# Streamlining T-cell engager

with diverse, fully human CD3-binding antibodies, bispecific engineering technology, and an integrated discovery engine

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REFERENCES 1. S Pessano et al. (1985). The T3/T cell receptor complex: antigenic distinction between the two 20-kd T3 (T3-delta and T3-epsilon) subunits. EMBO J. Feb;4(2):337-44. COPYRIGHT © ABCELLERA

#### THE CHALLENGE

CD3 T-cell engagers have the potential to be a cornerstone of immuno-oncology. However, a limited pool of CD3-binding antibodies and technological challenges in engineering bispecifics have hindered their development.

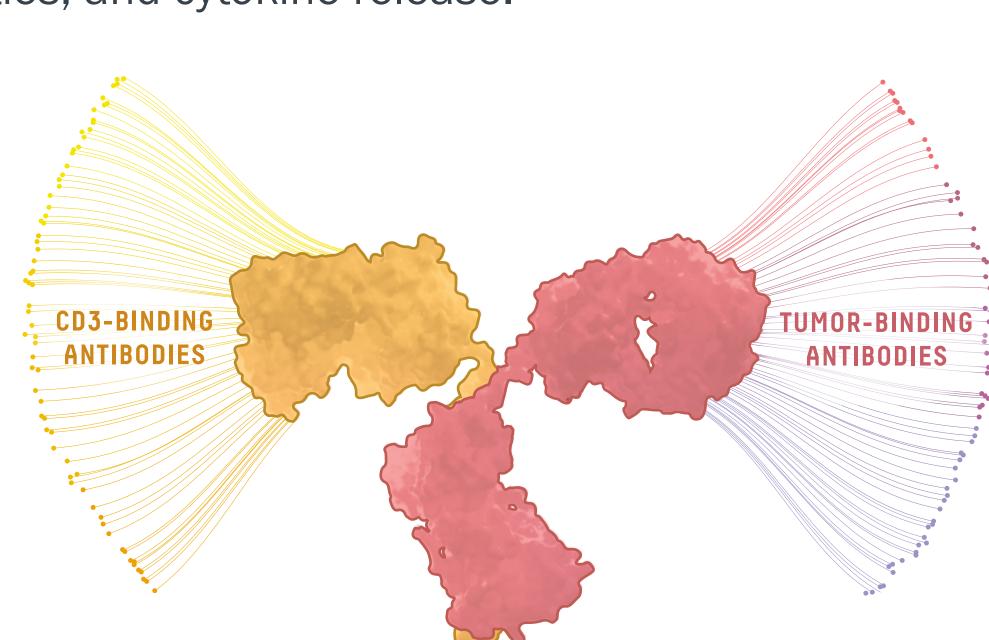
Discovering effective T-cell engagers requires identification of two target-binding arms — a CD3 arm that fine-tunes T cell activation and a tumor arm with high specificity for cancer cells optimized as a whole to work in concert with each other.

The intensity of T cell activation is a key predictor of safety and efficacy and is, in part, determined by the affinity and epitope of the CD3-binding arm. CD3 binders that strongly activate T cells can trigger dose-limiting toxicities, including cytokine release syndrome. On the other hand, CD3 binders that weakly activate T cells can lack potency. The result is a narrow therapeutic window for T-cell engager development.

# THE SOLUTION

Beginning with diverse panels of parental antibodies increases the probability of finding appropriately potent and developable T-cell engagers, reducing the need for downstream engineering to eliminate liabilities and improve functionality.

Our goal was to develop a large and diverse panel of fully human CD3-binding antibodies and partner to discover T-cell engagers with optimized specificities, potencies, biophysical properties, and cytokine release.



## THE OUTCOME

CD2 hinding antibodies that

A panel of fully human CD3-binding antibodies that are:					
DIVERSE	DEVELOPABLE	VALIDATED			
NINE different epitope bins	FAVORABLE biophysical properties	CD3 x EGFR proof-of-concept T-cell engagers			
<ul> <li>A range of CD3         affinities (K<sub>D</sub> from         ~1 nM - 1 µM)</li> <li>A broad range of T</li> </ul>	<ul> <li>Including high thermal stability and low hydrophobicity, self-association,</li> </ul>	<ul> <li>A broad range of</li> <li>T cell activation</li> <li>potencies (EC<sub>50</sub> from</li> <li>pM - 2 nM)</li> </ul>			
cell activation potencies (EC <sub>50</sub> from ~6 - 200 nM)	and polyspecificity	<ul> <li>A broad range of tumor cell killing potencies (EC<sub>50</sub> from ~0.01 - 1 nM)</li> </ul>			
<ul> <li>Multiple epitope bins containing human and cyno cross- reactive binders</li> </ul>		<ul> <li>Potent molecules that achieve &gt;90% cell killing with low cytokine release</li> </ul>			

All characterization data shown are for the subset of total antibodies that have been expressed and analyzed to date.

## DIVERSITY

- Hundreds of unique human antibodies with sequence diversity.
- Diverse antibody sequences from mature immune responses in humanized mice

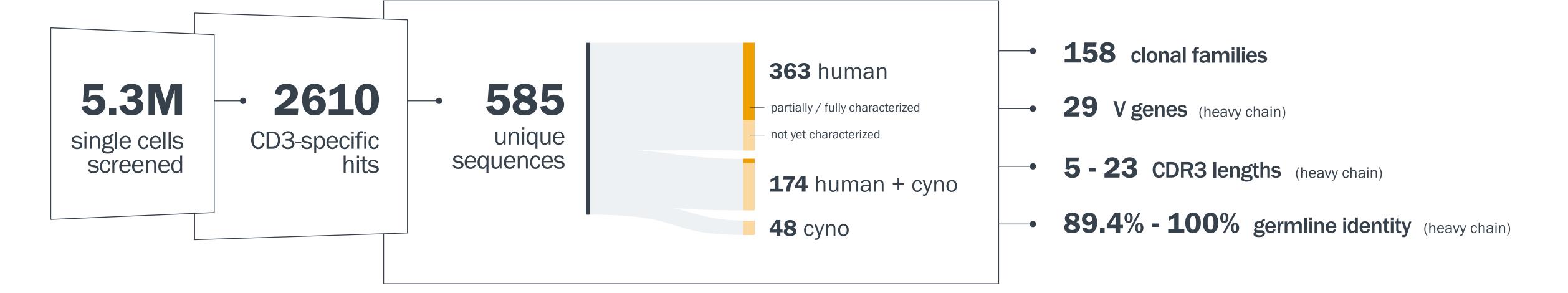


Figure 1. Proprietary immunization strategies using intact human and/or cyno CD3 complexes in humanized mice enriched antibody diversity from the start. CD3-specific antibody sequences had diverse V gene usage, CDR3 lengths, and identity to germline. Antibodies with specificities to human CD3, cyno CD3, and human + cyno CD3 were recovered.

- CD3-specific antibodies with binding and functional diversity.
  - Diverse CD3 affinity

KD from  $\sim$ 1 nM - 1  $\mu$ M

- Diverse target engagement times
- Broad T cell activation potencies A range of on/off rates EC<sub>50</sub> from ~6 - 200 nM

 $Log_{10}(K_D)(M)$ 

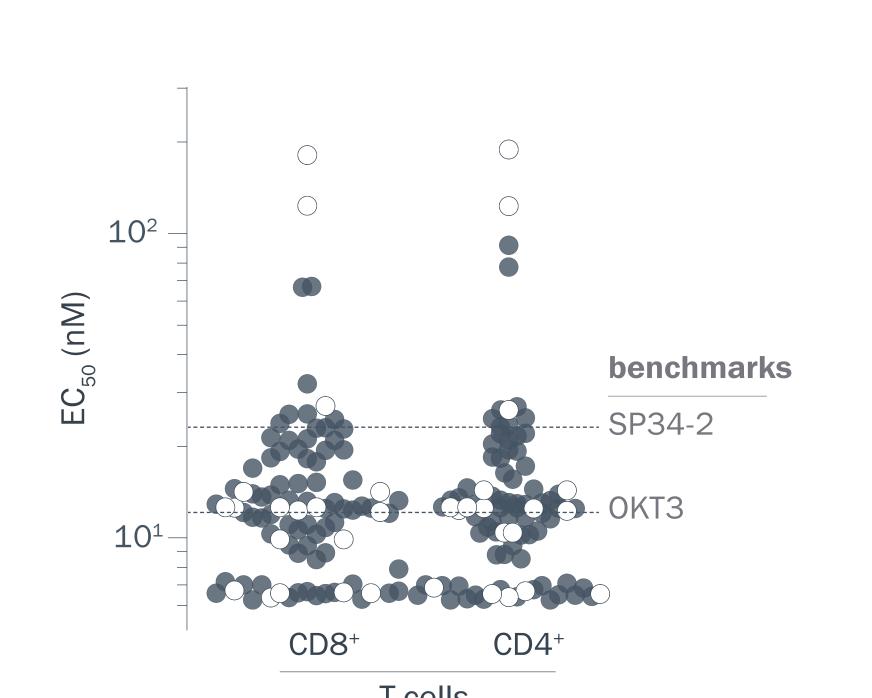


Figure 2. High-throughput antibody expression and analysis showed diverse affinites, target engagement times, and T cell activation potencies. (A) Surface Plasmon Resonance (SPR)-coupled kinetics analysis of antibody binding affinity to human CD3  $\epsilon\gamma$  and  $\epsilon\delta$  subunits.

(B) SPR-coupled kinetics of antibody binding to the human CD3 εδ subunit.

10-6 10-5 10-4 10-3 10-2 10-1 100

Off rate  $K_d$  (s<sup>-1</sup>)

(C) T cell activation (CD25 and CD69 upregulation) measured by flow cytometry. Naive primary human T cells were incubated with plate-bound anti-CD3 antibodies for 24 hours. Values are the concentration of each antibody needed to induce 50% of the maximal activation of primary human CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

• 9 epitope bins, 4 containing cross-reactive binders from the antibodies characterized to date

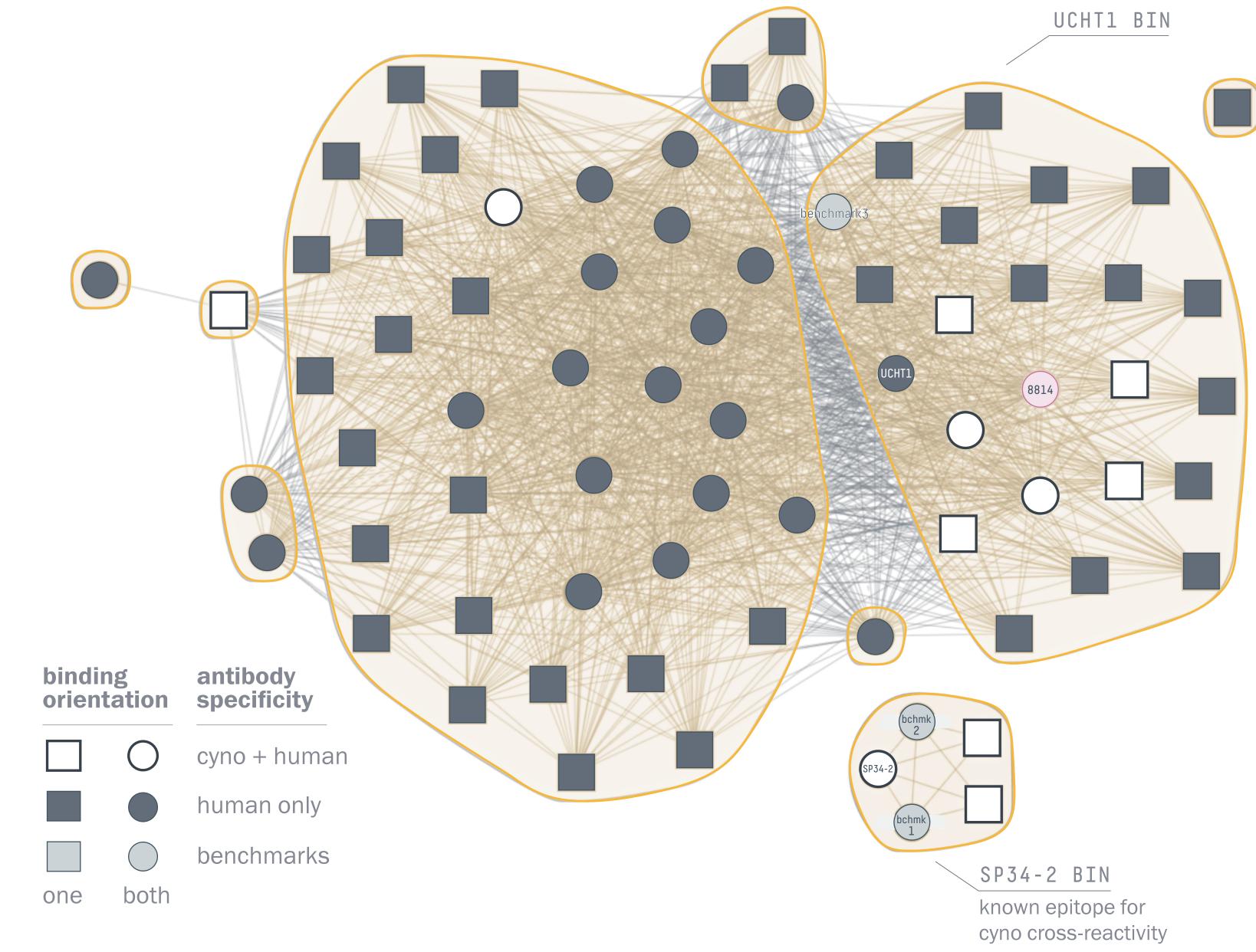


Figure 3. High-throughput SPR epitope binning of CD3-binding antibodies revealed nine epitope bins. Intibodies with cross-reactivity to human and cyno CD3 were found in four epitope bins, three of which were distinct from the SP34-2 bin. UCHT1 and SP34-2 are characterized, commercially available CD3-specific antibodies.

#### **MOST CD3 T-CELL ENGAGERS ARE DERIVED FROM SP34-2**

#### Finding novel CD3 binders is hard.

The CD3 complex on T cells is a challenging target. Finding antibodies that bind both human and cyno CD3 adds another layer of complexity due to the relatively low sequence similarity (~81%) between them.

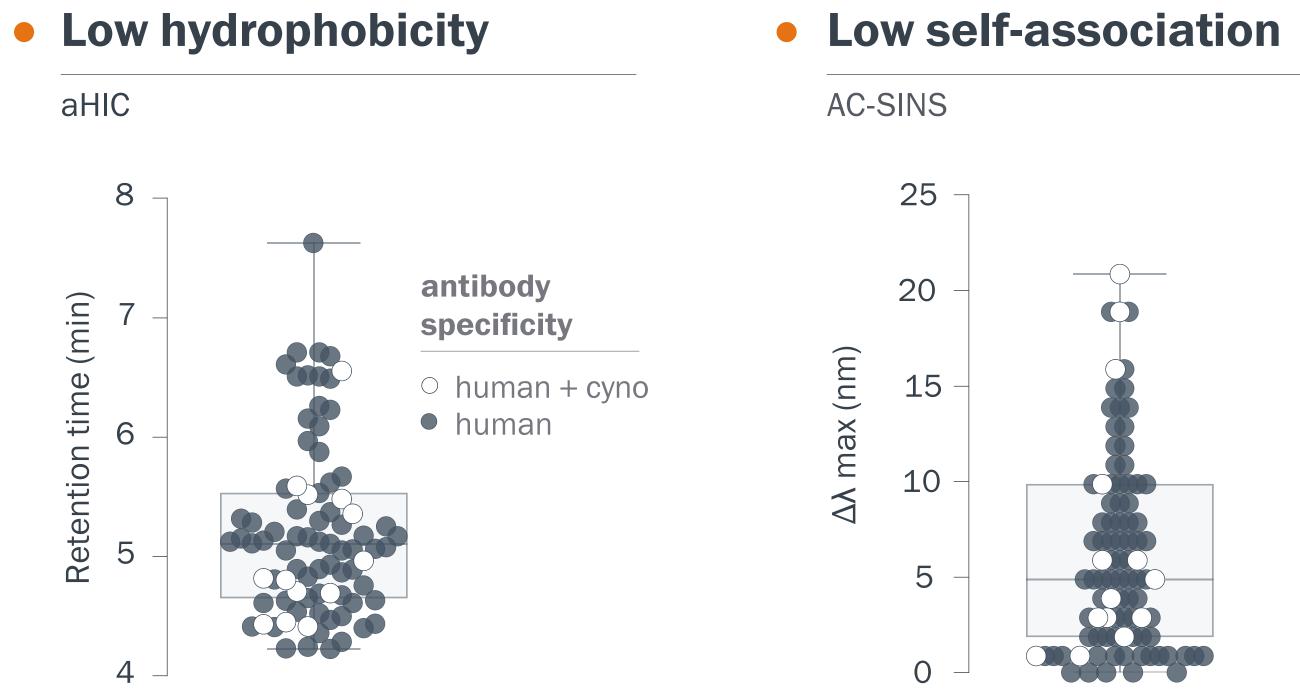
As a result, many drug developers have relied on the limited number of publicly disclosed human + cyno cross-reactive antibodies, with SP34-2 being the most common.

#### Commonly used CD3 binders have limitations.

SP34 was discovered in 1985 from mice immunized with SDS-denatured protein (1). It binds to a linear epitope on the N-terminus of the CD3 ε protein, leaving most of the conformational epitopes of the complex unexplored. In addition, the antibody is often heavily modified to mitigate liabilities. Novel, cross-reactive, fully human anti-CD3 antibodies that bind diverse epitopes greatly expand opportunities for development of CD3 T-cell engagers, fine-tuned for function.

## DEVELOPABILITY

## Scalable antibodies with favorable biophysical properties.



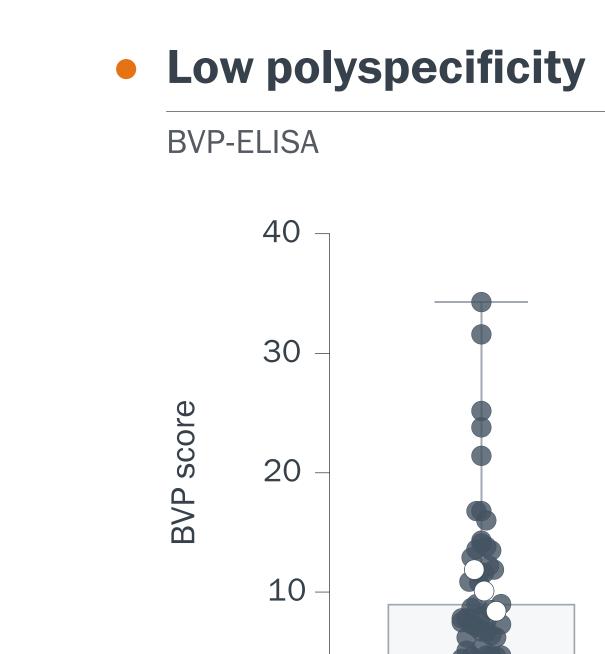


Figure 4. Biophysical analysis of CD3-specific antibodies revealed favorable developability properties. (A) Relative surface hydrophobicity Interaction Chromatography (aHIC). (B) Self-association assessed by Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS). (C) Polyspecificity assessed by Baculovirus Particle Enzyme-Linked Immunosorbent Assay (BVP-ELISA).

## VALIDATION

### ■ CD3-specific antibodies validated as proof-of-concept CD3 x EGFR bispecifics using OrthoMab.



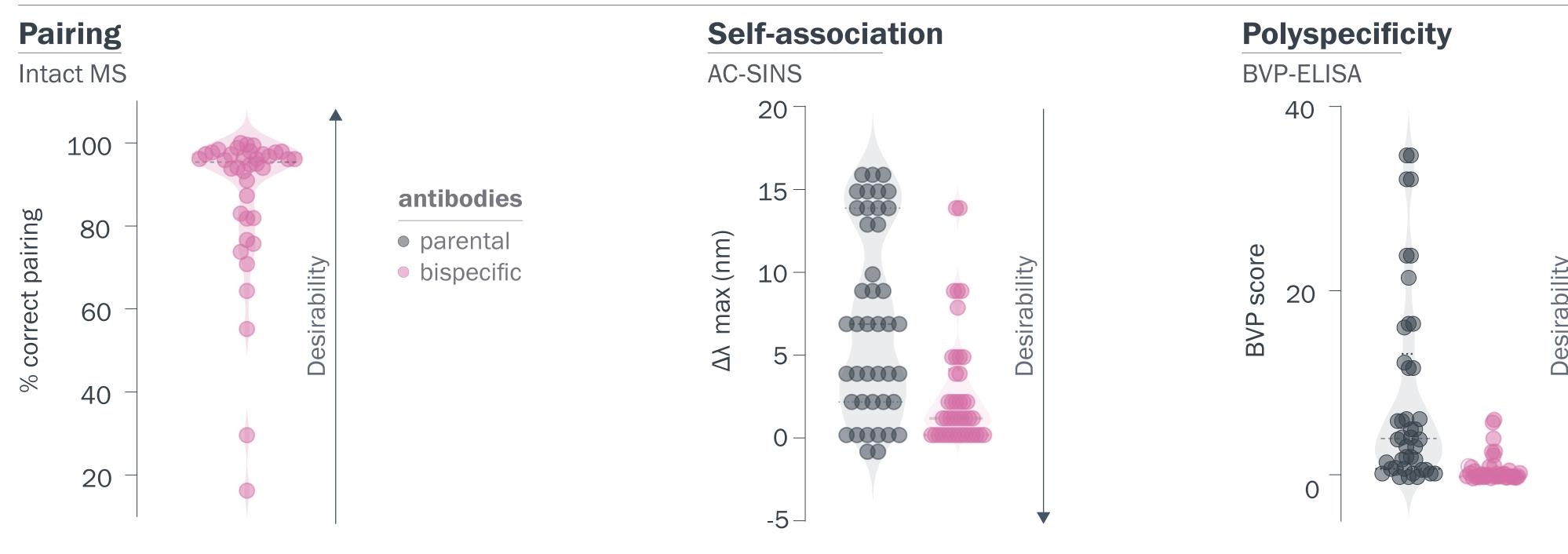


Figure 5. Biophysical analyses showed developability properties comparable to CD3-specific parental antibodies.

Bispecific IgG
POWERED BY OrthoMab™

The fractional percentage of correctly paired vs. incorrectly paired bispecifics was measured by mass spectrometry (MS). Self-association and polyspecificity were measured by AC-SINS and BVP-ELISA, respectively. Stability (measured by nano differential scanning fluorimetry) and aggregation (measured by aSEC) also remained comparable to CD3-specific parential antibodies (data not shown).

Broad T cell activation potencies

0.01 -

0.001

 $EC_{50}$  from ~2 pM - 2 nM

Figure 6. Functional analysis of CD3 x EGFR

bispecific antibodies showed broad potencie

antibody needed to induce 50% of the maxim

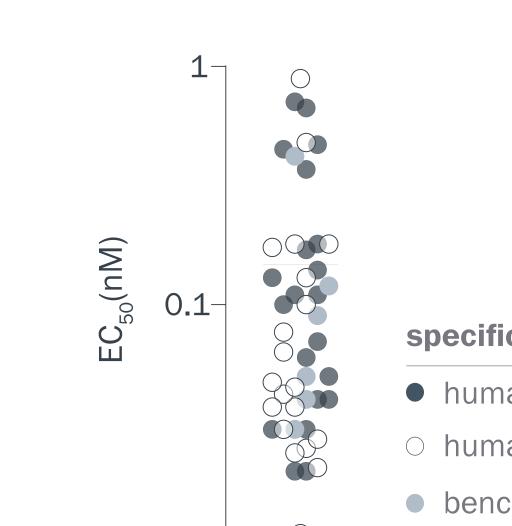
(A) The concentration of each bispecific

activation of NFAT reporter Jurkat T cells.

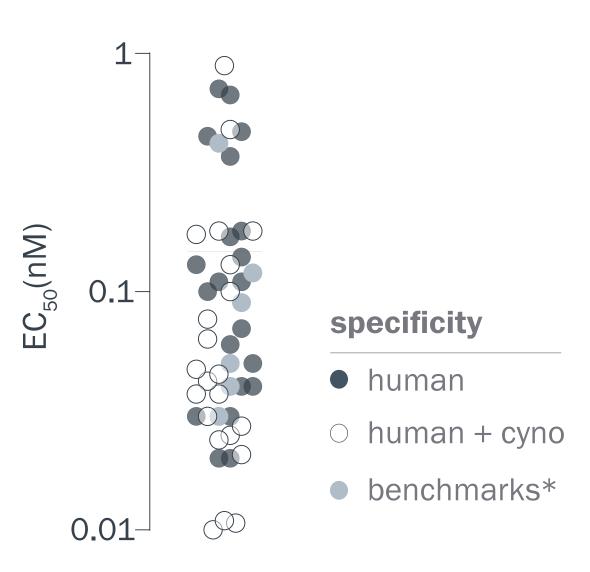
of EGFR for 6 hours at a ratio of 7:1.

Effector T cells were incubated with target

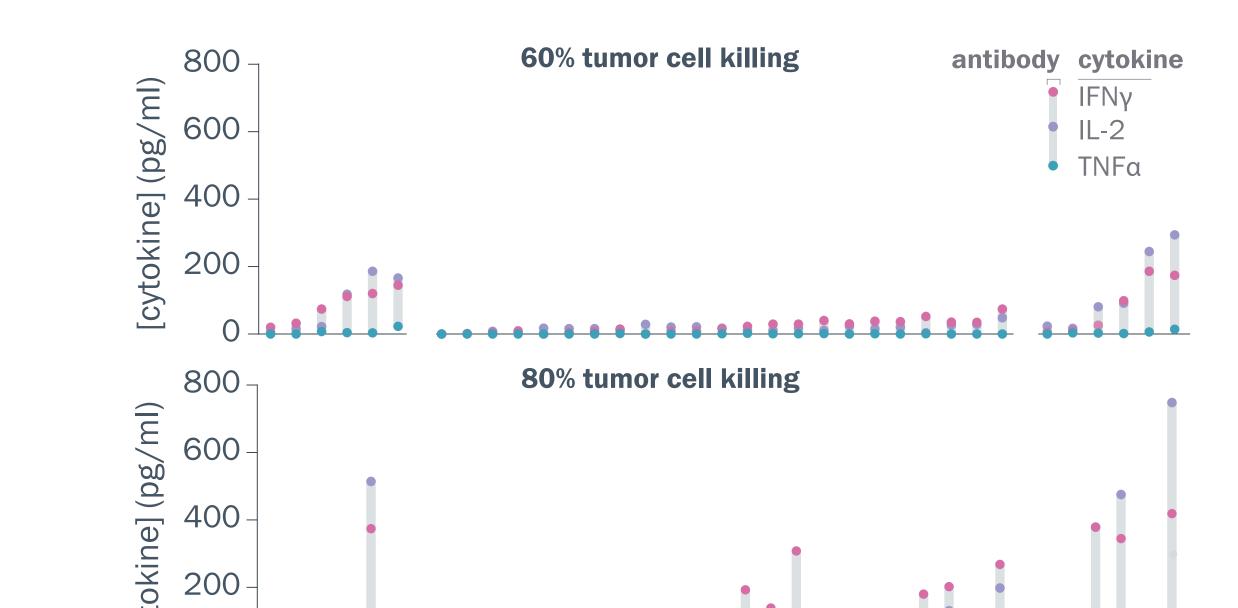
CHO-K1 cells expressing low or medium levels



Broad tumor cell killing potencies EC<sub>50</sub> from ~0.01 - 1 nM



(B) The concentration of each bispecific antibody needed to induce 50% of the maxima T cell-mediated killing of HeLa cells. Unactivated human T cells were incubated with target cells at a ratio of 10:1. Cell killing was measured at 48 hours using an xCELLigence assay.



Potency [bsAb] 0.02 - 5 nM at 60% | 0.07 - 5 nM at 80% tumor cell killing

A range of cytokine release

#### Figure 7. Functional analysis revealed potent bispecific antibodies with minimal cytokine release. In the same assay described in Figure 6B, cytokines were measured after 48 hours of incubation. Cytokine concentrations were calculated for each

antibody at 60% and 80% HeLa cell killing.

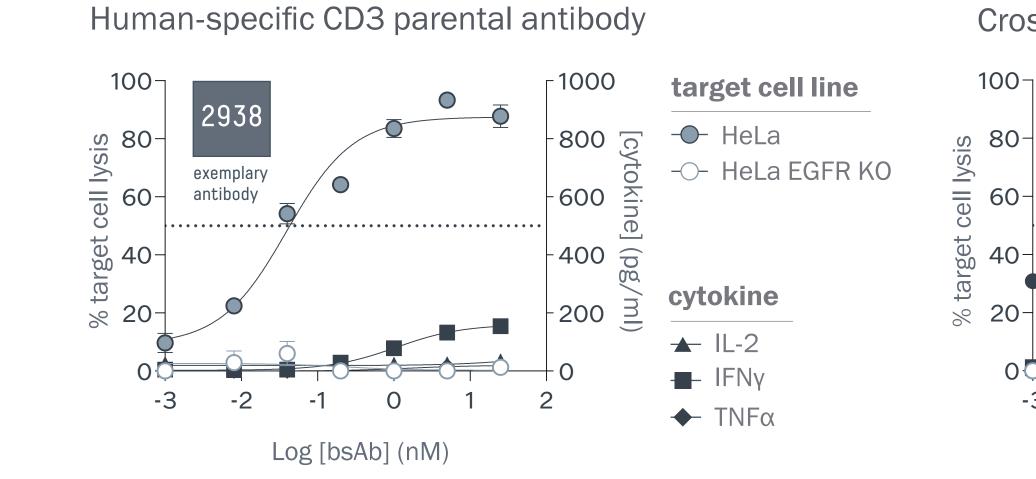
#### Proof-of-concept CD3 T cell engagers that strike the right balance.

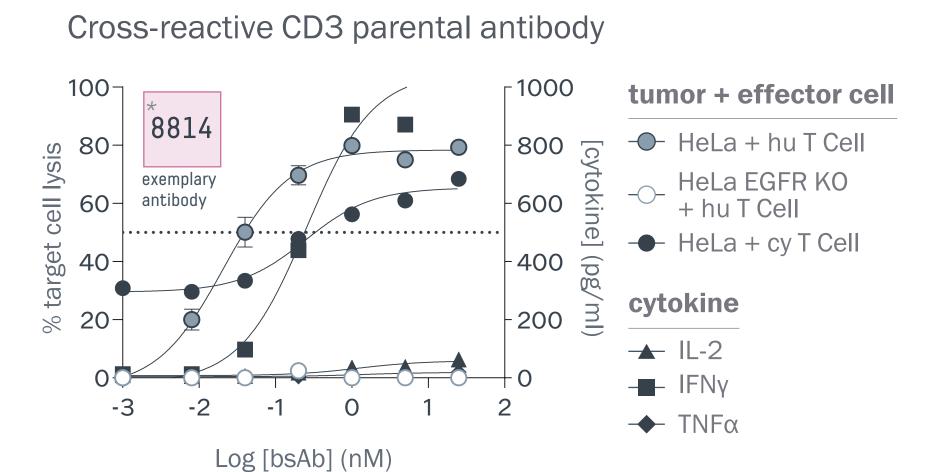
#### Potent tumor cell killing and low cytokine release

human

human + cyno

benchmarks\*





\*Parental antibody in epitope bin distinct from SP34-2

Figure 8. Pairing our CD3 panel with our bispecifics platform identified T-cell engagers that balance potency and cytokine release. Profiles of (A, left) a human-specific CD3 x EGFR tumor cell killing and minimal cytokine release.

	T cell activation (EGFR <sup>MED</sup> )	Tumor cell killing	<b>Affinity</b> (εδ CD3)
human + cyno 8814	EC <sub>50</sub> 15.1 pM	EC <sub>50</sub> 20.7 pM (human T cells)	K <sub>D</sub> 0.99 nN
3311		EC <sub>50</sub> 249 pM (cyno T cells)	
human 2938	EC <sub>50</sub> 23.4 pM	EC <sub>50</sub> 39.5 pM (human T cells)	K <sub>D</sub> 351 nN