Ampersand biomedicines

AB CHECK

AN AMPERSAND BIOMEDICINES COMPANY

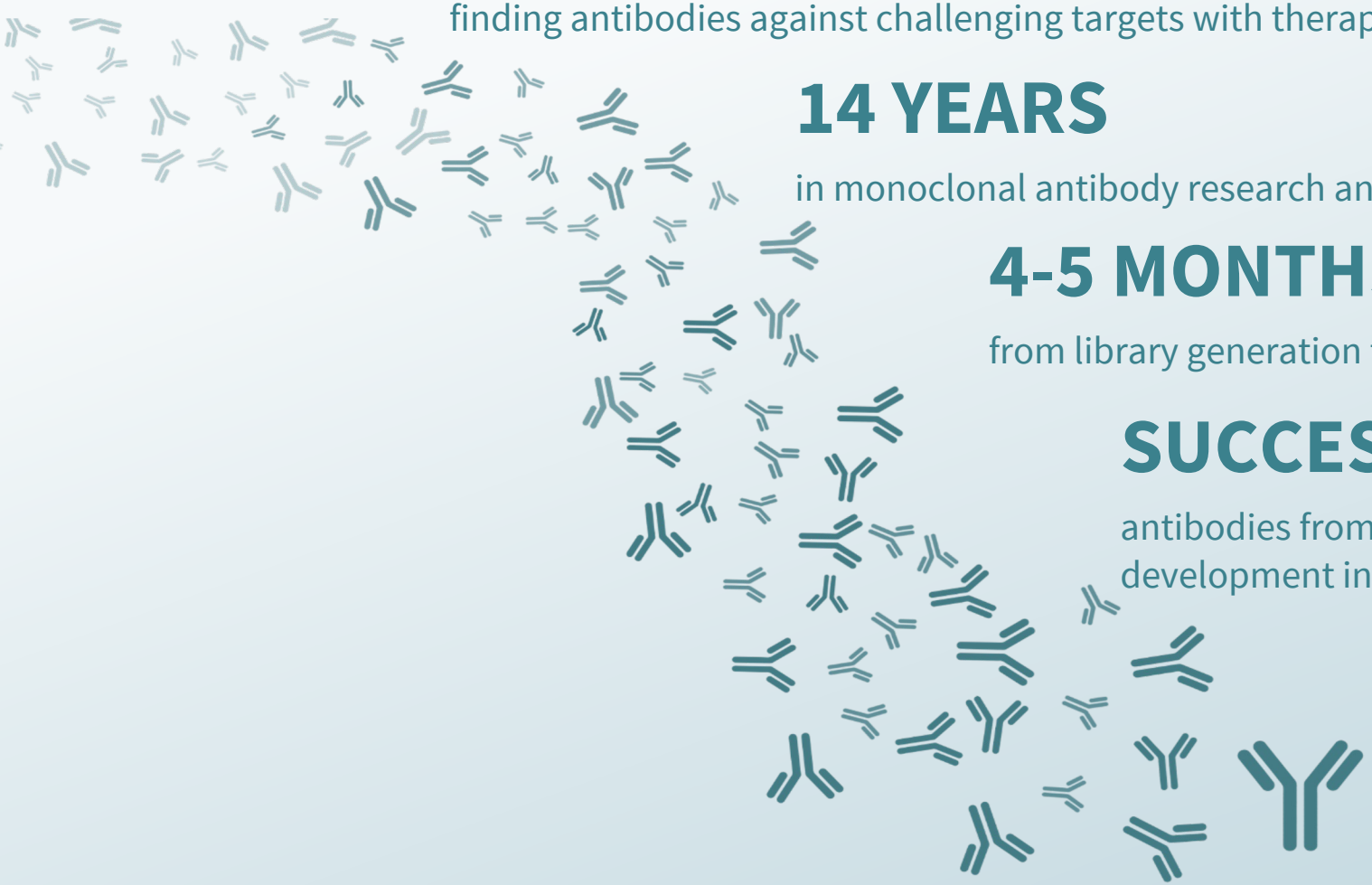
Disruptive Antibody Discovery & Development Solutions for Challenging Targets

Company Presentation

Key company metrics

HIGH-END TECHNOLOGY BOUTIQUE

finding antibodies against challenging targets with therapeutically relevant functions



14 YEARS

in monoclonal antibody research and development

4-5 MONTHS

from library generation to lead selection

SUCCESSFUL DRUG DISCOVERY

antibodies from our discovery platform currently in clinical development in high medical need indications

45+ COLLABORATIONS

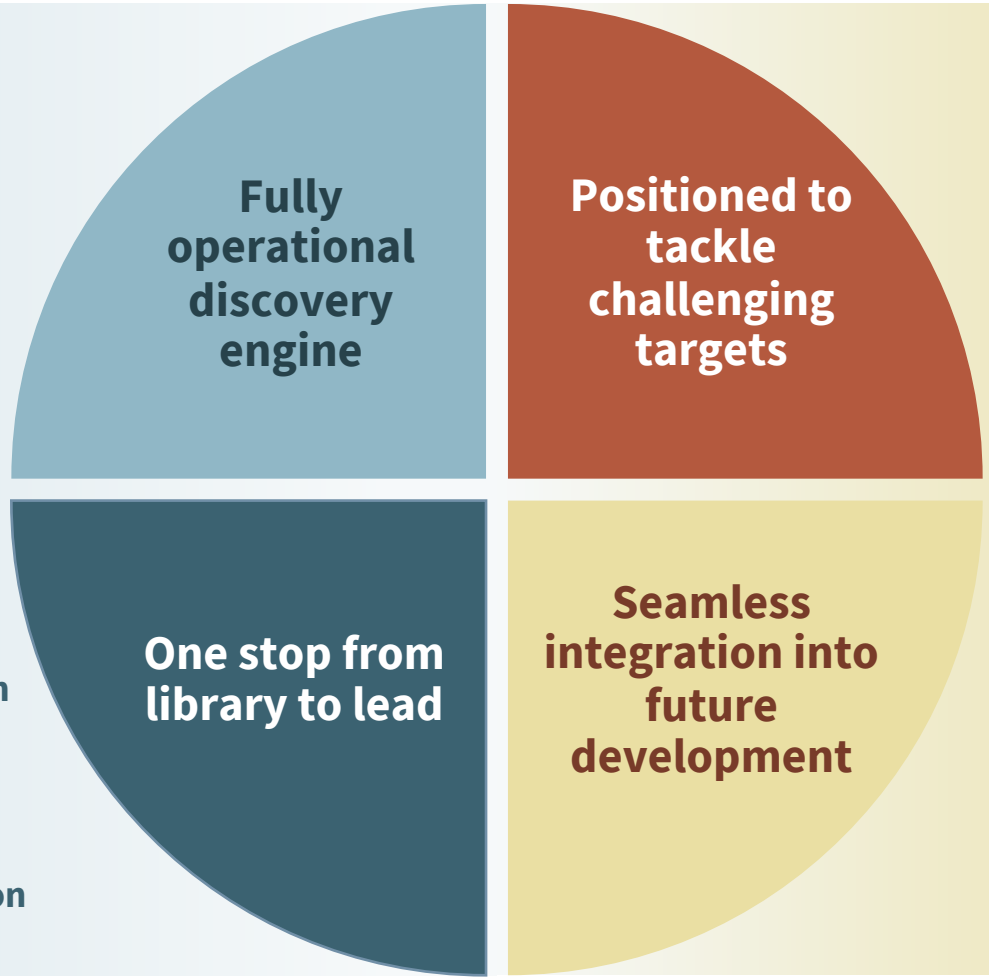
with pharmaceutical companies in US, EU, and Japan

Unique positioning for agile antibody discovery

CAPABILITIES

- **Fully-functional laboratories and office space (540m² lab space, 725m² total)** located in the Czech Republic
- Team (9 PhDs, 15 MSc, 2 BSc) with sophisticated expertise in **immunization, sensor cell line and microfluidics engineering**
- **Project management capabilities** with international track record (45+ completed discovery projects)

- Offers **state-of-the-art and cutting-edge techniques in library generation**
- In-house **microfluidics design and manufacturing** for **high-throughput functional screening**
- Established processes for **humanization and optimization**



POTENTIAL

- Proprietary sensor cell lines enable **discovery of functional antibodies,** e.g. agonistic, internalizing mAbs
- Integrated library and screening approaches enable **targeting of historically challenging targets** e.g. GPCRs or MHC/Peptide complexes

- **Highly customizable processes** evidenced by long-standing industry collaborations **ensures seamless integration** into existing drug development programs
- **Established high-throughput methods** answers demands of cutting-edge antibody-based drug discovery

AbCheck overcomes the challenges of various, ‘difficult-to-develop’ targets

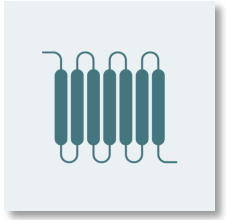
Opportunity:

- GPCRs are one of the largest receptor families. Currently, mostly targeted by small molecules.

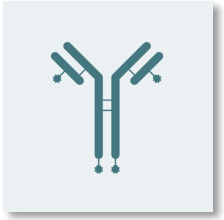
Challenge and AbCheck solution:

- GPCR antibodies are difficult to develop as GPCRs have only small accessible extracellular regions and epitopes

➢ **AbCheck combines proprietary immunization protocols and the microfluidics platform for early discovery of rare binders as millions of antibody-secreting cells can be screened per day.**



GPCRs



ADCs

Opportunity:

- ADCs combine an antibody and an active payload primarily used in oncology.

Challenge and AbCheck solution:

- ADCs require both high affinity as well as high internalization to be functional

➢ **AbCheck’s microfluidics platform simultaneously screens for both affinity and internalization**

Opportunity:

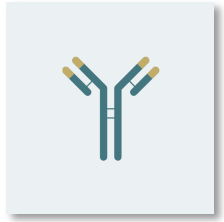
- Most antibodies in oncology target extracellular proteins. Targeting MHC-I peptide complexes also allows targeting of internally expressed oncoproteins increasing number of potential targets.

Challenge and AbCheck solution:

- Targeting MHC-I/peptide complexes requires high specificity due to toxicity risk

➢ **AbCheck’s narrow-specificity sorting enables selecting highly specific candidates with pM Kd**

MHC-I/Peptide Complexes



Binders/agonists/ antagonists

Opportunity:

- The majority of antibodies developed so far have been antagonists rather than agonists.

Challenge and AbCheck solution:

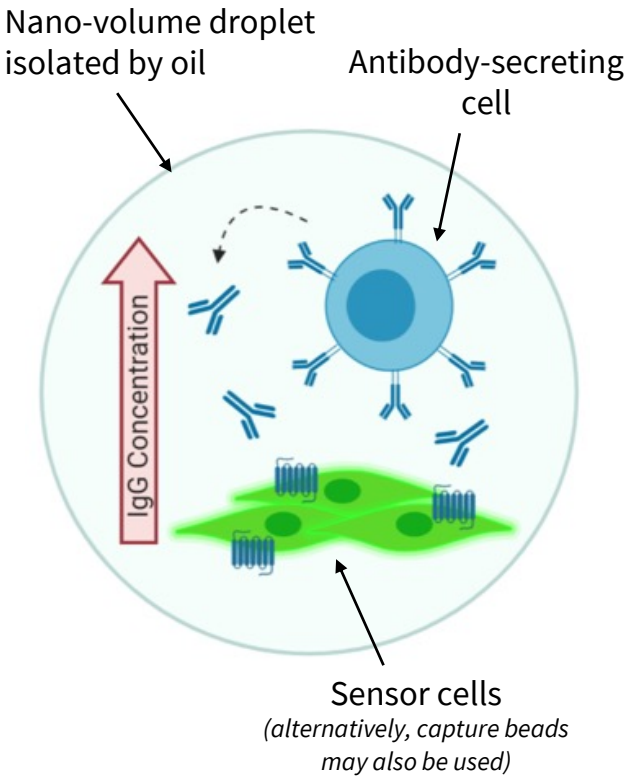
- Agonist and antagonist antibodies have historically been difficult to develop as affinity ≠ functionality

➢ **Using microfluidics AbCheck combines screening for affinity and function in single step**

High-throughput microfluidics system for fast and more efficient antibody discovery

Tailored, high throughput method for sampling/sorting of immune plasma cell repertoires with functional resolution at single-cell level:

Fastest and most efficient way to isolate antibodies with therapeutically relevant biological functions

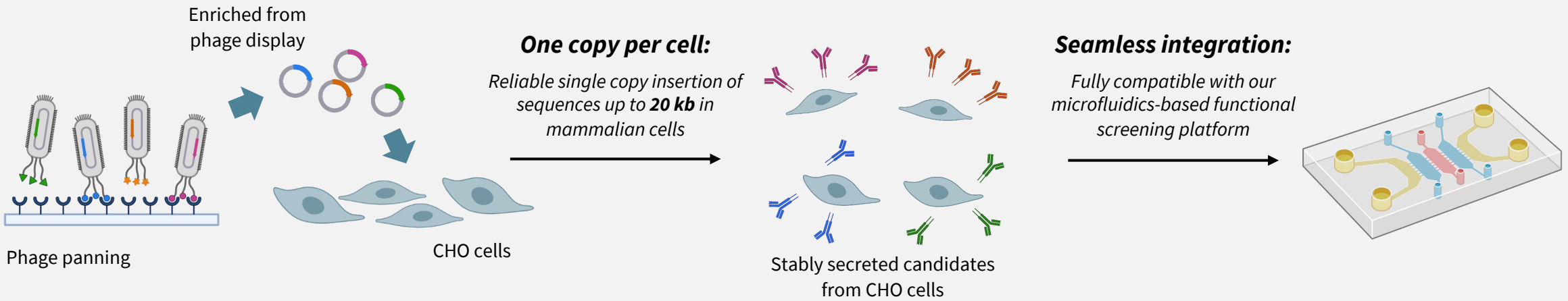


Advantages:

- Direct **sorting for function** and/or other critical criteria can be **combined *in one step!***
- High throughput of ***Millions* of droplets per day**
- No amplification during library construction resulting in **clonal diversity *as high as natural repertoire***

AbCheck's Single Copy Integration Site technology enables efficient, streamlined discovery of novel functional antibody candidates

Repertoire Generation | **Screening** | **Lead Selection**



- Mammalian libraries enable expression and secretion of antibodies with **native post-translational modifications and folds**
- Single copy integration ensures **stable expression** of candidate mAbs and possibility of **functional sorting**

➔ **Process is independent of immunization**

➔ **Proof-reading is integrated in the secretion step, enriching for developable candidates**

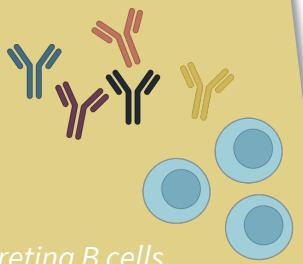
➔ **Enables a highly cost- & time-efficient path to lead selection and characterization**

AbCheck's platform enables high throughput screening of previously 'difficult-to-develop' targets with unprecedented cost & time efficiency

REPERTOIRE GENERATION (1-2 MONTHS)

Immunization

B-cell isolation

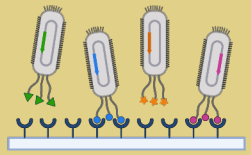


Classical Immunization

→ *Conventional approach to acquire antibody-secreting B cells which can be further screened for desired target product profile*

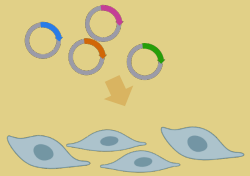
Mammalian libraries

Phage Panning



Single Copy Integration Site

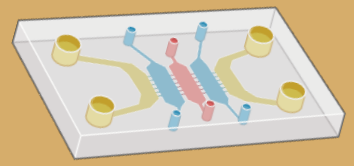
NEW



Library-based technologies combined with single copy integration site → *Increase probability of success for challenging targets by maximizing repertoire size while still allowing combination with microfluidics for increased throughput*

SCREENING (DAYS-WEEKS)

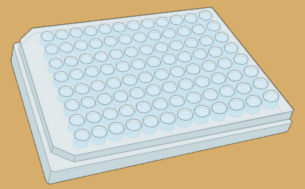
Microfluidics



High-Throughput Cell Sorting

→ *ensures high-throughput screening and can combine affinity + functional screening in single assay*

LEAD SELECTION / CHARACTERIZATION (6-8 WEEKS)



ELISA, FACS, SPR, etc.

Classical Ab Characterization

→ *Further characterization of hits for desired target product profile*

LEAD OPTIMIZATION (OPTIONAL, 9 MONTHS)

AbAccel®

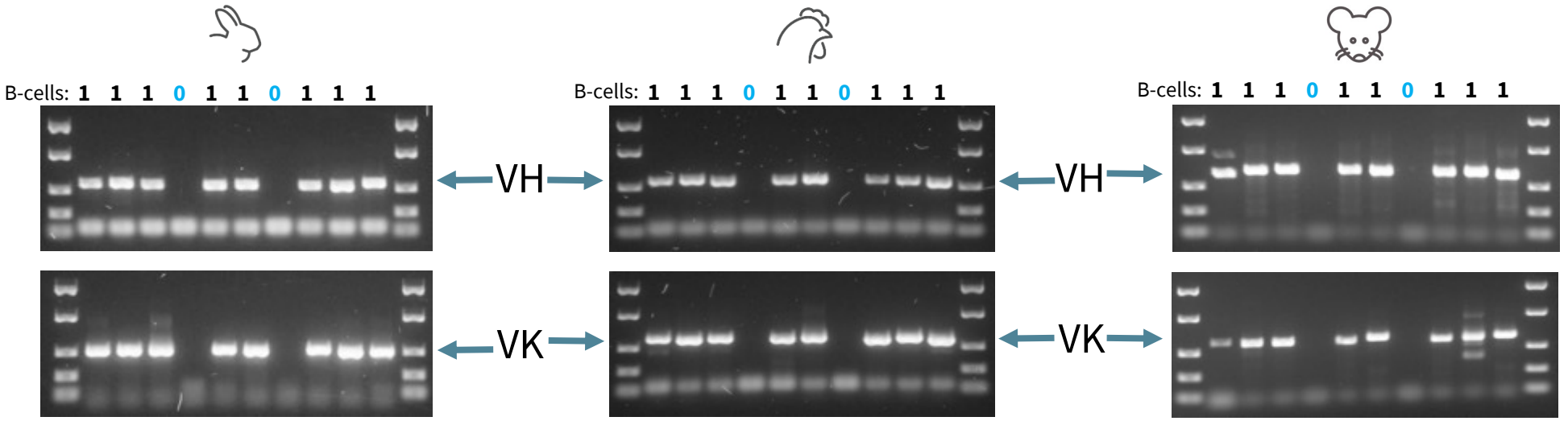
Antibody optimization for lead candidate

→ *Efficient generation of mAbs with superior drug-like properties*

Abcheck's reliable cloning and antibody production process enables subsequent thorough characterization

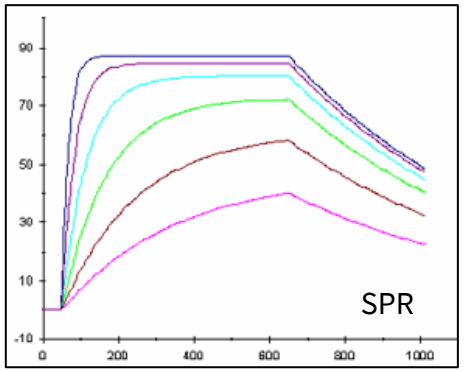
Step 1

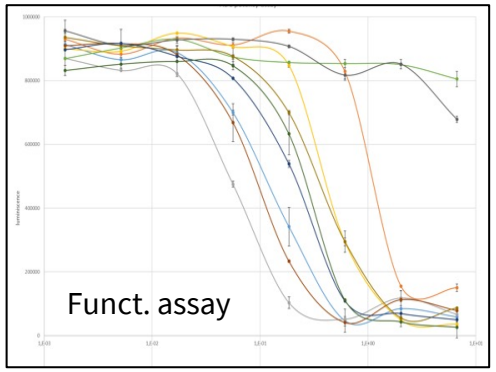
Ab genes from each sorted cell are amplified, sequenced and cloned in parallel for expression of full-length Abs



Step 2

Binding properties of MAbs are confirmed by plate and FACS-based assays, and positive clones are taken forward for functional characterization and full binding kinetics (SPR)





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Technology applications

Microfluidics allows screening of current as well as next-generation targets for both classical antibodies and ADCs

Repertoire Generation | Screening | Lead Selection

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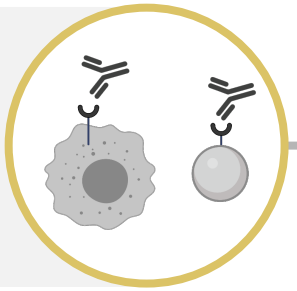
Classical Binding

Key for Antibody Discovery: AbCheck's microfluidics platform can be used for both detection of binders and functional sorting. The platform can detect binding to sensor cells or to recombinant protein on capture beads

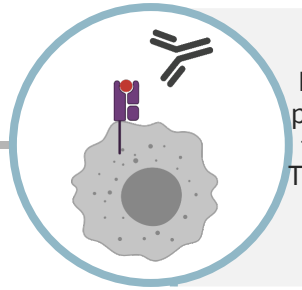
Key Therapeutic Areas: Oncology, Immunology, Metabolic Diseases

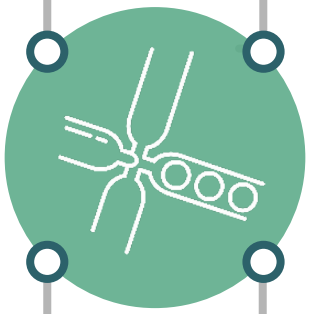


Binding of Novel Targets

Key for next-gen Ab discovery: AbCheck has developed a process for the discovery of Antibodies for peptide-specific targeting of MHC-I Complexes for next-gen tumor therapy. The high specificity could also potentially be used for other targets such as mutated vs. non mutated receptors

Key Therapeutic Areas: Oncology

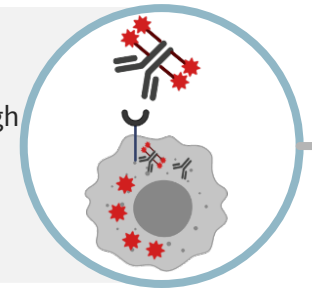




Internalization

Key for ADC discovery: ADCs need to be selective for a given target and also trigger high internalization rates.

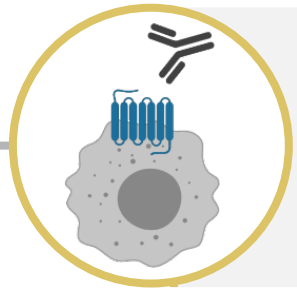
Key Therapeutic Areas: Oncology, Immunology, Infections



GPCR Functionality

Key for GPCR Agonist/Antagonist Ab Discovery: Target binding & activation are not linked. AbCheck's platform can determine both in a single step, saving cost & time to get to fully functional candidates

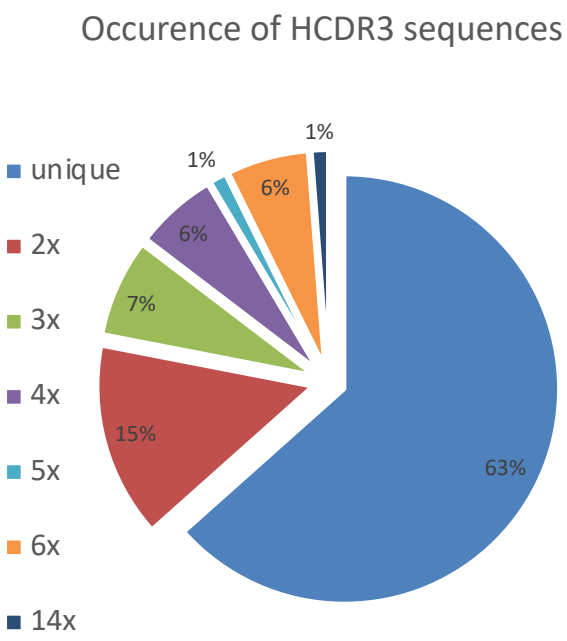
Key Therapeutic Areas: Oncology, Immunology, Metabolic Diseases



We have demonstrated the reliable discovery of a large number of clones with high affinity using our microfluidics platform

Repertoire Generation | **Screening (Classical Binding)** | Lead Selection

Discovery of antibodies to a non-challenging, classical tumor target



Target: Transmembrane protein with extracellular domain, validated target overexpressed in many carcinomas

Microfluidics Solution

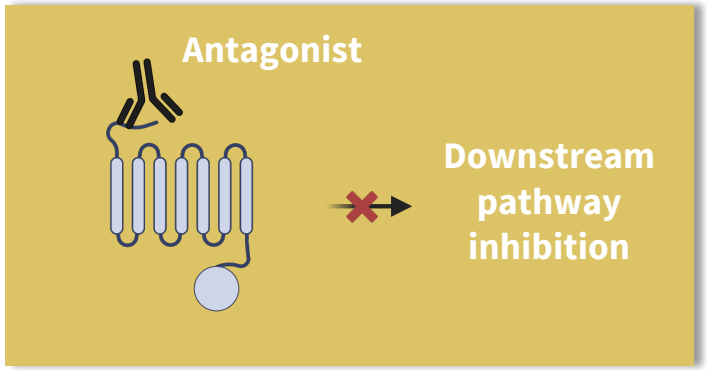
- Isolation of Antibody-secreting cells from spleens of chicken immunized with DNA or protein and screening of 1.5 million droplets
- Sorting of droplets with a positive signal (range 0.2% - 1% per droplet, depending on the respective chicken splenocyte) and amplification of VH and VL genes via single-cell RT-PCR

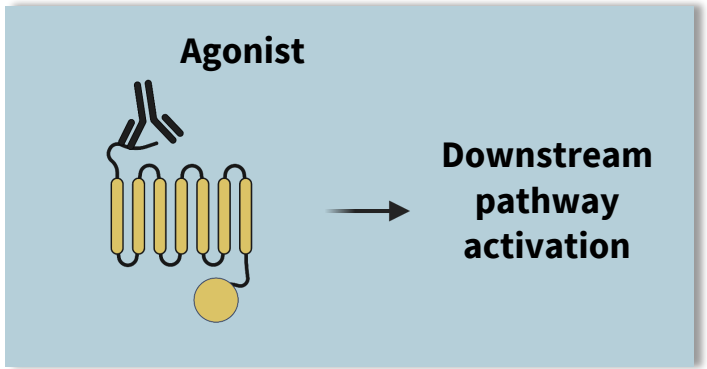
Powerful Results

- Enabled selection for specific criteria out of **3,000-15,000 Antibodies**
- Antibodies from 168 droplets selected for further analysis, successful cloning, **sequencing and testing of 153 (93%) Antibodies, >90% of tested clones confirmed as positive. Monomeric affinities of selected clones in the picomolar to single-digit nanomolar Kd range**

Agonist mAb development is initially focused on binding, however this has led to many discontinuations as binding ≠ functionality

Repertoire Generation | **Screening (Agonistic GPCR Antibodies)** | Lead Selection





Opportunity

- The majority of antibodies that have been developed so far have been antagonists targeting overactive pathways, activating pathways has been less explored due to development hurdles

Challenge

Historically the **development of agonist mAbs is challenging**, unlike antagonist mAbs their activity can be:

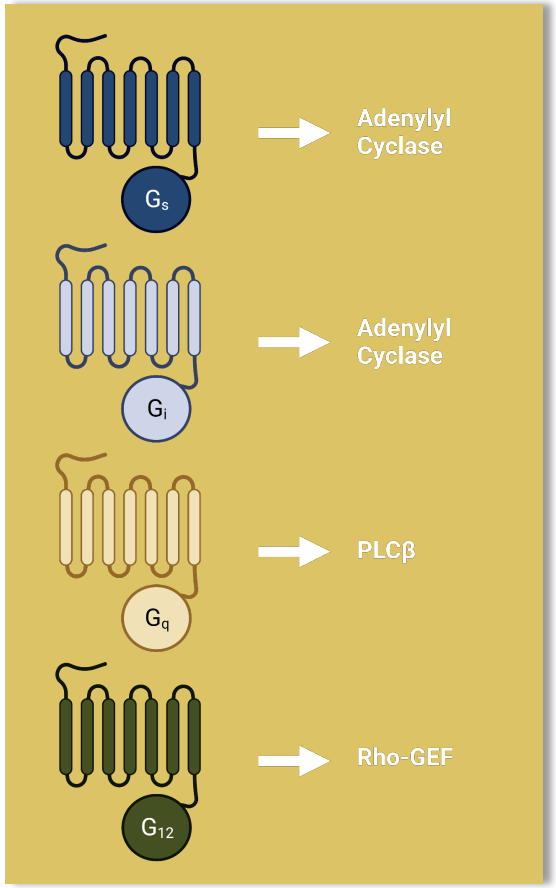
- inversely correlated with affinity
- inversely correlated with receptor occupancy
- driven by propensity to induce super clustering

AbCheck Solution

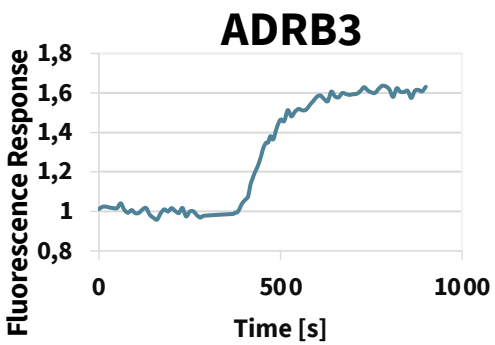
- The platform is based on **microfluidic droplet sorting** for functional signalling that screens for affinity and function simultaneously
- We employ a dual signal selection, which virtually **eliminates false positives**
- A typical 'run' screens of **2.5 hrs yields 5-10 confirmed <u>functional</u> agonists**

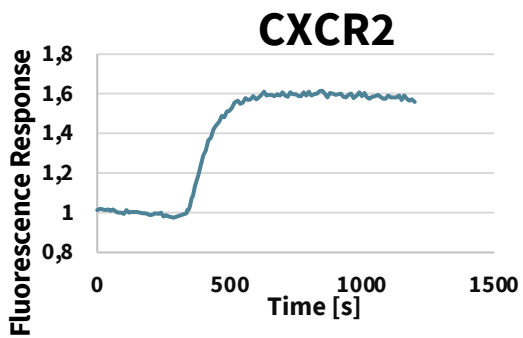
We have developed a universal system to analyze GPCR targets independent of their Gα subunits to identify agonists

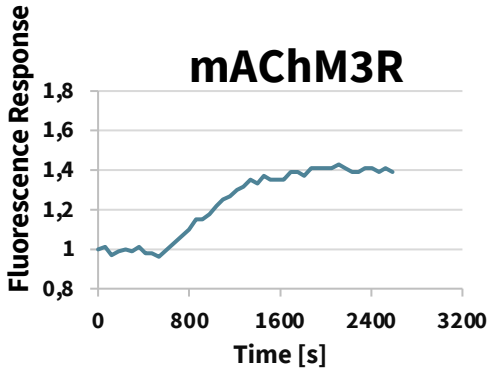
Functional screening of all GPCRs regardless of G protein subunit

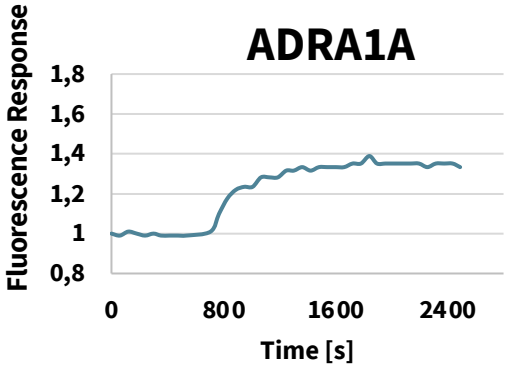


*Responses measured on transiently transfected cells **prior to optimization***



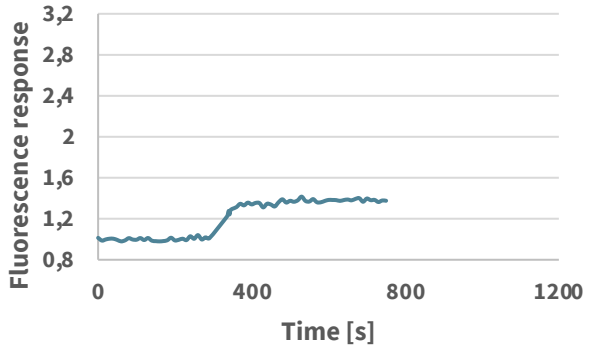




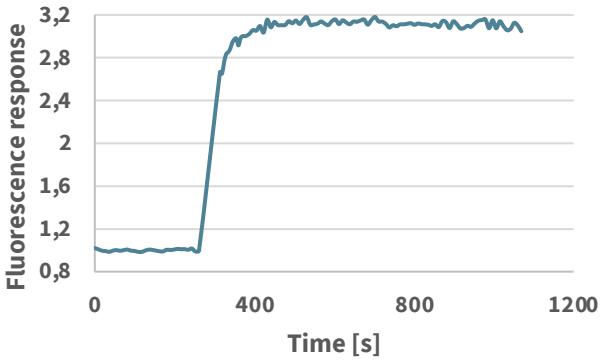


Assay windows can be further optimized by monoclonal selection of stably-expressing cells

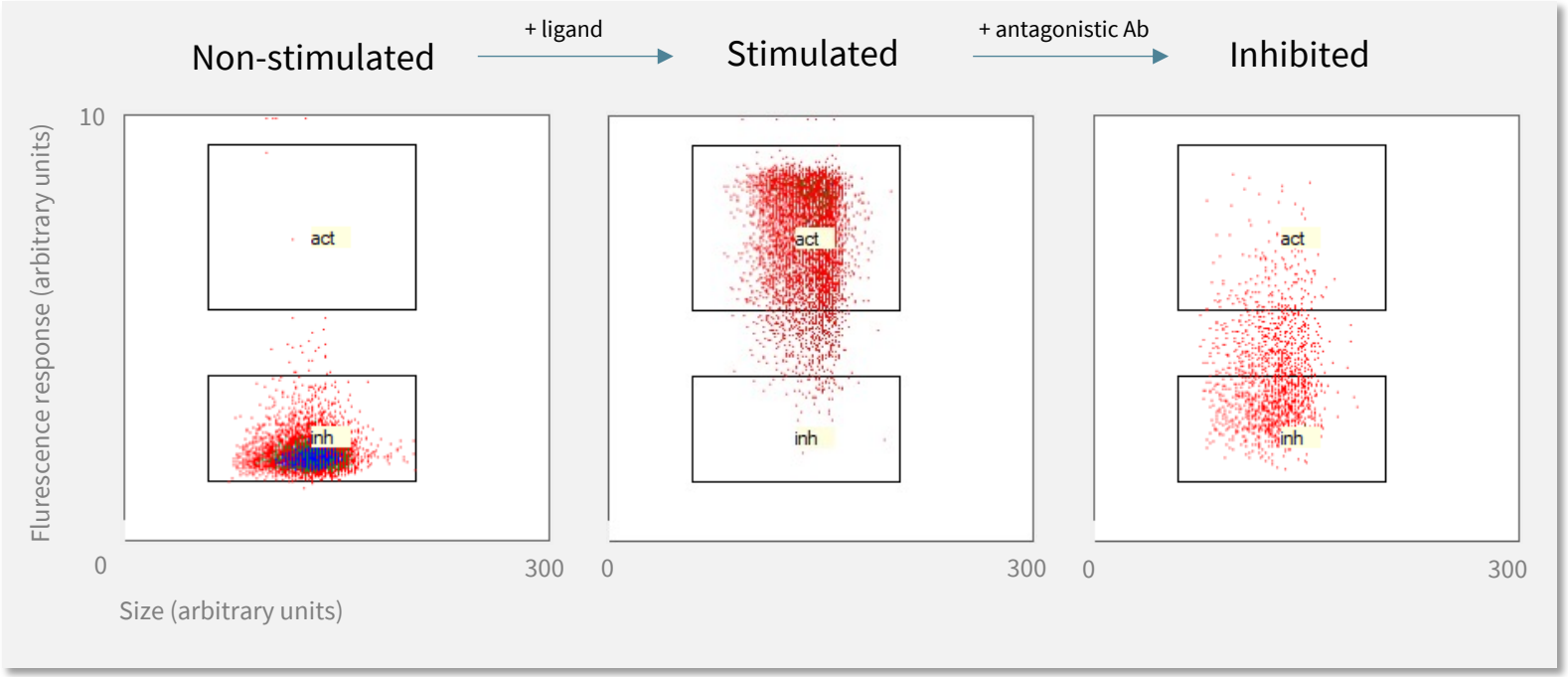
Unoptimized assay

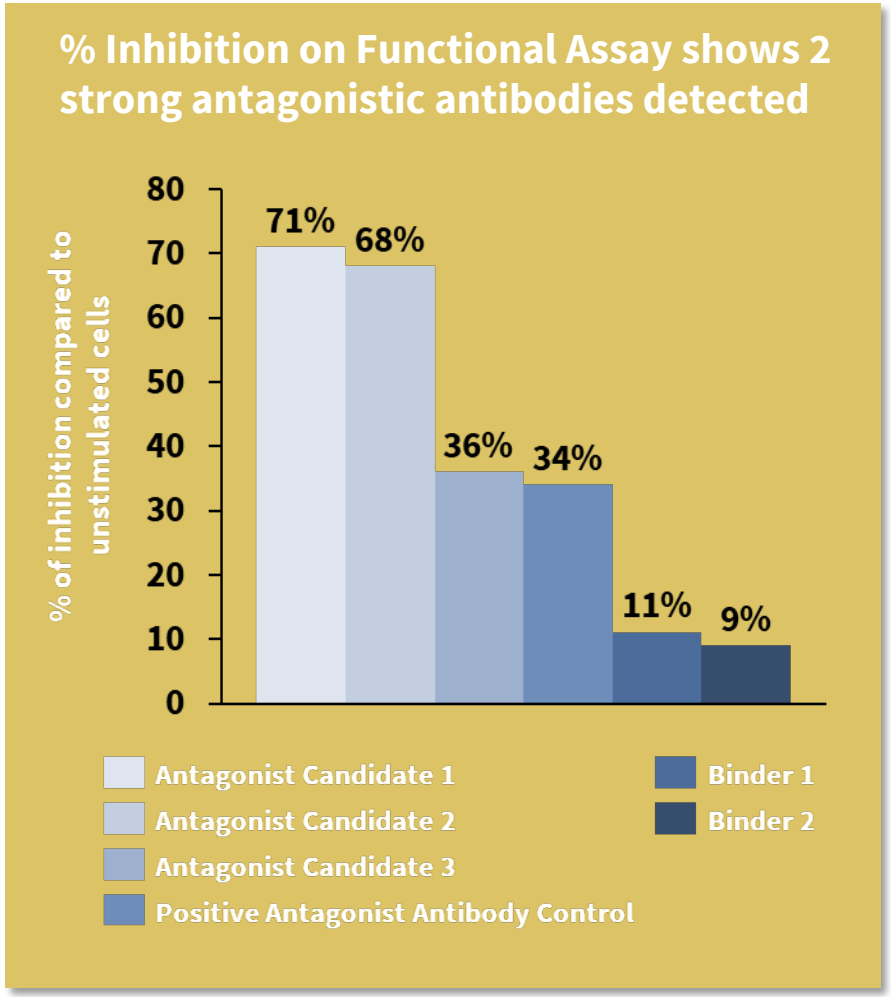


Optimized for screening



Our Microfluidics platform can also be used for antagonistic antibodies by pre-stimulating the target with agonist





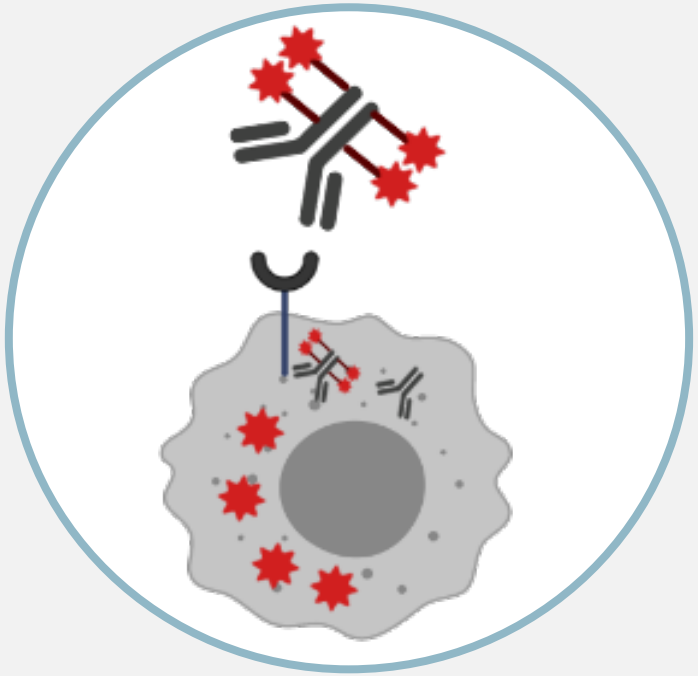
Our Microfluidics platform is designed towards both key requirements for potent ADCs: selectivity and internalization

Repertoire Generation | Screening (Internalization / ADCs) | Lead Selection

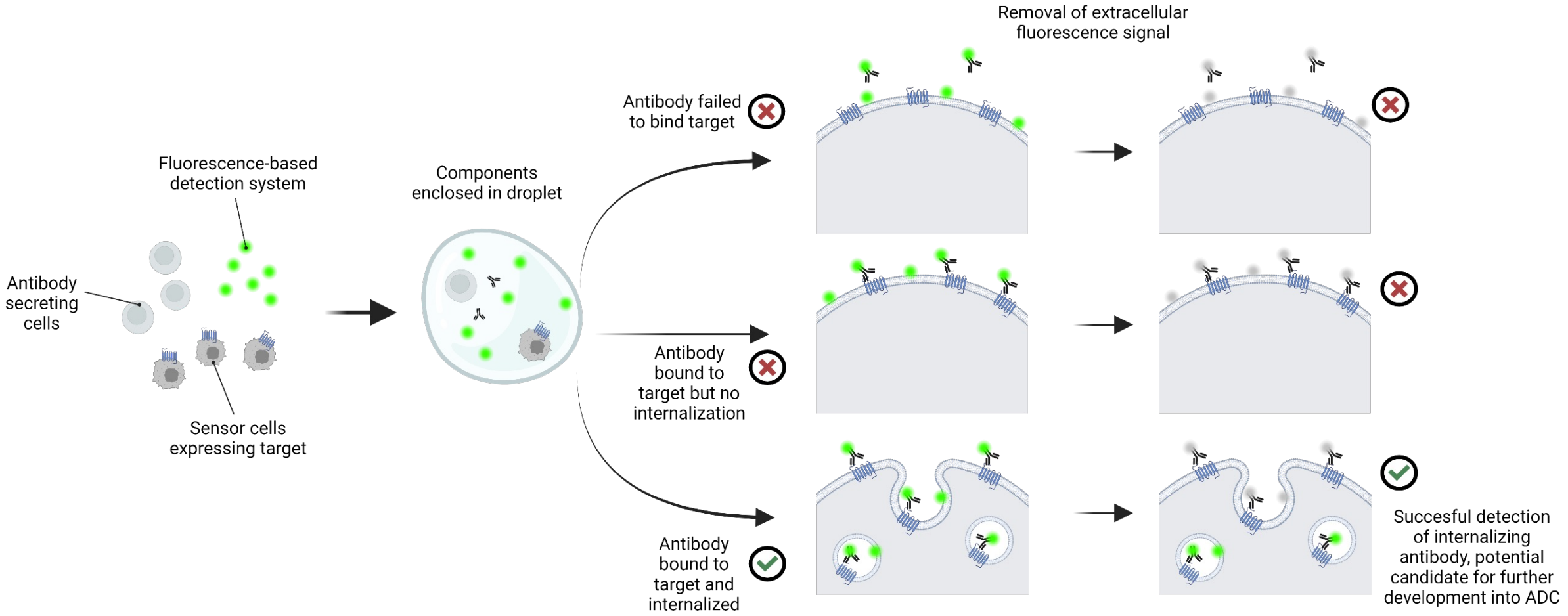
Internalization

Key for ADC discovery:
Antibody-drug conjugates (ADCs) are a recognized potent class of targeted therapeutics. ADCs need to be selective for a given target and also trigger high internalization rates for their functionality.

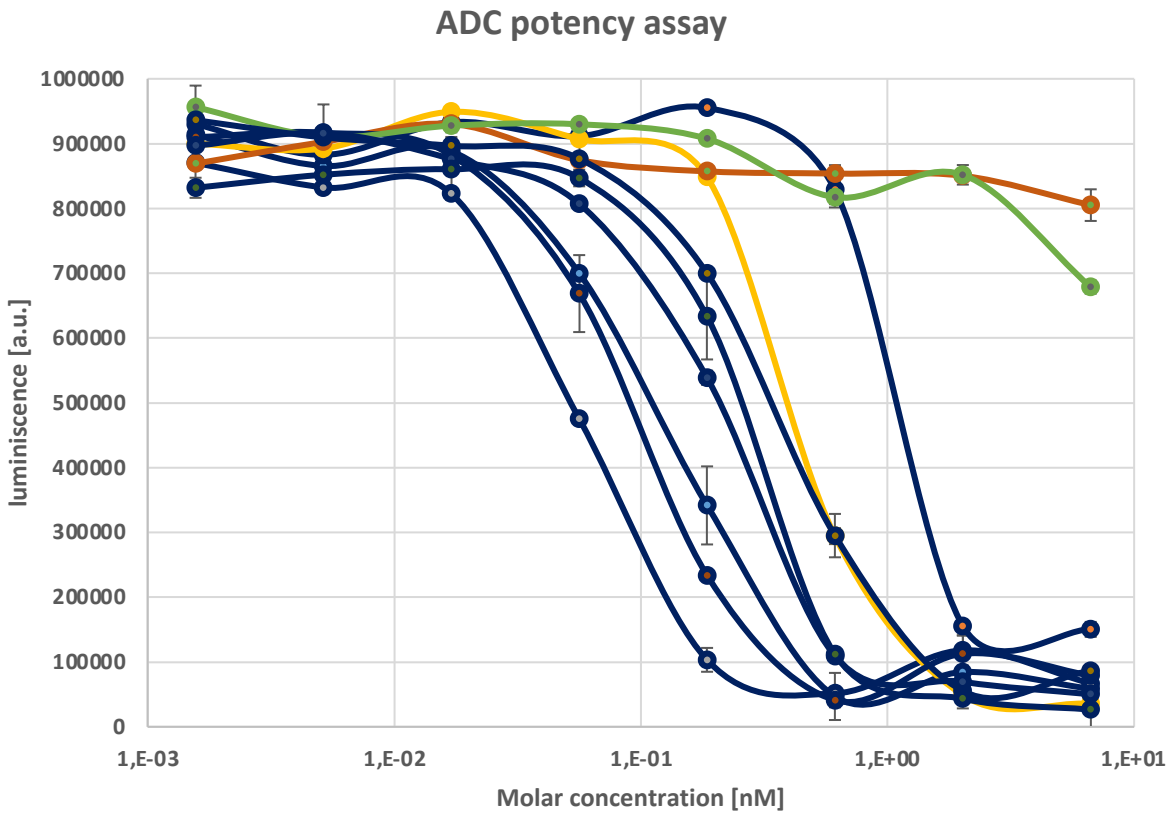
Key Therapeutic Areas:
Oncology, Immunology, Infections



Our Internalization Assay allows for high-throughput screening of internalizing antibodies for ADC development



Our platform was able to detect multiple ADCs with higher potency compared to clinical stage-candidates



Sample	EC50 (nM)
clone 1	0.06
clone 2	0.10
clone 3	0.12
clone 4	0.22
clone 5	0.24
clone 6	0.36
pos.ctrl	0.45
clone 7	0.81
binder	n/a
neg.ctrl	n/a

Positive Control Ab
Clinical-stage ADC

Negative Control Ab

Internalizing Abs
New ADC Candidate

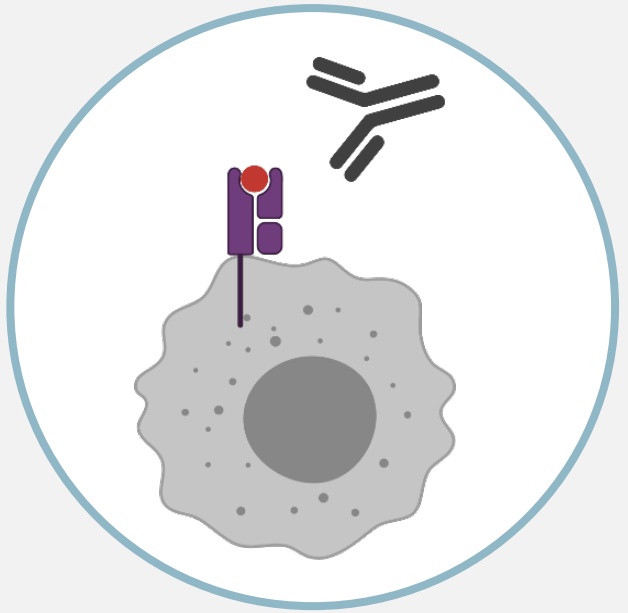
Non-internalizing Ab
Not an ADC candidate

We are also capable of screening antibodies for next-generation tumor therapy targets such as MHC-peptide complexes

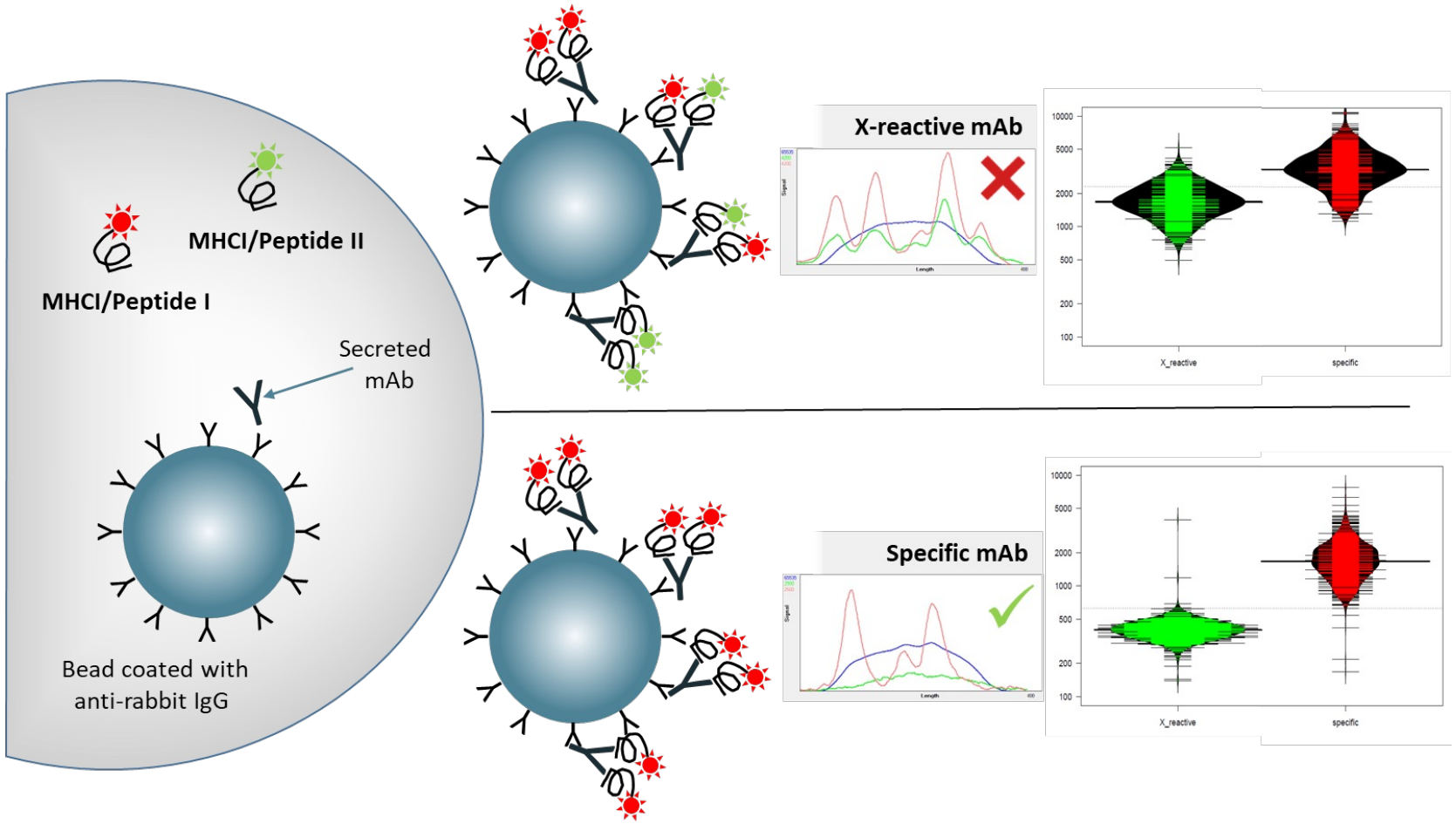
Repertoire Generation | **Screening (Novel Targets)** | Lead Selection

Novel Targets

- MHC complexes display intracellular peptides on the surface of cells and alert the immune system to ongoing tumorigenic processes inside a cell. Antibodies specifically targeting MHC-peptide complexes open up the therapeutic drug target space to otherwise unattainable tumor-specific cytosolic proteins
- AbCheck's microfluidics / dual-staining-based narrow-specificity sorting offers an easy and efficient way of **generating highly specific, high-affinity antibodies to MHC-I-peptide complexes** in our proprietary transgenic rabbit model
- High selectivity in this process can also be used for other targets that require a high degree of selectivity, e.g., **mutated vs. non-mutated receptors**, species cross-reactivity → **significant upside potential**
- AbCheck generated a **transgenic rabbit model** carrying the human MHC-I gene; immunization of the transgenic rabbits with a hMHC-I-peptide-complex induced a **very robust immune response** and **significantly increased specificity** compared to WT



Differential sorting for high-specificity antibodies



Dual staining of distinct antigens enables direct detection of mAbs with high specificity against homologous targets such as MHC-peptide complexes, mutated variants of receptors or orthologues

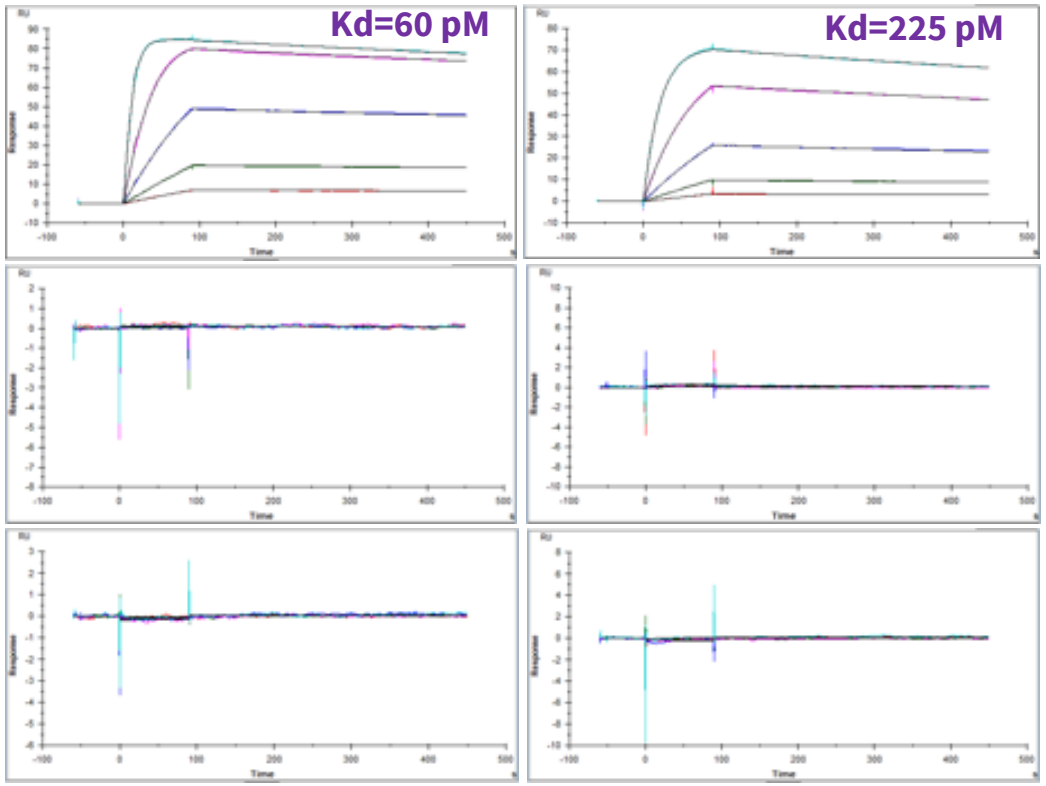
Our platform allows generation of specific picomolar affinity antibodies to MHC-I-peptide complexes

Exemplary specific mAbs

MHCI/ Peptide I (specific)

MHCI/ Peptide II (off target)

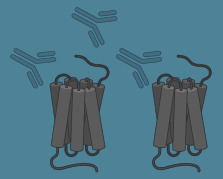
MHCI/ Peptide III (off target)



- AbCheck's microfluidics / dual-staining-based narrow-specificity sorting offers an easy and efficient way of generating highly specific, high-affinity antibodies to MHC-I-peptide complexes in our proprietary transgenic rabbit model
- High selectivity in this process can also be used for other targets that require a high degree of selectivity, e.g., mutated vs. non-mutated receptors, species cross-reactivity → significant upside potential

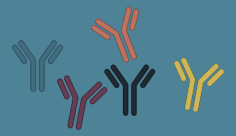
Our platform technology presents an all-in-one solution for current needs to address multiple historically ‘challenging’ targets

REPERTOIRE GENERATION
1-2 months



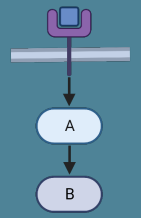
Increased binding affinity
→ *Reduce need for optimization*

+



Diversified repertoire
→ *Increase probability of success for challenging targets*

SCREENING
Days-weeks



Functional screening
→ *Increase hit rate of functional antibodies*

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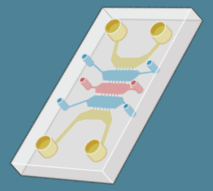

High throughput
→ *Rapid, extensive screening even for challenging targets*

LEAD CHARACTERIZATION
6-8 weeks

Lead Sequence Generation
→ *Cloning, expression, sequencing, and characterization of hits*

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