Multi-parametric antibody discovery approaches to accelerate therapeutic lead selection

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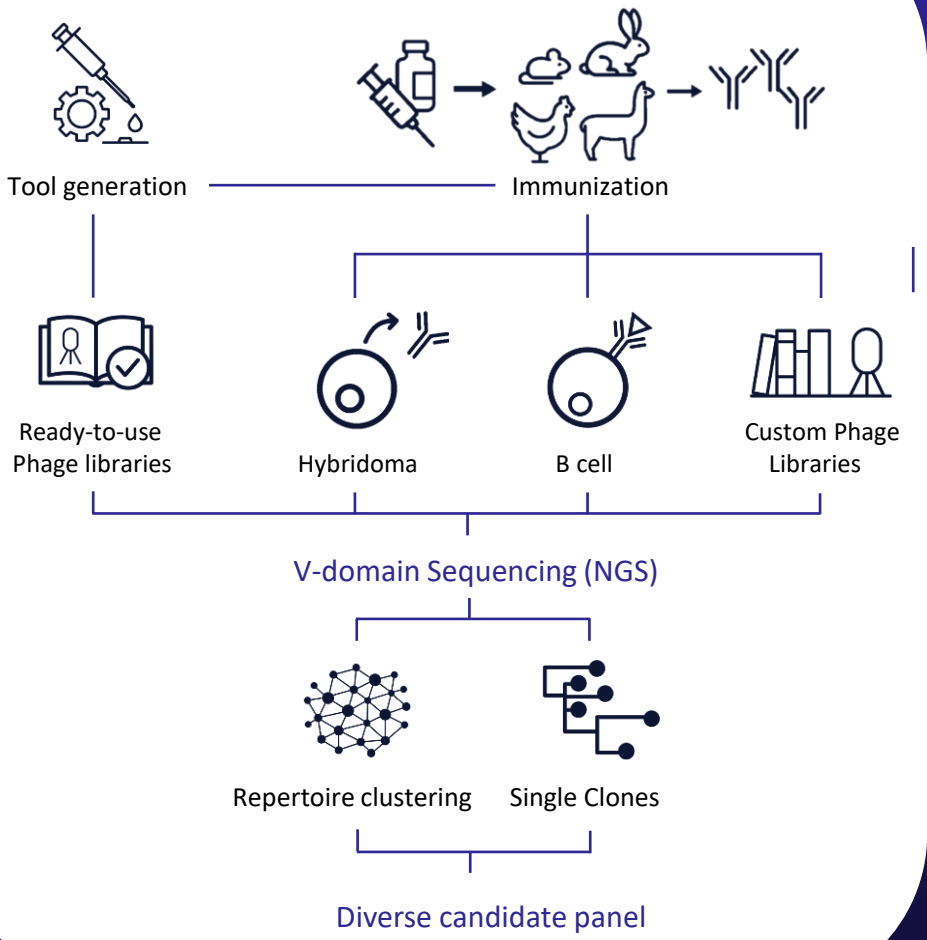
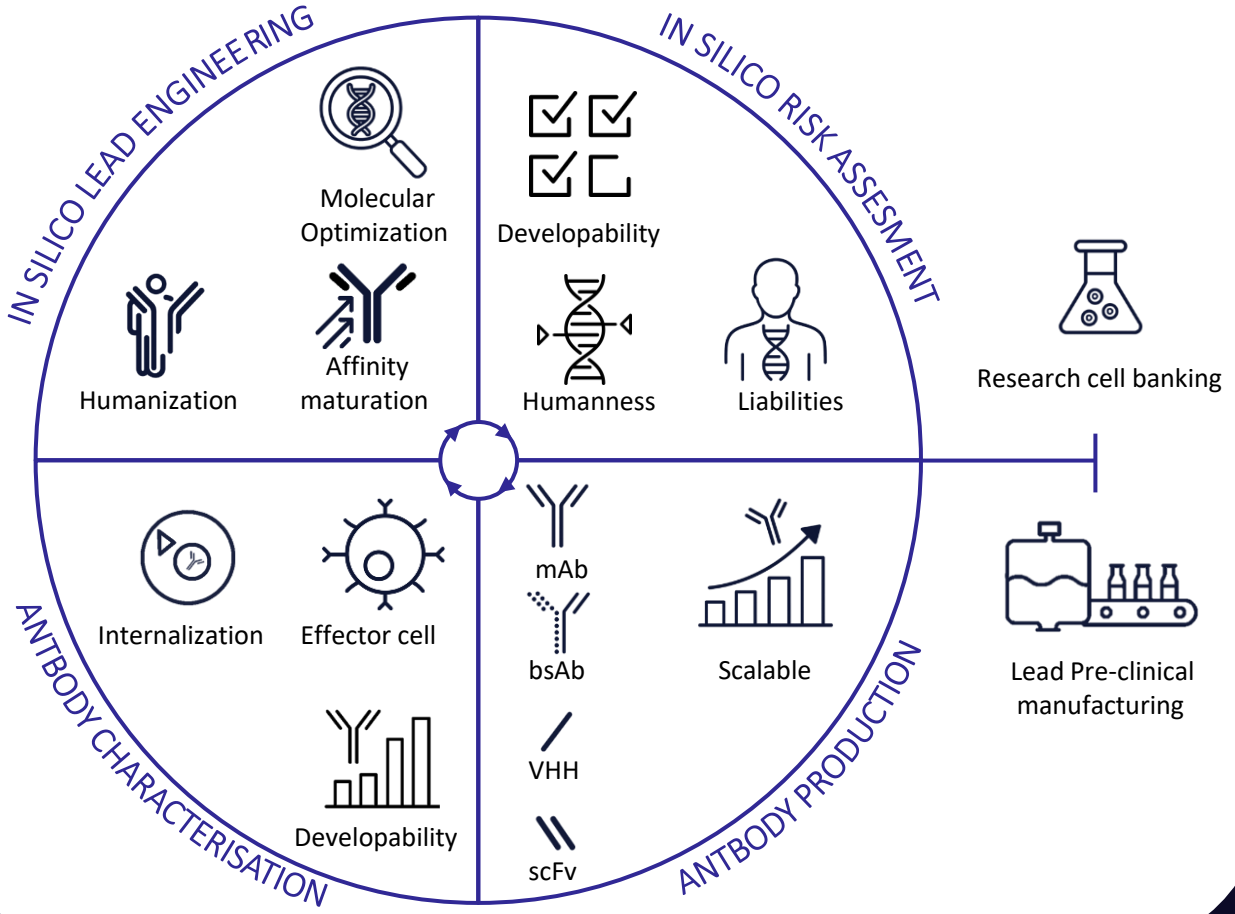
End-to-end antibody discovery and development

From research to clinical lead panel

Discovery – Diversity



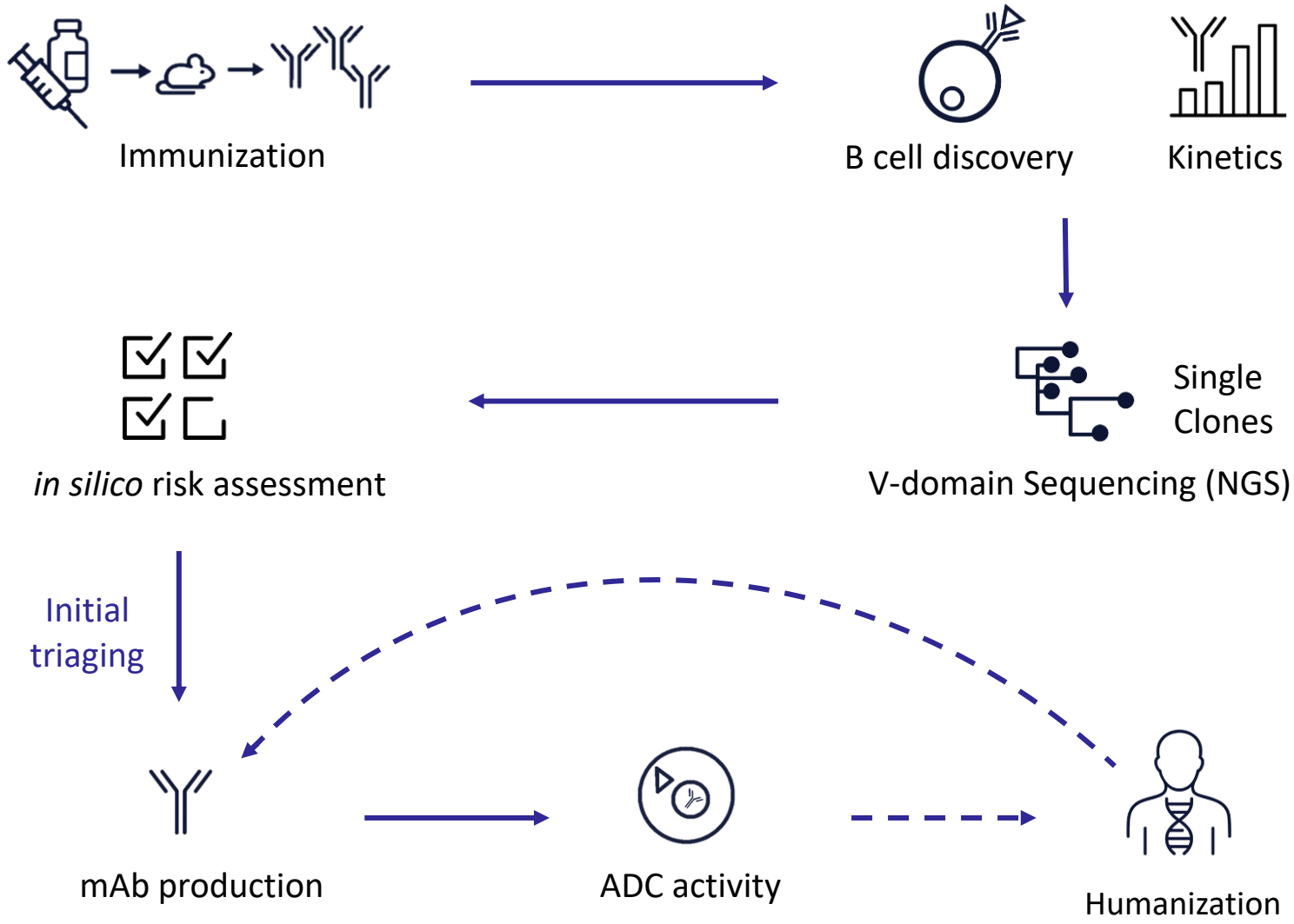
Development – Down-selection & De-risking



Integrated end-to-end workflow – client study

Empowering diversity-focused discovery and data-driven down-selection

- **A tumor cell target**
  Antibody drug conjugate (ADC)
- **Antibody discovery**
  Diversity-focused
- **Initial triaging**
  in vitro binding / kinetics
  in silico developability profiling
- **mAb production, conjugation, and functionality profiling**
  ADC potency
- **Humanization and validation**
  High throughput



Targeting cancer cells

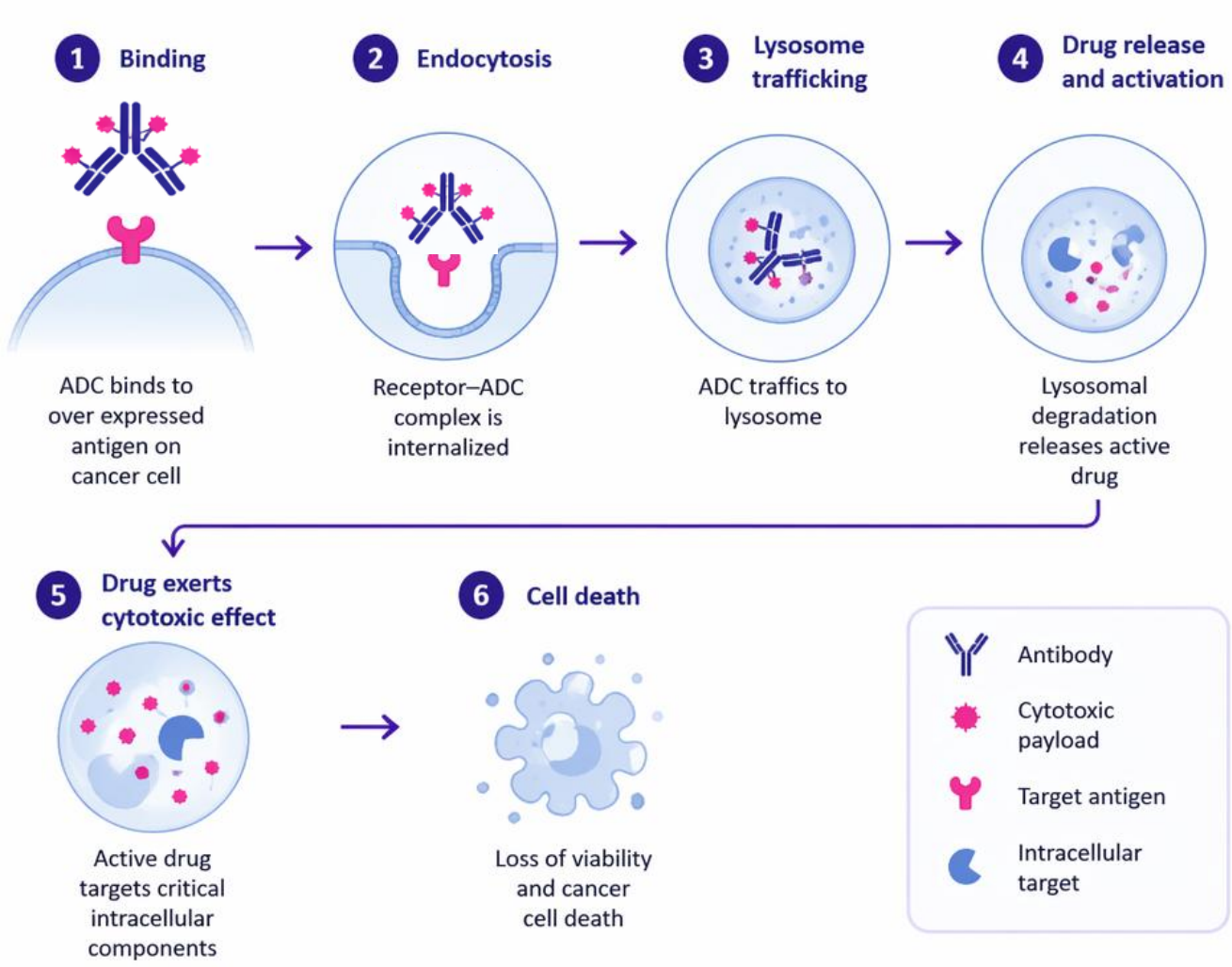
Maximizing therapeutic potential of antibody drug conjugates

Antibody drug conjugate:

- Transmembrane tumor target
- Internalization upon mAb binding
- Intracellular drug release

Aim:

- Identify diverse set of molecules with different affinities and favorable developability profile



Diversity-focused discovery

Discovery to obtain hit diversity

avsbio

Mouse immunization

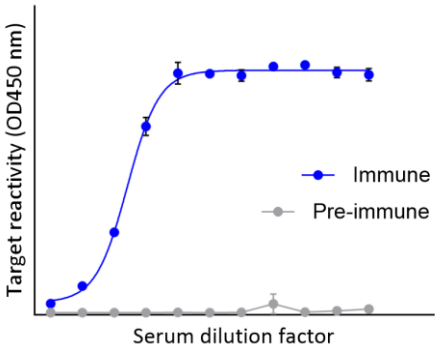
- *In vivo* antibody maturation with diversity

Robust B cell selection platform

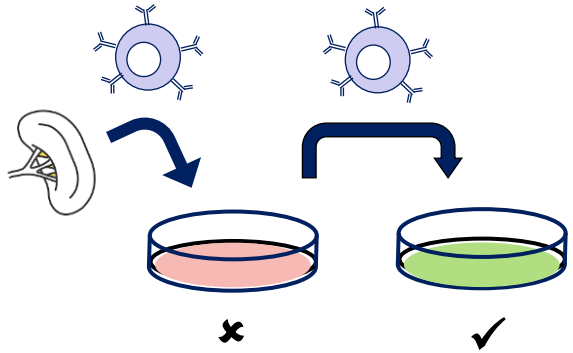
- Target enrichment
- High throughput binding characterization
- NGS-based v-domain recovery of B cell clones

Selection of broad panel of target specific hits

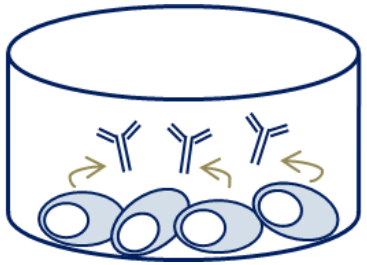
Serum screening



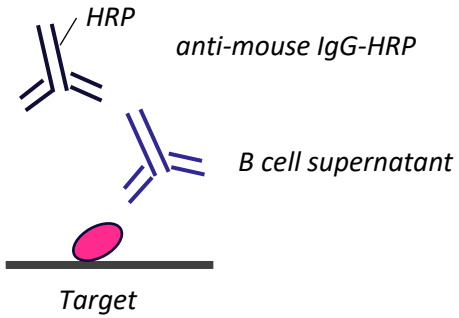
B cell selection

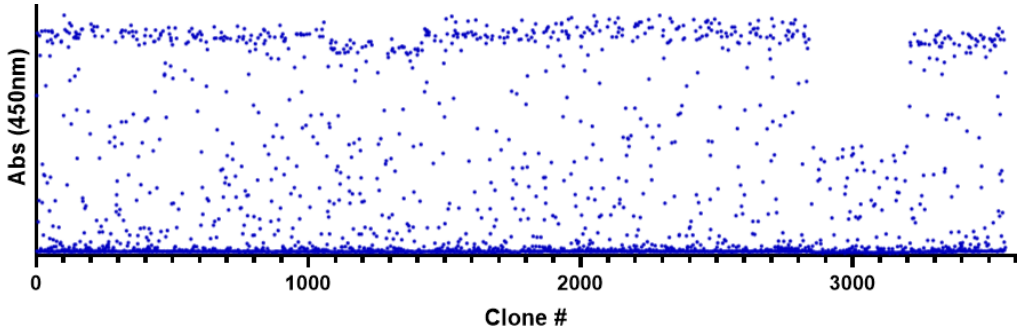


B cell culture



ELISA-based reactivity screening of 3760 B cell supernatants yielded **1135 hits**





Diversity-focused discovery

Screen for affinity diversity

Mouse immunization

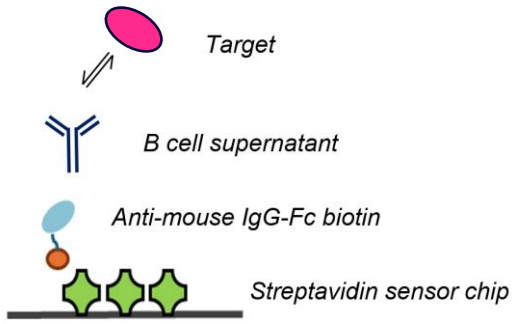
- *In vivo* antibody maturation with diversity

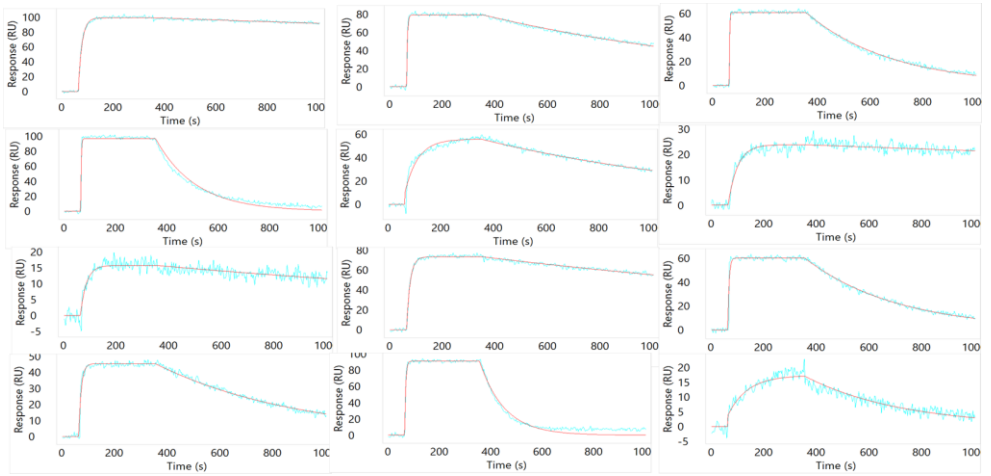
Robust B cell selection platform

- Target enrichment
- High throughput binding characterization
- NGS-based v-domain recovery of B cell clones

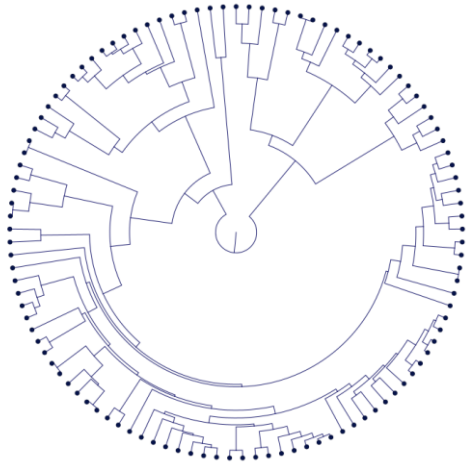
High throughput analyses supporting data-driven lead candidate selection

SPR-based high-throughput analysis of B cell supernatants to determine Ab off-rates





up to 1100 crude supernatants in a single experiment can be screened for association and dissociation rates



A subset of clones showing diversity in kinetics was selected for sequencing

NGS-based v-domain recovery was successful for ~85%

High degree of sequence diversity was obtained

Diversity-focused discovery

Structure-focused *in silico* risk assessment

AbRefine™

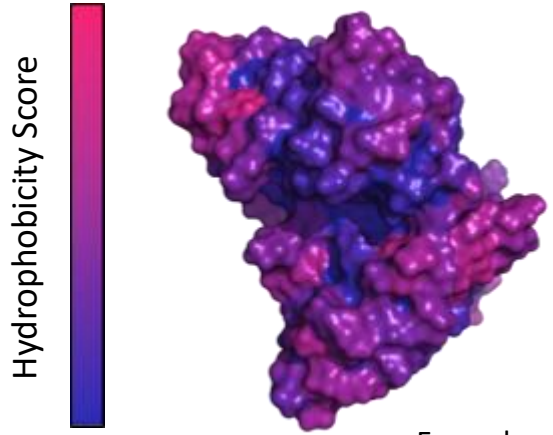
High throughput *in silico* platform:

- Fv Homology modelling
- Biophysical properties
- Chemical liabilities
- Humanness
- Compare with database of clinical therapeutic mAbs

Lead **candidate panel** selections was based on **affinity diversity** (SPR) and a favorable *in silico* **developability profile** for subsequent recombinant production and functional analysis

In silico profiling complementing data-driven lead candidate selection

1	CDR Length
2	CDR Hydrophobicity
3	CDR Positive Patch
4	CDR Negative Patch
5	Fv charge symmetry
6	VH Liability score
7	VL Liability score
8	VH Humanness pct
9	VL Humanness pct
10	Fv Humanness pct



Example

Snapshot of output

Diversity-focused discovery

Lead candidate panel production and mode of action analysis

Recombinant production of selected candidates (rPEx®)

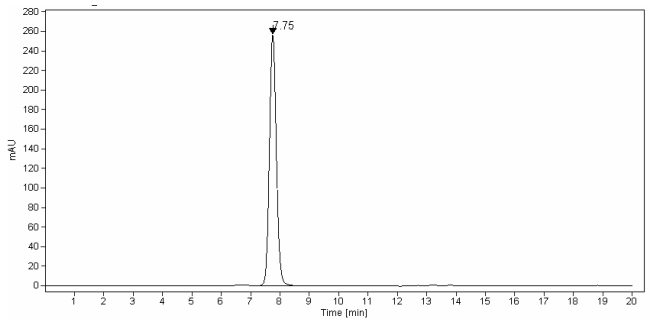
- Recombinant cloning, production and purification
- QC including HP-SEC and CE-SDS

Antibody drug conjugate generation and testing

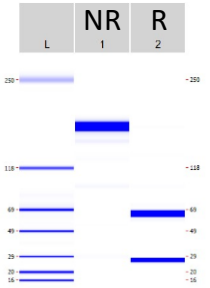
- Functional validation in cancer cell line killing assay

High quality proteins to advance mode of action (MoA) analysis

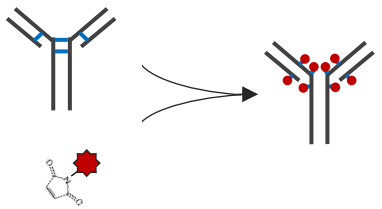
Example HP-SEC chromatogram



Example CE-SDS chromatogram

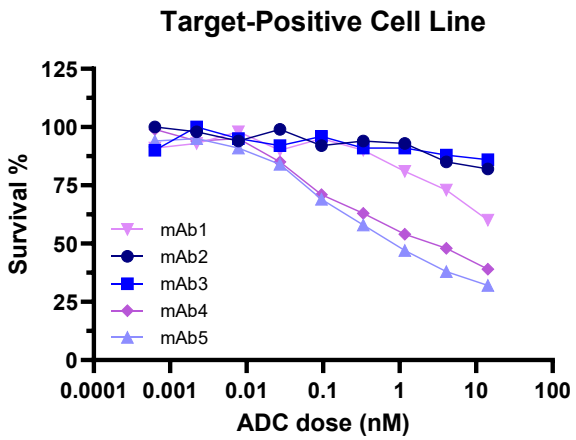


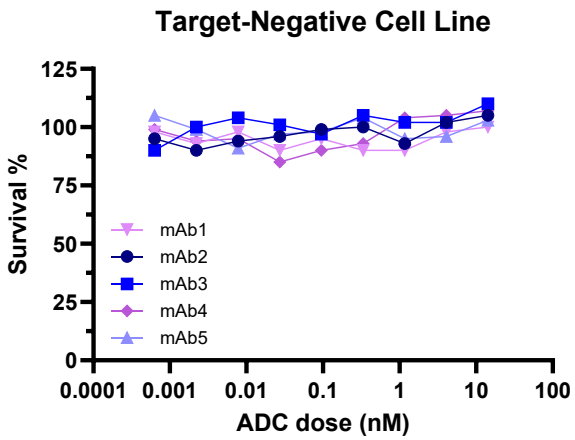
ADC preparation



B cell clone	k_{dis} (1/s)
mAb1	1.49
mAb2	0.88
mAb3	0.45
mAb4	0.44
mAb5	0.08

ADC killing assay





Integrated end-to-end workflow – client study

Empowering diversity-focused discovery and data-driven down-selection

- **A target overexpressed on cancer cells**
- **Discovery**
  Diversity-focused
  Affinity and *in silico* guided
- **Functionality profiling**
  Antibody drug conjugate testing

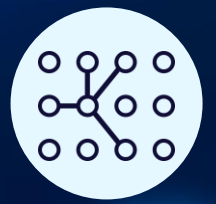
Further development
Data-driven decision making

Further *in vitro* testing

Mode-of-Action

Species-agonistic lead humanization
Rodent
Rabbit
Chicken
Llama

Highly Scalable



De-risking analysis

Liability analysis
Immunogenicity screening
Developability profiling
Molecular optimization

Integrated/ Interactive with Humanization

Recombinant optimized lead screening
Binding characterization
Functionality verification
Developability profiling

High-Throughput Platforms