The Hummingbird Abvantage™

Engineering immune responses for epitope-focused antibody discovery

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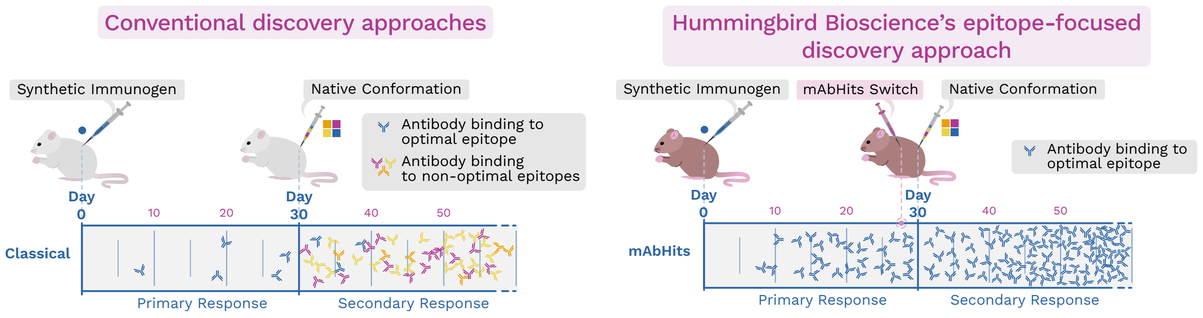
Epitope-focused Rational Antibody Discovery (RAD)

Hummingbird Bioscience's RAD platform uses proprietary AI-enabled algorithms, immunization strategies and Abvantage™ mice to accelerate discovery of epitope-specific, high affinity antibodies

- Conventional discovery methods produce antibody pools of which a large percentage bind to undesirable, non-optimal epitopes on target proteins
- Hummingbird Bioscience's epitope-focused discovery approach yields antibodies that bind specific sites on targets to elicit desired functional outcomes
- The Hummingbird Bioscience RAD platform is an integrated workflow designed to focus antibody responses to optimal functionally-relevant epitopes
- **mAbPredictAI:** AI-enabled design and scaffolding of epitopes aligned to antibody design goals
- **mAbHits:** immunization toolbox to drive affinity maturation of antibodies against optimal epitopes
- **Abvantage™ mice:** proprietary transgenic mice allow temporal modulation of B cell responses



Hummingbird Bioscience's RAD platform enables generation of highly differentiated antibodies against challenging targets with high clinical development potential

- Epitope-focused antibody discovery can unlock complex and difficult drug targets to enable the development of next-generation modalities including ADCs, T-cell engagers, and CAR-Ts
- Multiple RAD-derived therapeutics have entered clinical trials

Applications of Hummingbird Bioscience's RAD platform

Specific Protein Isoforms

HMBD-011 (VH4-34)

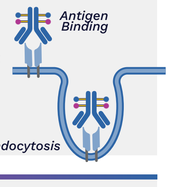
Targets unique motif found in an encoded heavy chain IgG

Out-licensed

Enhanced target internalization

HMBD-803

Binds to epitope which effectively triggers target internalization

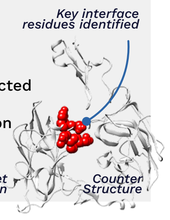


Antagonists

HMBD-002 (VISTA)

Binds a specific site predicted to be essential for ligand-binding and function

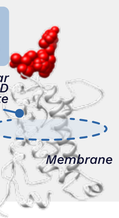
Currently in Phase I



Multi-pass Transmembrane Proteins

D-015

Binds a small extra-cellular domain in a complex target



Agonists

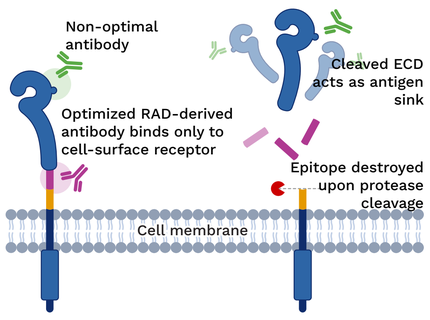
D-013

Mimics binding of conformational epitope spanning two subunits

Case Study I – Surface receptor-specific antibody

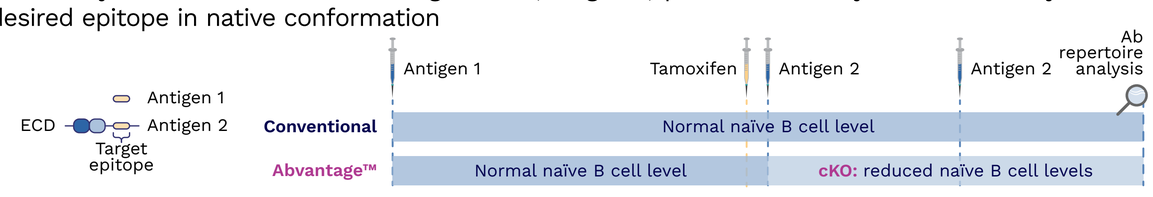
Generating antibodies against epitope in extracellular domain (ECD) of a transmembrane receptor

- Cleaved ECDs present a common antigen sink challenge
- The RAD platform was used to target an epitope at the protease cleavage site in the ECD of the target receptor
- RAD-derived antibodies only bind to cell surface receptors
- Can be adapted to multiple next-generation modalities e.g. ADCs, T-cell engagers, CAR-Ts

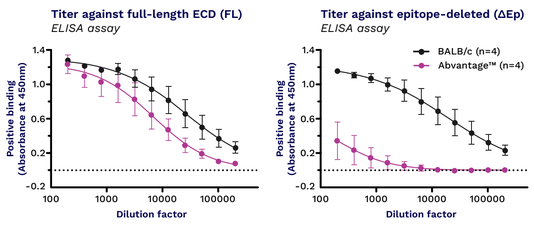


1. RAD campaign

- Primary immunization with synthetic immunogen against epitope at cleavage site in ECD (Antigen 1)
- Injection of tamoxifen in Abvantage™ mice shuts down new primary B cell responses to immunizations
- Secondary immunization with full-length ECD (Antigen 2) promotes affinity maturation only to desired epitope in native conformation



2. Robust epitope-specific titers are obtained in Abvantage™ mice



- *High epitope-differentiated titers are generated only in Abvantage™ mice*
- *Low sera titer against ΔEp proteins indicated inhibition of responses to new epitopes during secondary immunizations with the full-length ECD*

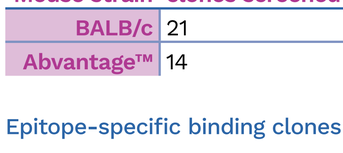
3. Strong lead antibodies with high binding affinity and epitope specificity

Screening of single B-cell derived clones

Mouse strain	Number of clones screened	% Epitope specific
BALB/c	21	0
Abvantage™	14	21

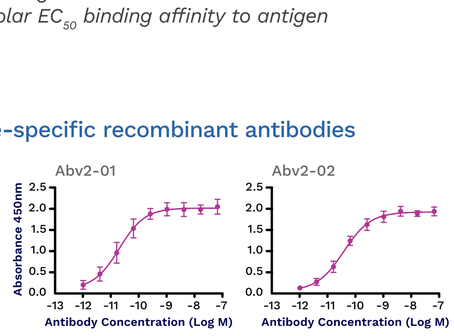
- *High hit rates for epitope-specific antibody clones in Abvantage™ mice*
- *Successful generation of RAD-derived antibodies with nanomolar EC_{50} binding affinity to antigen*

Epitope-specific binding clones



Binding affinity of epitope-specific recombinant antibodies

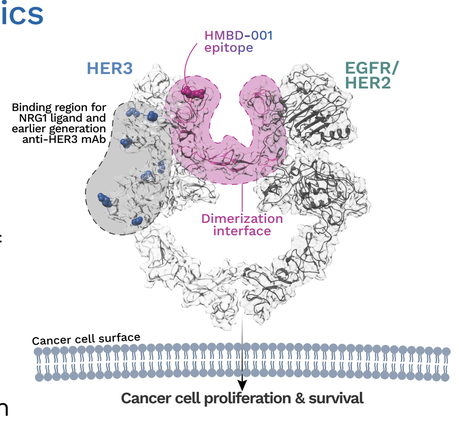
RecAb	EC_{50} (M) *Full-length ECD*
Abv2-01	2.01E-11
Abv2-02	3.81E-11



Case Study II – Anti-HER3 mAb and ADC

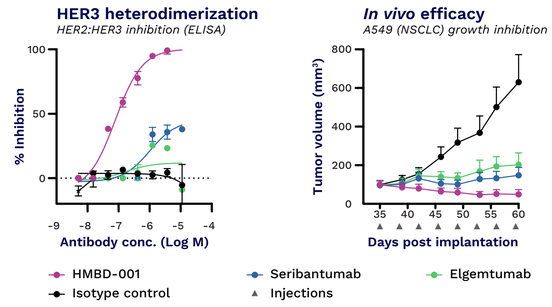
Developing clinical stage anti-HER3 therapeutics

- Anti-HER3 antagonists and ADCs developed with conventional approaches have demonstrated sub-optimal efficacy because of competition with HER3's ligand, or HER3 conformation, occluding binding epitopes
- The RAD platform was used to obtain antibodies that bind a species-conserved epitope in the heterodimerization interface, accessible regardless of ligand or conformation of the HER3 receptor
- Epitope-specific antibody, HMBD-001, blocks heterodimerization induced signaling of HER3, leading to potent tumor growth inhibition
- Epitope-specific ADC, HMBD-501, show optimal binding even in the presence of high concentrations of HER3 ligand, leading to superior tumor growth inhibition

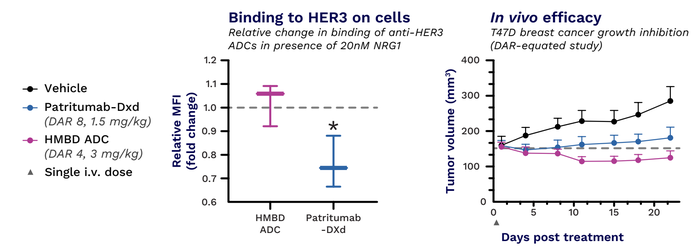


HMBD-001 mAb blocks HER3 heterodimerization and inhibits tumor growth

Binding to HER3 on cells *(assessed by Octet)*	Binding to HER3 in absence of NRG1 (K_d)	Binding to HER3 in presence of NRG1 (K_d)
HMBD-001 (Blocks heterodimerization)	**<100 pM**	**<100 pM**
Seribantumab (Blocks ligand binding)	<100 pM	16.8 nM
Elgemtumab (Blocks conformation change)	0.73 nM	1.92 nM



HMBD-501, anti-HER3 ADC, shows superior binding and tumor suppression in presence of ligand

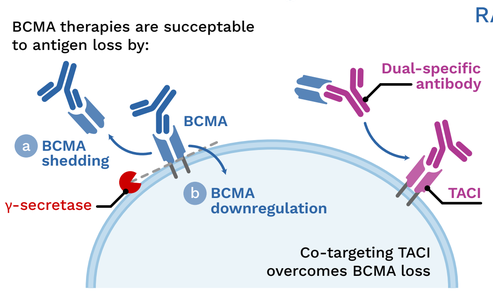


Case Study III – Dual-specific anti-BCMA/TACI x CD3 TCE

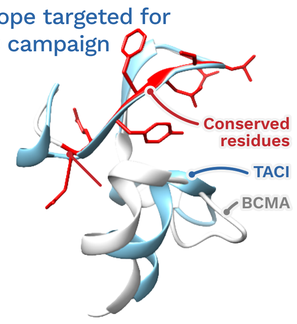
Targeting a common epitope in two proteins

- B cell antigens are restricted to specific B cell types and may also be cleaved leading to antigen loss that limits the efficacy of therapies targeting single antigens
- Targeting two B cell specific antigens, such as the BCMA and TACI receptors, allows for target binding redundancy and broadens binding to more B cell subpopulations
- The RAD platform enabled discovery of a dual-specific monoclonal antibody that binds a 3D epitope conserved on both BCMA and TACI

Mechanisms of BCMA antigen loss



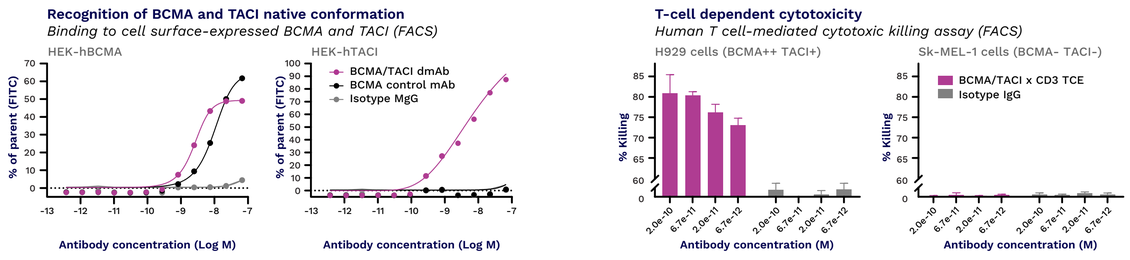
Epitope targeted for RAD campaign



- *BCMA and TACI share a similar 3D structure in the ligand binding domain*
- *mAbPredictAI generated a 3D epitope conserved on both BCMA and TACI for RAD campaign*

Development of dual-specific antibody in T-cell engager (TCE) format

- RAD-derived antibody binds both BCMA and TACI receptors in their native conformation
- Bispecific BCMA/TACI x CD3 antibodies induced potent killing of BCMA- and TACI-expressing cells via T-cell mediated mechanisms *in vitro*



Conclusions

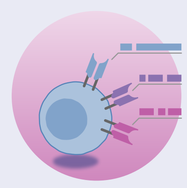
Hummingbird Bioscience's epitope-focused rational antibody discovery platform can unlock a broad range of next-generation antibody-based modalities

mAbPredictAI

AI-enabled design and scaffolding of desired epitopes

mAbHits Abvantage™

Enriched response of antibodies against epitopes of interest via immunization



mAbSelect

Single B-cell screening to identify binders and rapid expression system

- The RAD platform is a well-validated antibody discovery approach that enables the development of ADCs, TCEs, CAR-Ts and other next-generation modalities against hard-to-drug targets
- Multiple RAD-derived therapeutics are currently in clinical development or have been partnered
- Contact bd@hummingbirdbio.com or visit us at www.hummingbirdbioscience.com to learn more about our platform and the Abvantage™ mouse models or collaborative partnership opportunities