinity towards target

ADC validation
- Efficacy validation *in vitro* & *in vivo* (mouse)
- Developability assessment (pre-CMC package):
  - Thermal stability (T_m & T_{agg}),
  - Hydrophobicity (HIC-HPLC)
  - Stress tests (temperature / pH / freeze-thaw)
  - *Ex vivo* plasma stability (DAR over time)
  - *In vivo plasma* stability (DAR over time)
  - Solubility & formulation

THE COMBINED EXPERTISE IN ADC DESIGN AND PHARMACOLOGY ASSESSMENT

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

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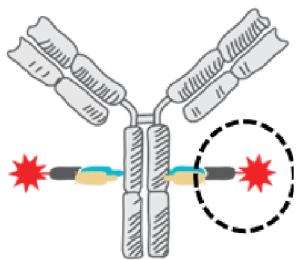
Choose from a wide range of clinically validated drug-linkers (DLs).

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

efficacy

safety
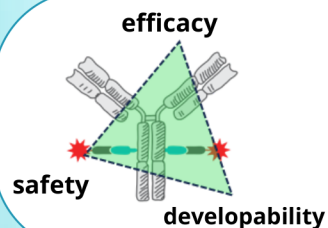
developability

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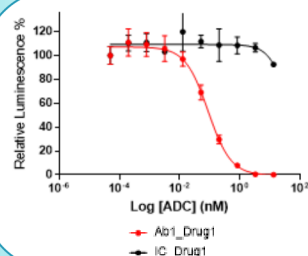
The best cell and mouse models to validate ADC efficacy in vitro and in vivo.

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.

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

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
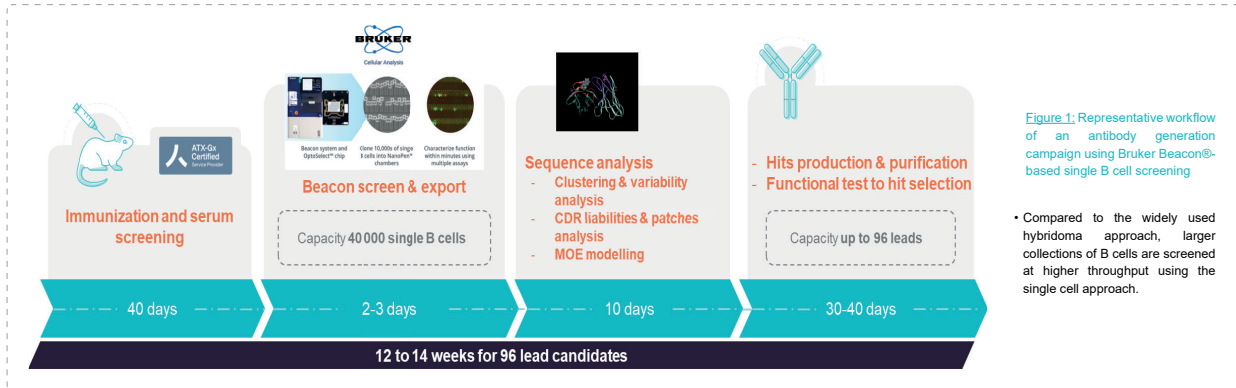
MI mAbs
ANTIBODIES FOR MEDICINE

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.
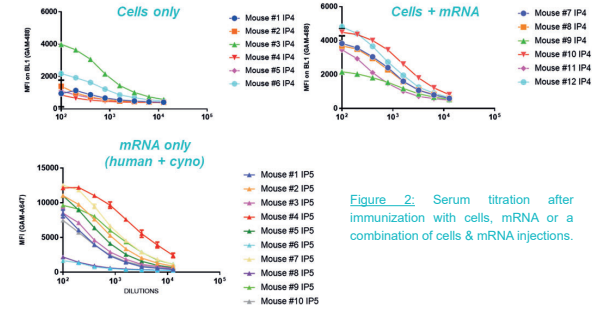
MImAbs ANTIBODIES FOR MEDICINE

Workflow

BRUKER Cellular Analysis

Immunization and serum screening

Beacon screen & export
Beacon system and OptoSelect™ chip
Clone 10,000s of single B cells into NanoPen™ chambers
Characterize function within minutes using multiple assays

Capacity **40 000 single B cells**

Sequence analysis
- Clustering & variability analysis
- CDR liabilities & patches analysis
- MOE modelling

- Hits production & purification
- Functional test to hit selection

Capacity **up to 96 leads**

40 days | 2-3 days | 10 days | 30-40 days

12 to 14 weeks for 96 lead candidates

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.
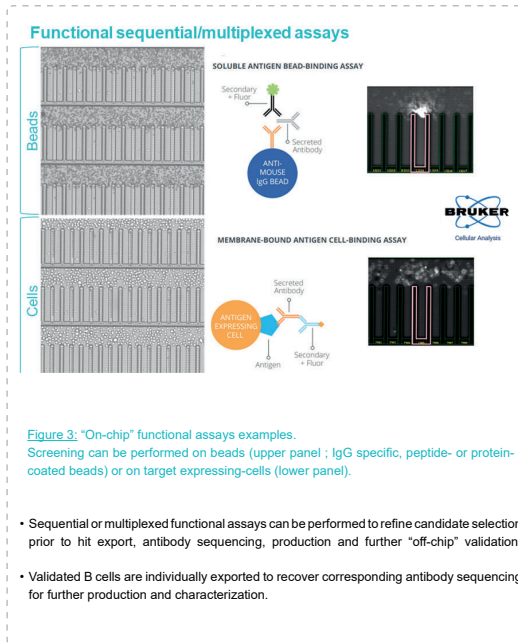
mRNA Immunization

Current challenges in immunization:
- Possible issues in recombinant protein production
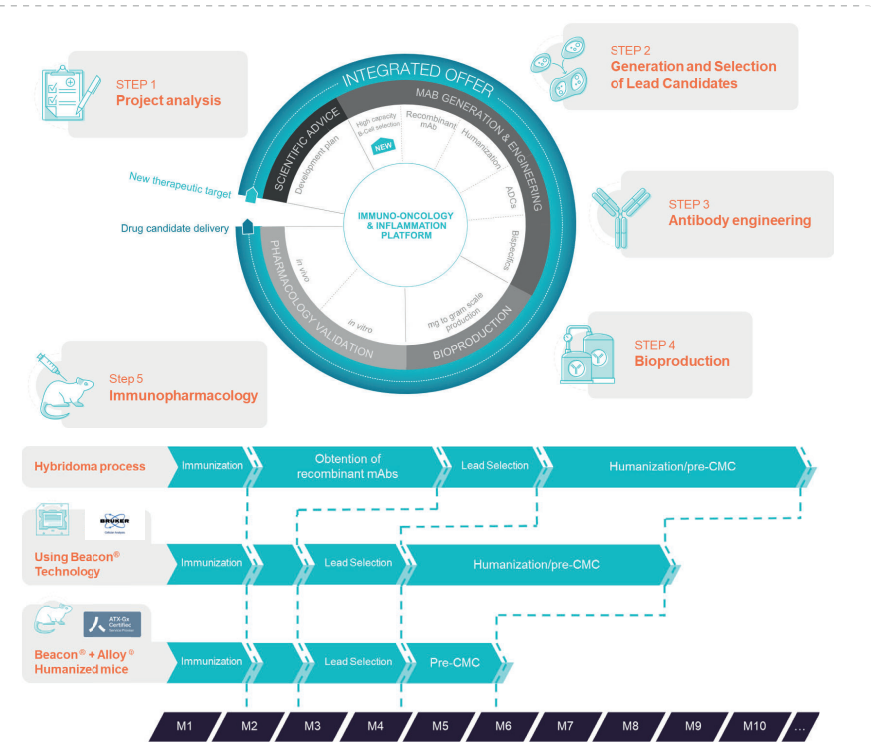- Poor immunogenicity / cross-reactivity

Cells only — Mouse #1 IP4, Mouse #2 IP4, Mouse #3 IP4, Mouse #4 IP4, Mouse #5 IP4, Mouse #6 IP4

Cells + mRNA — Mouse #7 IP4, Mouse #8 IP4, Mouse #9 IP4, Mouse #10 IP4, Mouse #11 IP4, Mouse #12 IP4

mRNA only (human + cyno) — Mouse #1 IP5, Mouse #2 IP5, Mouse #3 IP5, Mouse #4 IP5, Mouse #5 IP5, Mouse #6 IP5, Mouse #7 IP5, Mouse #8 IP5, Mouse #9 IP5, Mouse #10 IP5

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

SOLUBLE ANTIGEN BEAD-BINDING ASSAY
Secondary + Fluor
Secreted Antibody
ANTI-MOUSE IgG BEAD

BRUKER Cellular Analysis

MEMBRANE-BOUND ANTIGEN CELL-BINDING ASSAY
Secreted Antibody
ANTIGEN EXPRESSING CELL
Antigen
Secondary + Fluor

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**

B) **EC50 Cell +mRNA** — HYB, Ab10, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10

C) **EC50 mRNA** — HYB, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20

D) **HEK293T cyGPCR** — HYB, Ab10, Ab17, Ab13, Ab16

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

STEP 1 **Project analysis**

STEP 2 **Generation and Selection of Lead Candidates**

STEP 3 **Antibody engineering**

STEP 4 **Bioproduction**

Step 5 **Immunopharmacology**

INTEGRATED OFFER
SCIENTIFIC ADVICE
MAB GENERATION & ENGINEERING
IMMUNO-ONCOLOGY & INFLAMMATION PLATFORM
PHARMACOLOGY VALIDATION
BIOPRODUCTION

New therapeutic target
Drug candidate delivery

Hybridoma process — Immunization → Obtention of recombinant mAbs → Lead Selection → Humanization/pre-CMC

Using Beacon® Technology — Immunization → Lead Selection → Humanization/pre-CMC

Beacon® + Alloy® Humanized mice — Immunization → Lead Selection → Pre-CMC

M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 | ...

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. 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.