Data-rich discovery and optimization to expedite lead antibody generation

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End-to-end antibody discovery and development

Through a powerful integration of *in vitro*, *in vivo*, and *in silico* technologies

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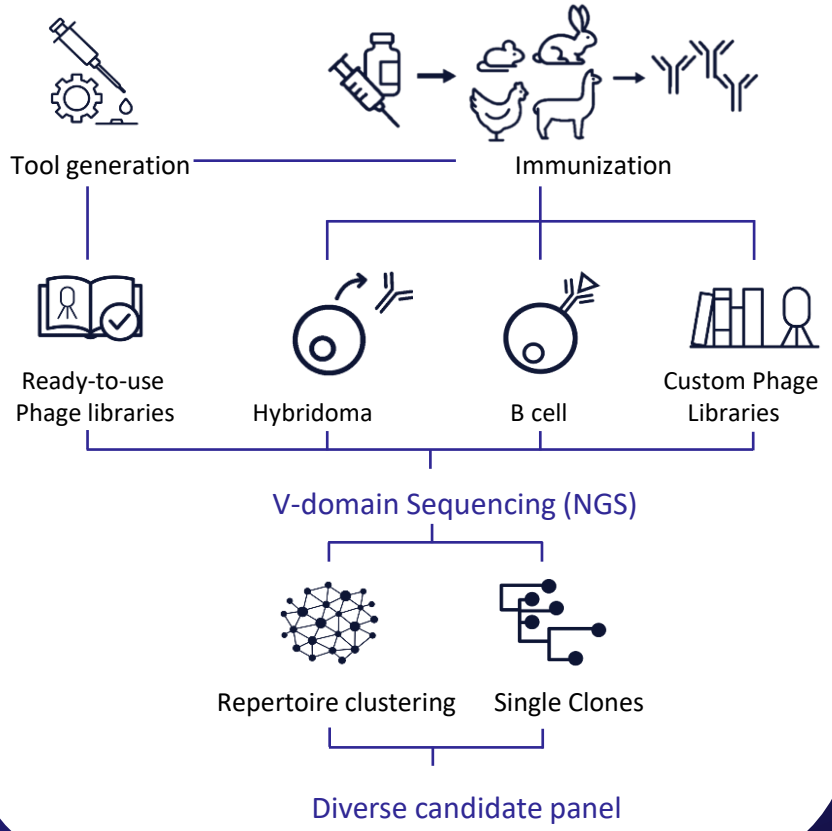
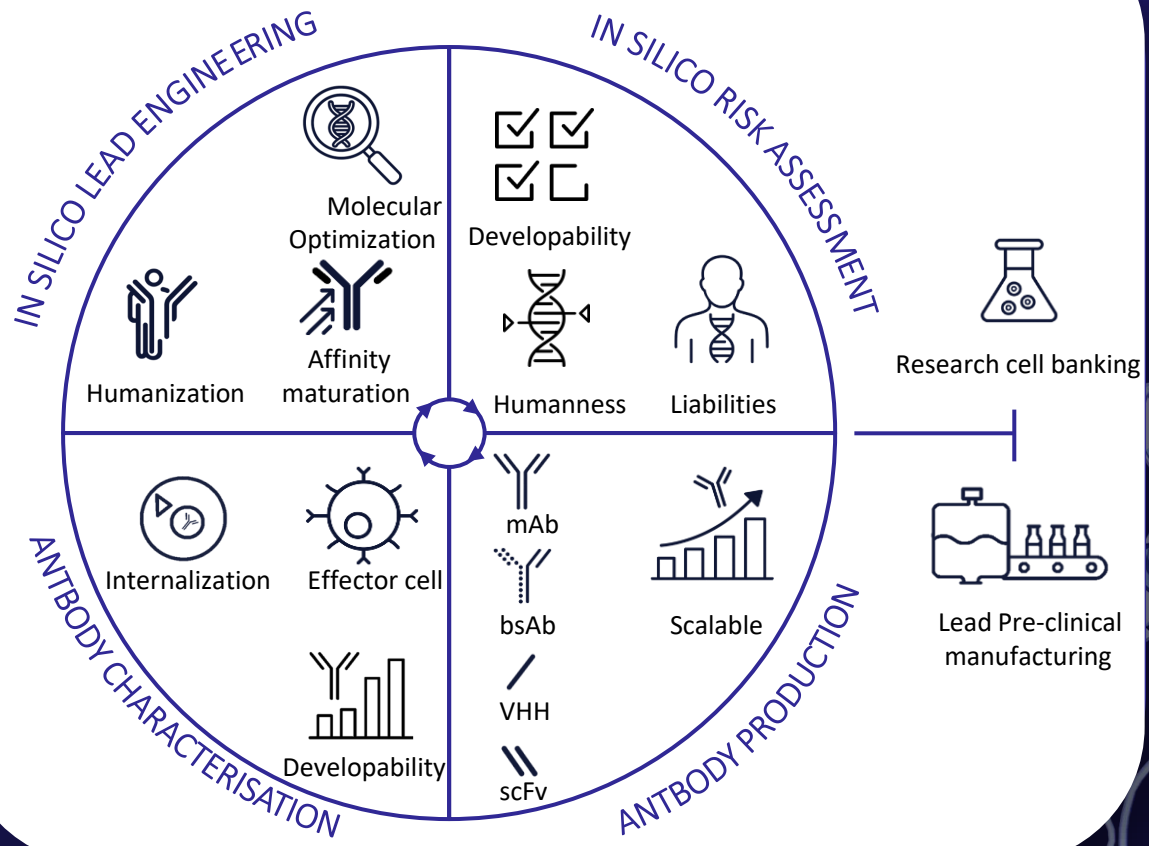
Discovery – Diversity



Development – Down-selection & De-risking



20+ Years of experience

150+ Discovery projects

80+ Development projects

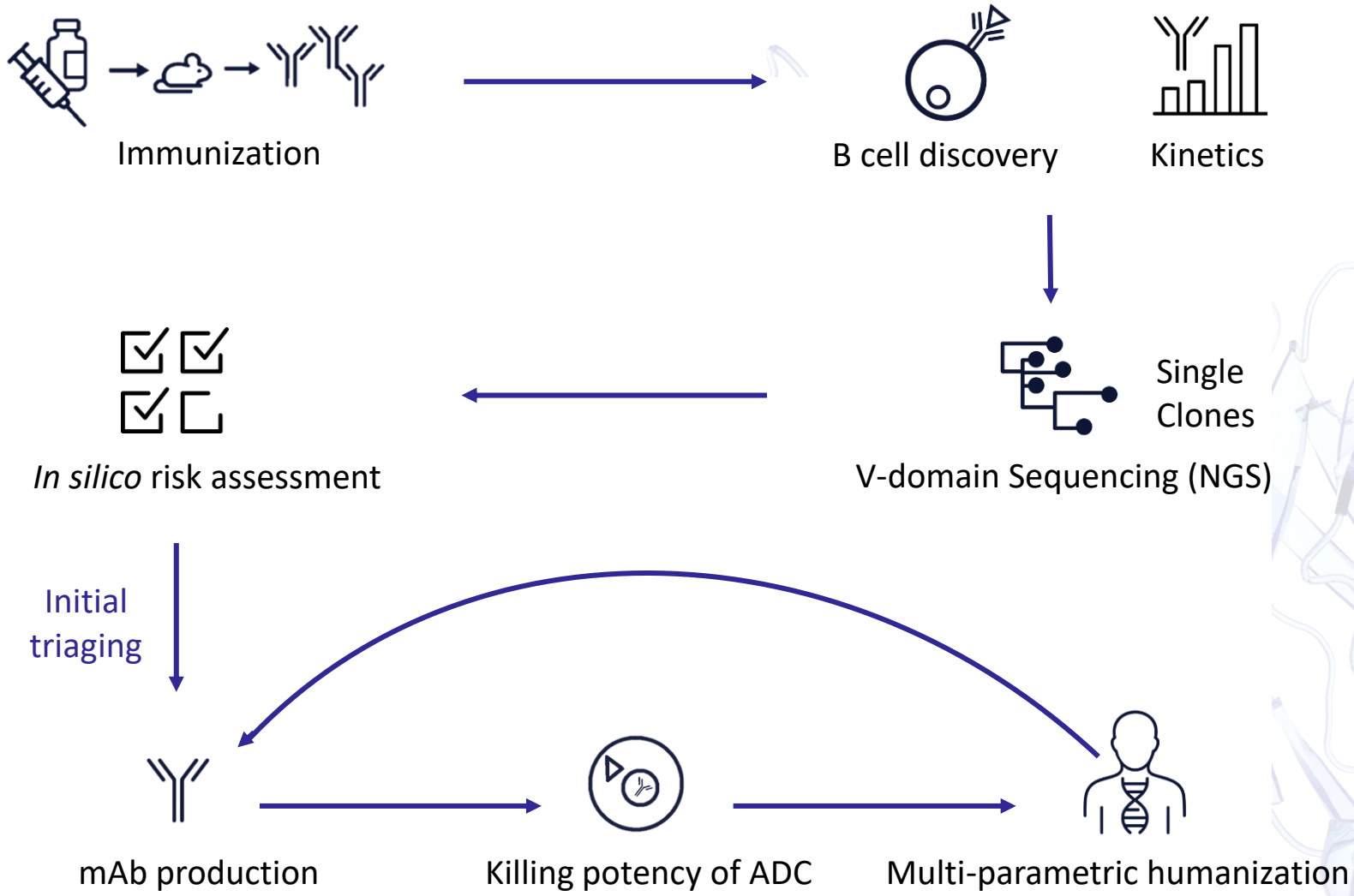
10+ Clinical Abs supported

25000+ Proteins produced

Integrated end-to-end workflow – case study

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

- **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**
  Killing potency
- **Humanization and validation**
  High-throughput and early de-risking



Targeting cancer cells

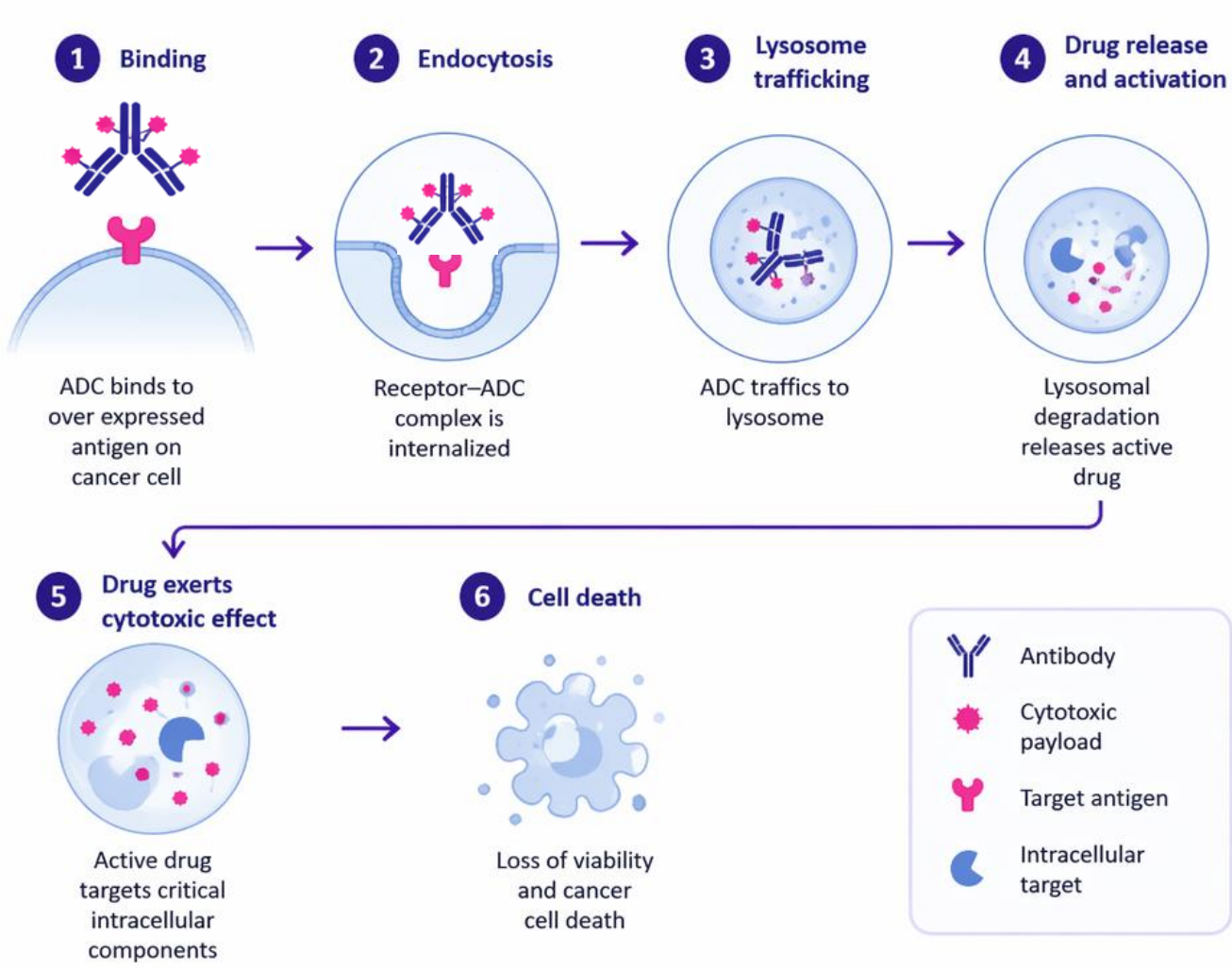
Maximizing therapeutic potential of antibody drug conjugates

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

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Mouse immunization

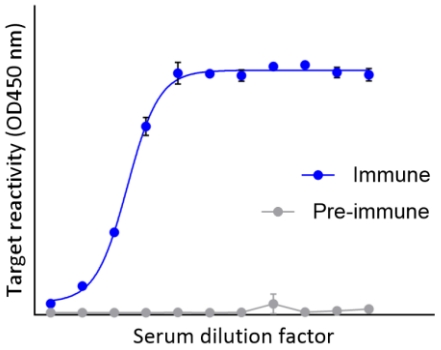
- *In vivo* antibody maturation with diversity

Robust B cell selection platform

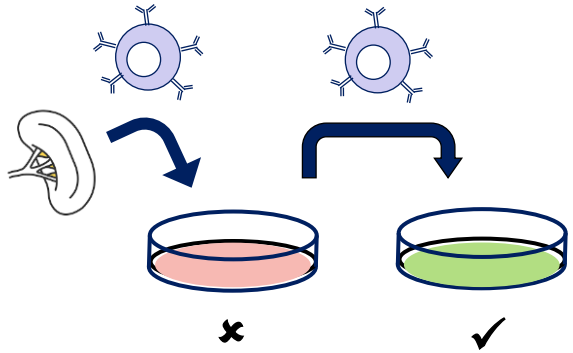
- Target enrichment
- High-throughput binding characterization
- NGS-based v-domain recovery of B cell clones

Selection of broad panel of target specific hits

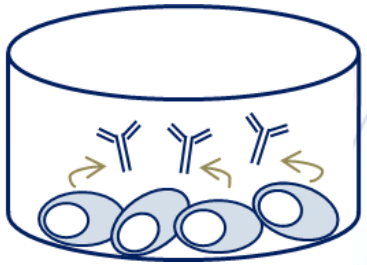
Serum screening



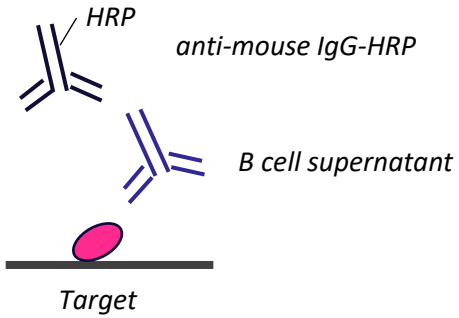
B cell selection

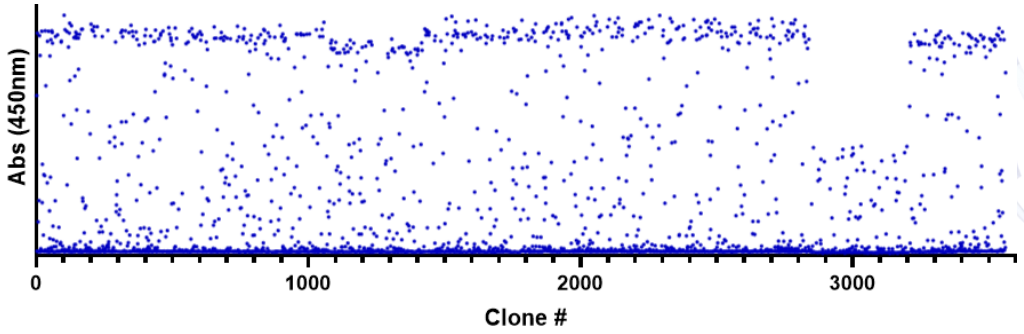


B cell culture



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





Diversity-focused discovery

Screen for affinity diversity

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Mouse immunization

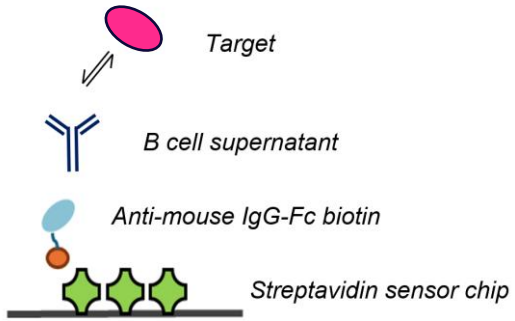
- *In vivo* antibody maturation with diversity

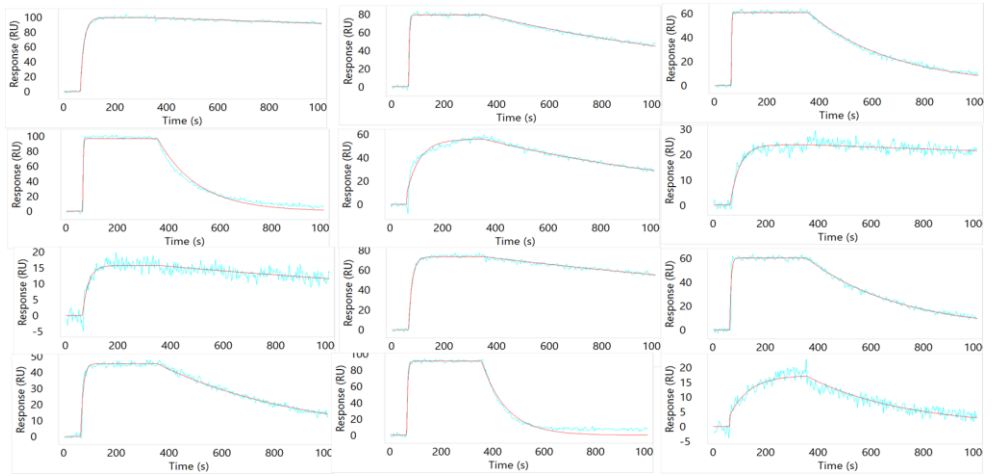
Robust B cell selection platform

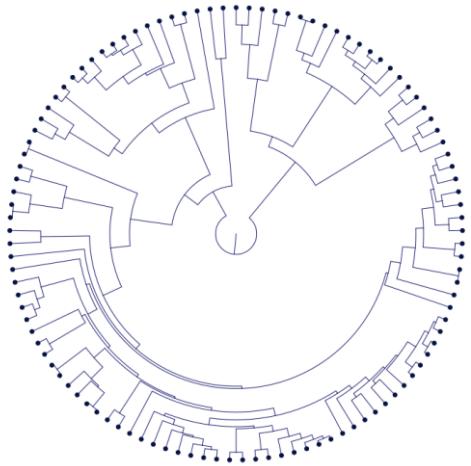
- Target enrichment
- High-throughput binding characterization
- NGS-based v-domain recovery of B cell clones

High-throughput analyses supporting data-driven candidate selection

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







Up to **1100** crude B cell supernatants can be screened for association and dissociation rates in a single experiment

k kinetics

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™

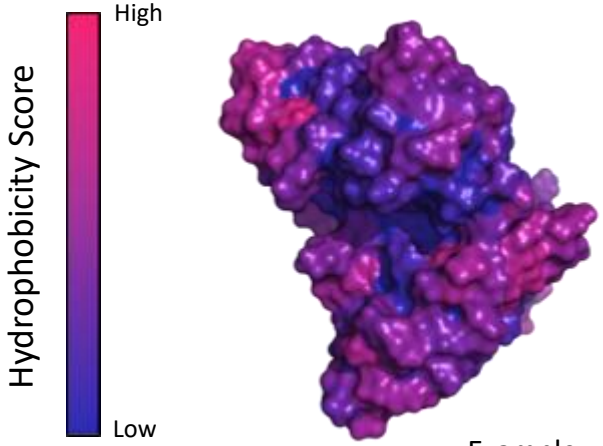
Highly scalable *in silico* platform:

- 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

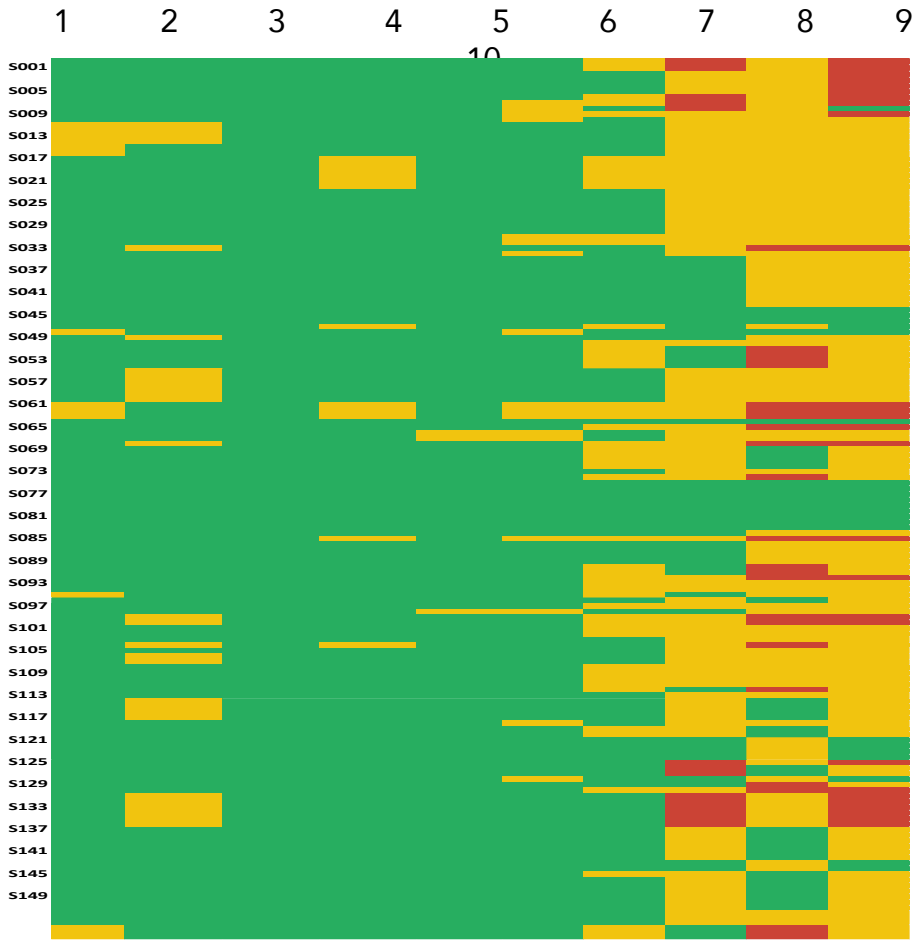
In silico profiling complementing data-driven lead candidate selection

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 AbRefine™ output



Diversity-focused discovery

Lead candidate panel production and mode of action analysis

Recombinant production of selected candidates (rPEx®)

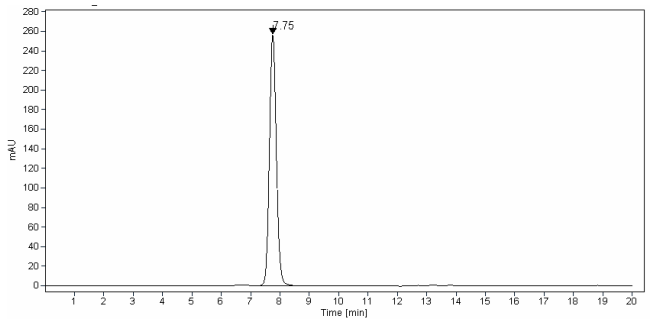
- Recombinant cloning, production and purification
- QC including HP-SEC and CE-SDS

Antibody drug conjugate generation and testing

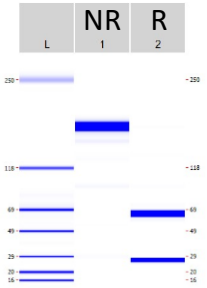
- Functional validation in cancer cell line killing assay

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

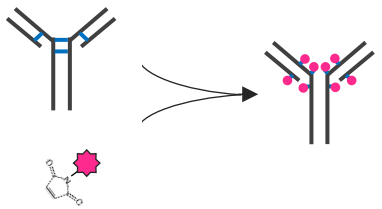
Example HP-SEC chromatogram



Example CE-SDS chromatogram

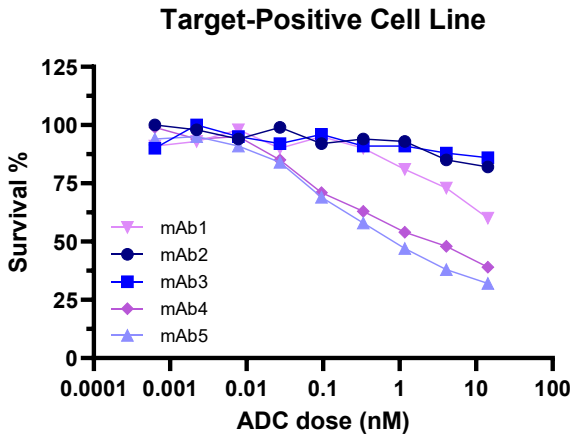


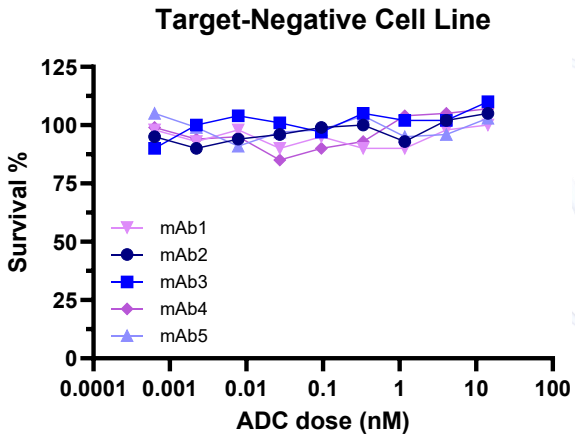
ADC generation



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





Scalable, integrated de-risking platform

Throughput empowering data-driven down-selection

Integrated *in silico*

Species-agonistic humanization

Rodent

Rabbit

Chicken

Llama

Highly scalable

De-risking

Liability analysis

Humanness scoring

Developability profiling

Molecular optimization

Integrated with humanization

In vitro

Recombinant optimized lead(s)

Binding characterization (SPR, flow)

Functionality verification

Developability profiling

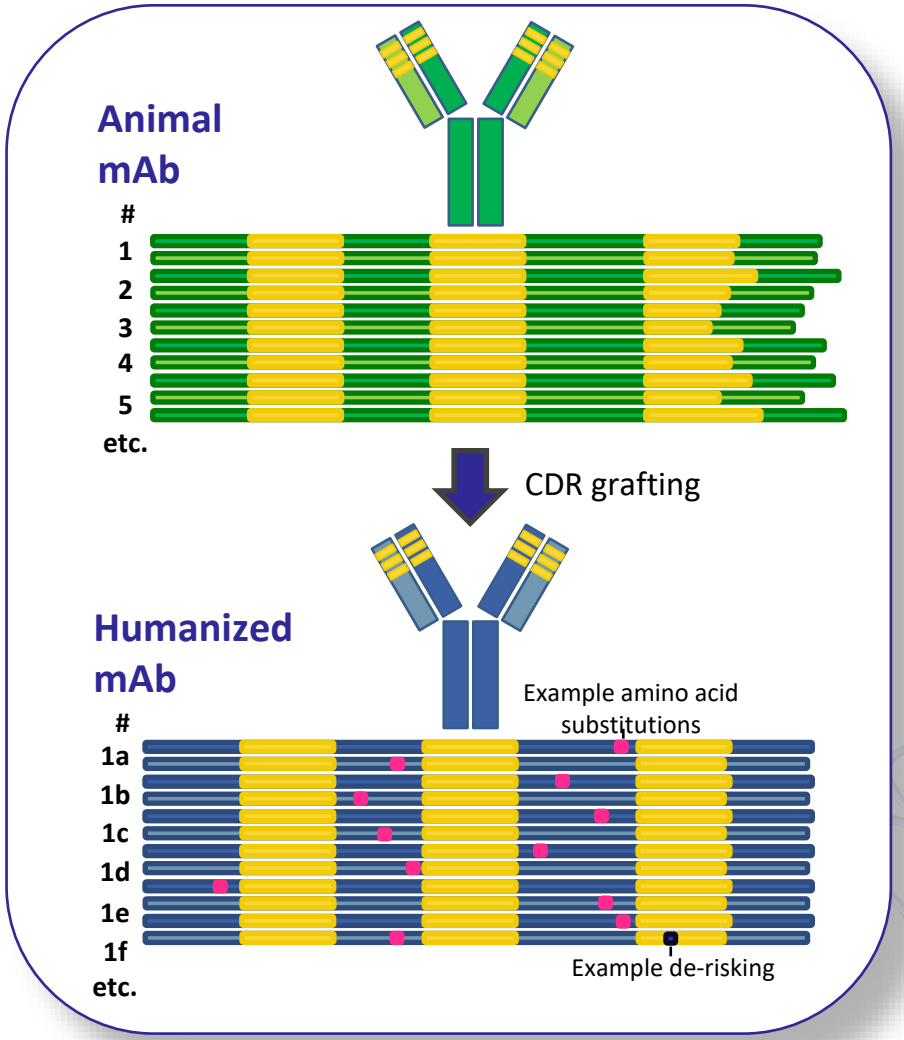
High-throughput platforms

Multi-parametric humanization for single-step engineering

Species agnostic workflow allowing for humanizing multiple lead candidates in parallel

Scalable *in silico* humanization

- CDR grafting to prioritized human germlines
- Amino acid substitutions based on advanced structural analysis
- Early de-risking addressing high risk liabilities



Highly scalable technologies advancing lead selection

Multiple lead candidates in parallel

- Scalable *in silico* design of variants
- High-throughput *in silico* risk assessment
  - Humanness
  - Developability
- High-throughput *in vitro* characterizations
  - Kinetics
  - Developability

Highly scalable *in silico* risk assessment

In silico developability profiling enabling data-driven decision making

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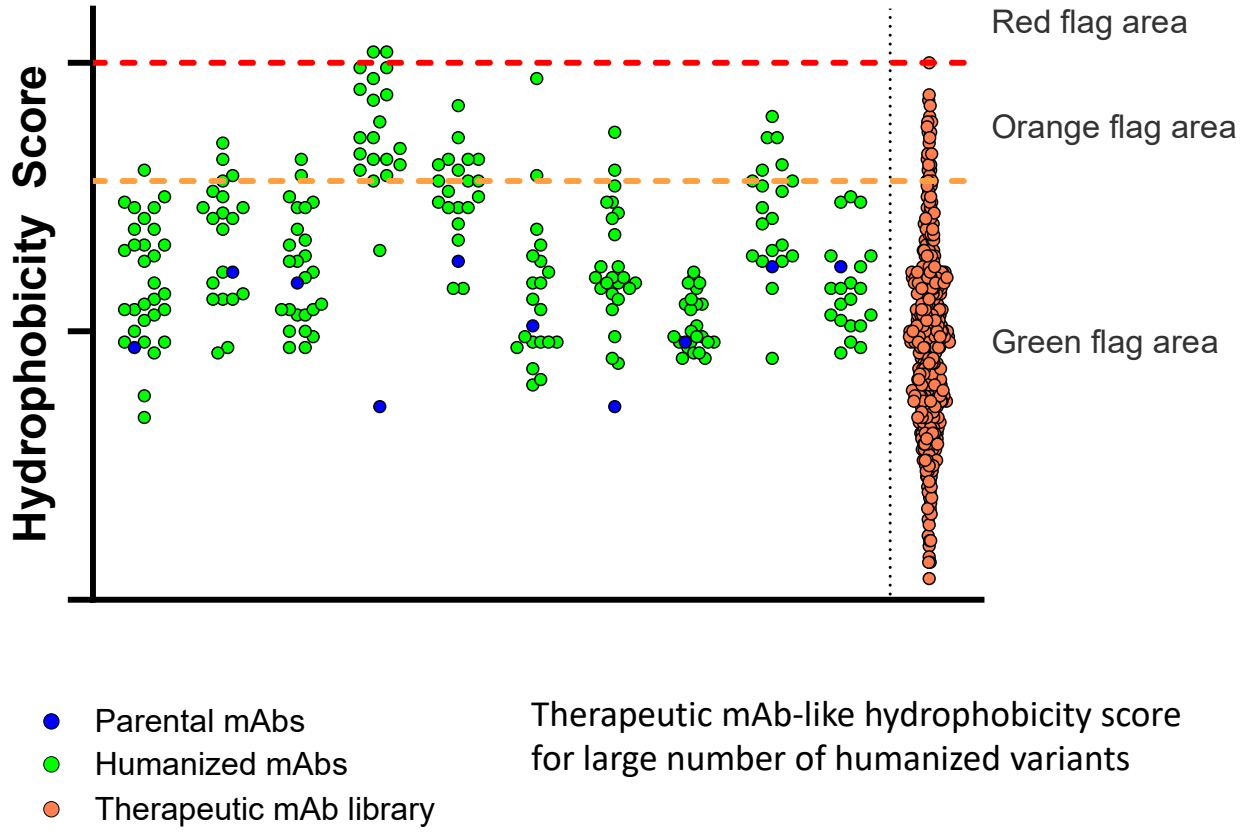
Fv hydrophobicity score of candidate and therapeutic mAbs



***In silico* biophysical characterization**

Hydrophobicity

- Scoring for aggregation-prone regions in antibodies based on hydrophobic patches
- Relative ranking towards a clinical benchmark mAb library

Fv charge symmetry, pI, CDR + and – patch energy, CDR length

Highly scalable *in silico* risk assessment

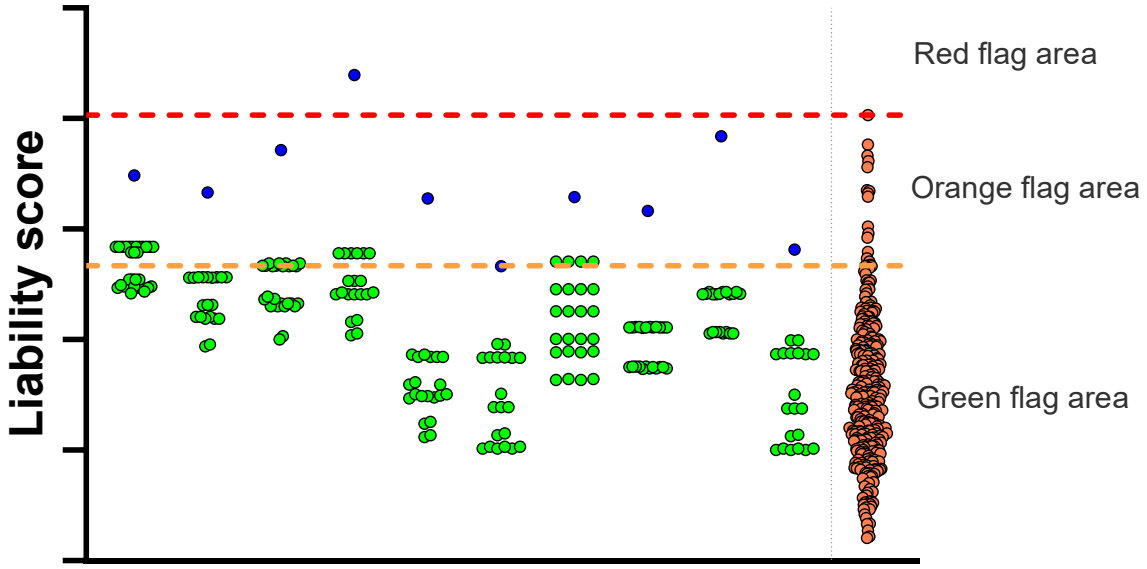
In silico developability profiling enabling data-driven decision making

Solvent-exposed liability scores of candidate and therapeutics mAbs

***In silico* structure-based liability analysis**

Liability score

- Scoring for solvent-exposed sequence liabilities
- Relative ranking towards a clinical benchmark mAb library



- Parental mAbs
- Humanized mAbs
- Therapeutic mAb library

Therapeutic mAb-like solvent-exposed liability score for majority of humanized variants

High-throughput *in vitro* affinity screening of variants

Empowering diversity-driven discovery during lead candidate development

HT SPR-based affinity determination

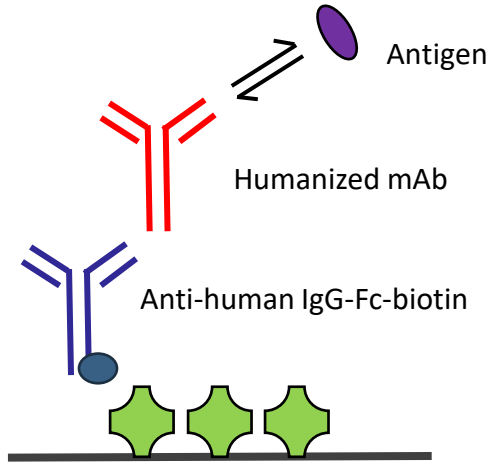
- Crude small-scale recombinant production sup
- High-throughput

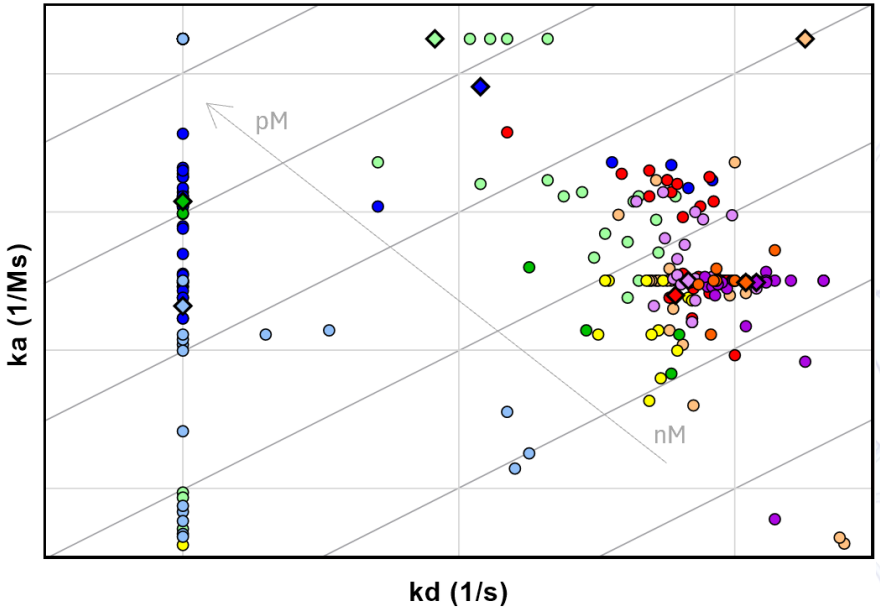
LSA^{XT}

In-depth insights in kinetic parameters using monovalent Ag

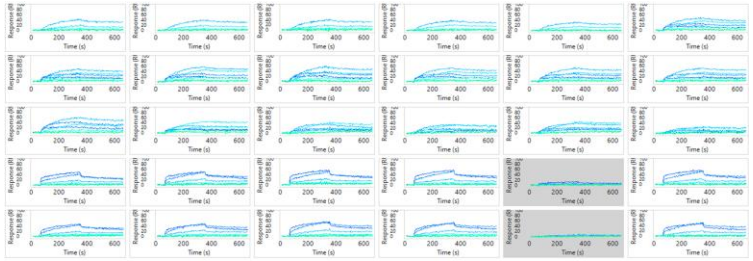
carterra

kinetics





Example unprocessed sensorgrams



High-throughput *in vitro* physicochemical profiling

Empowering diversity to mitigate risk for clinical development

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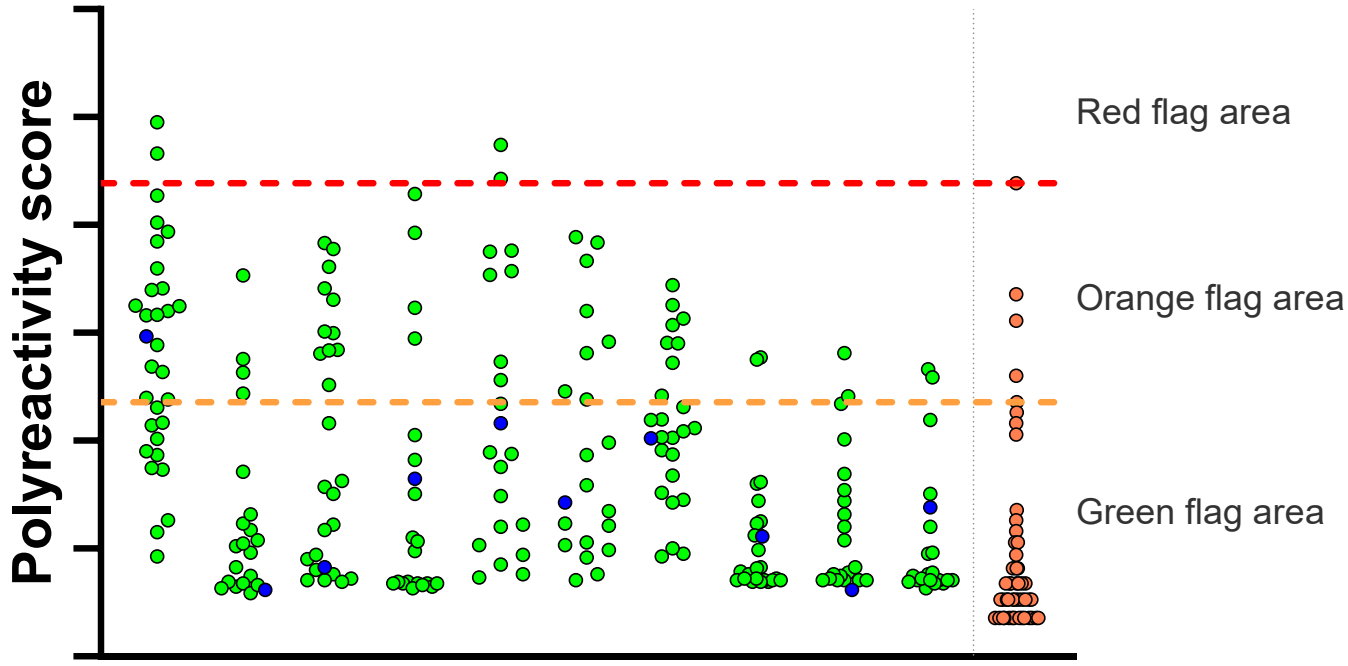
Polyreactivity scores of candidate and therapeutic mAbs



Therapeutic mAb-like polyreactivity scores for significant set of humanized variants

***In vitro* developability profiling**

More informed decision making for clinical development

- High-throughput profiling of physicochemical properties
- Rank candidates against >125 clinical mAbs (CHO-expressed)

SEC-HPLC Aggregation	**HIC-HPLC** Hydrophobicity	**DSF** Thermal stability	**Polyreactivity** dsDNA, cardiolipin
SMAC-HPLC Colloidal stability	**CIEX-HPLC** Charge	**PEG solubility**	**DLS** Diffusion interaction
CIC-HPLC Poly Ig interaction	**FcRn- HPLC** mAb clearance	**AC-SINS** Self-interaction	**Mass spec**

Case study highlights

Integrated end-to-end discovery and development

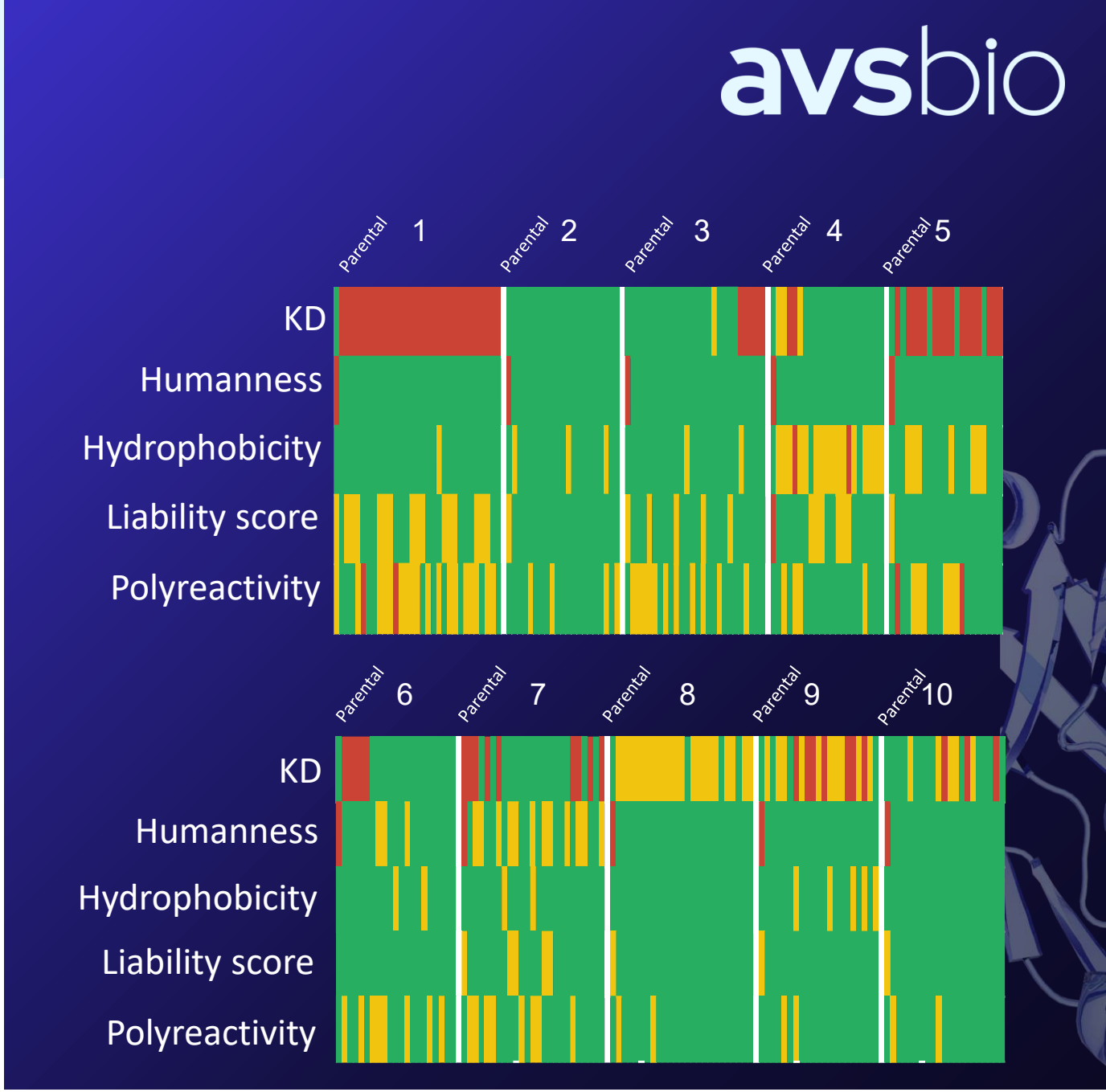
Synergy between *in silico* and *in vitro* technologies

Diversity-focused antibody discovery

- Multiple data points at an early-stage
- HT methods/technologies facilitating triaging for MoA screening
    - Binding, sequencing, kinetic profiling, risk assessment

Advancing and de-risking lead development

- Scalable/efficient lead candidate humanization
- More informed decision making
    - Highly scalable *in silico* assessments, high-throughput *in vitro* characterizations



Case study highlights

Integrated end-to-end discovery and development

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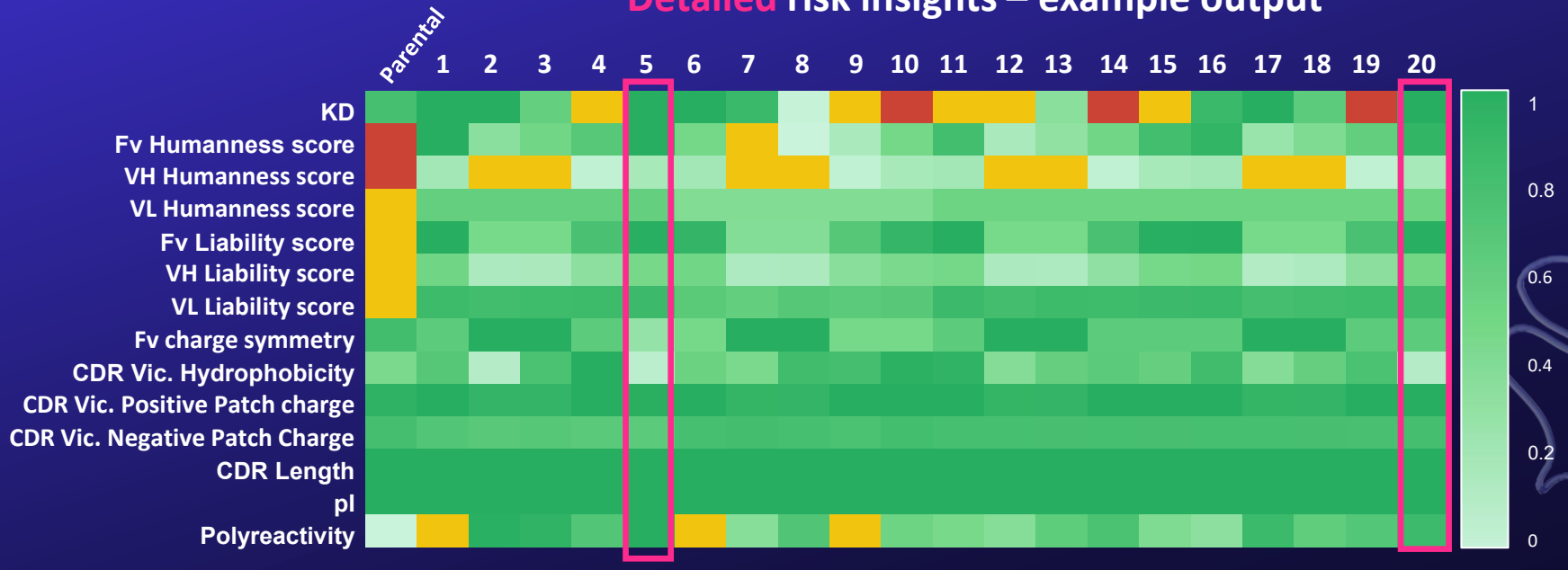
Synergy between *in silico* and *in vitro* technologies

Data-driven lead selection

- Combining scalable engineering and detailed risk insights
- Start at advanced
- Avoid extensive engineering

Detailed risk insights – example output



Empowering the value of hit diversity by highly scalable *in silico*-driven de-risking

Fusion of *in silico* and wet lab empowers multi-parametric approaches

More informed decision making to amplify lead selection

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Customized program design

- ✓ Target insights
- ✓ Therapeutic lead requirements

Diversity-focused discovery

- ✓ Functional diversity:
  From sequence to kinetics to MoA
- ✓ High-throughput technologies empowering triaging of large antibody panels

Data-driven decision making

- ✓ Early engineering combined with in-depth risk assessment
- ✓ Highly scalable *in silico* technologies matched with high-throughput *in vitro* techniques

In conclusion

In today's ever-evolving clinical landscape, uniting the scalability of *in silico* technologies with the experimental depth of high throughout *in vitro* studies amplifies therapeutic lead generation with precision by advancing and enabling data-driven decision making.

Advanced antibody technologies providing speed without sacrificing quality