# NOVEL ANTI-CD3 HEAVY CHAIN-ONLY ANTIBODIES FOR USE IN T CELL-ENGAGING THERAPEUTICS

ADIMAB

*Paul Khalifé, Hannah C. Watkins, Elizabeth A. McGurk, Elizabeth R. Parker, Cameron M. R. Henkel, Todd M. Boland, Hailey M. Heston, Whitney E. Renaud, Christin R. Strong, Jingfu Zhao, Carissa A. Metzger, Irina Burnina, Michael E. Brown, Jessica P. Dawson, Andrew D. Avery, C. Garrett Rappazzo, Beth H. Sharkey, Morgan B. Morrill, Cory L. Ahonen, Michael B. Battles, and Noel T. Pauli*

## BACKGROUND

T cell-engagers (TCEs) utilizing CD3 are an increasingly validated class of multispecific antibodies that have shown promise in treating oncologic conditions. From *in vivo* murine discovery followed by humanization and yeast-based optimization, we have generated a broad affinity anti-CD3 IgG panel with excellent developability properties and tunable potency[1].

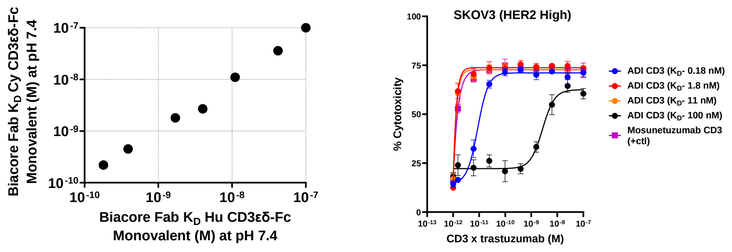


**Figure 1. Adimab's anti-CD3 conventional IgG lineage has human-cyno cross-reactivity across a broad range of affinities, binds to the N-terminal portion of CD3ε, and shows potent killing function as a TCE as exemplified in a T cell dependent cellular cytotoxicity (TDCC) assay on SKOV3 (HER2 high) with anti-CD3 x anti-HER2 bispecific antibodies[1].**

While current TCEs demonstrate great clinical efficacy, their molecular complexity is a challenge for drug manufacturability, developability, and obtaining desirable PK/PD properties. Heavy chain-only antibodies (HCAbs), which use single-domain (VHH) as binding moieties, are emerging as attractive alternatives across therapeutic areas due to their unique structural properties. Notably, the absence of a light chain can simplify multispecific development[2]. Here, we report the discovery and engineering of a panel of anti-CD3 HCAbs, providing a versatile platform to explore new formats and modalities within the TCE space.

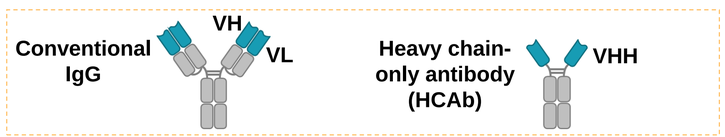


**Figure 2. Comparison of conventional IgG to HCAb**

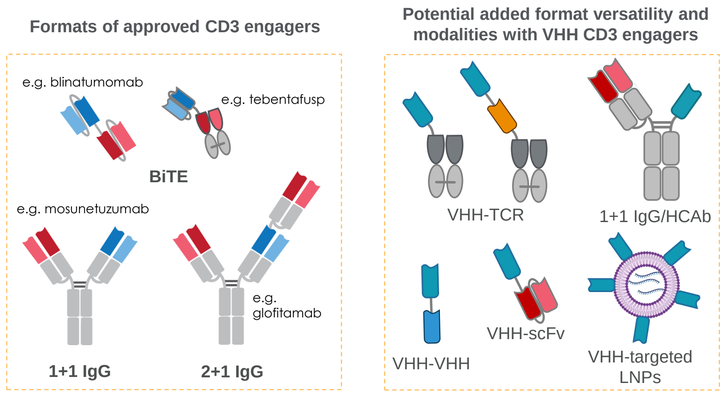


**Figure 3. Advancing TCEs: From IgG to VHH-Based Formats**

## ADIMAB'S LLAMA-DERIVED HCAB DISCOVERY PLATFORM

Camelids naturally produce two classes of antibody: conventional (HC+LC) and HCAbs (HC-only)[3]. Adimab's immunization expertise, combined with its proprietary yeast-based immune library platform facilitates discovery of llama-derived HCAbs against complex targets.

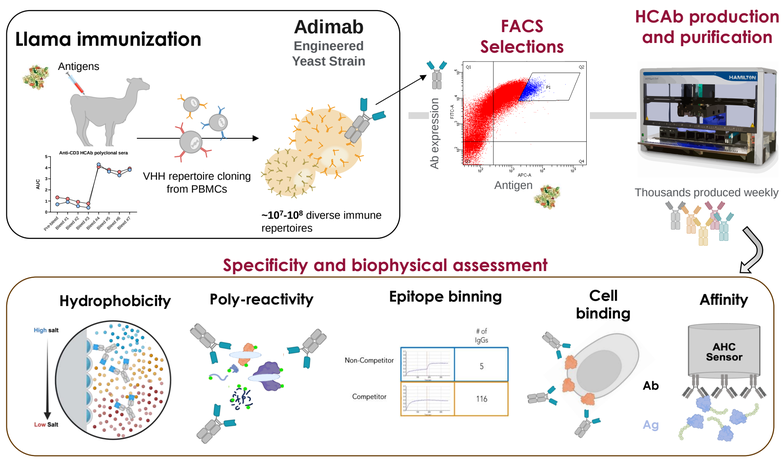


**Figure 4. Adimab's llama HCAb discovery platform**

## SELECTION ROUNDS PERFORMED TO ISOLATE ANTI-CD3 SPECIFIC HCABs

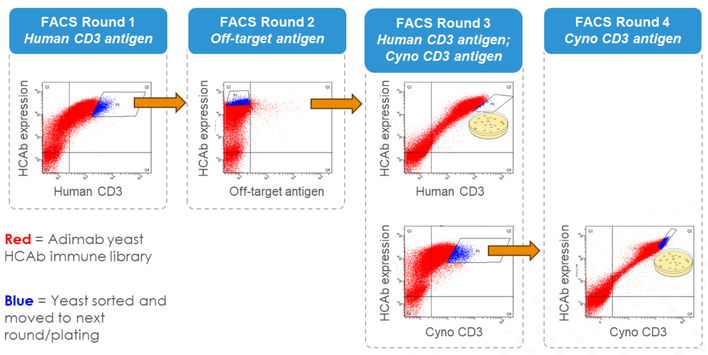


**Figure 5. Multiple rounds of selections were performed on the Adimab yeast immune libraries to isolate anti-CD3 HCAbs with desirable properties. Selections were performed to push for cyno cross-reactivity, wide affinity range, and favorable developability. *Note: figure displays only one representative selection*.**

## IMMUNE LIBRARY SELECTIONS DISCOVERED HUNDREDS OF ANTI-CD3 HCABs

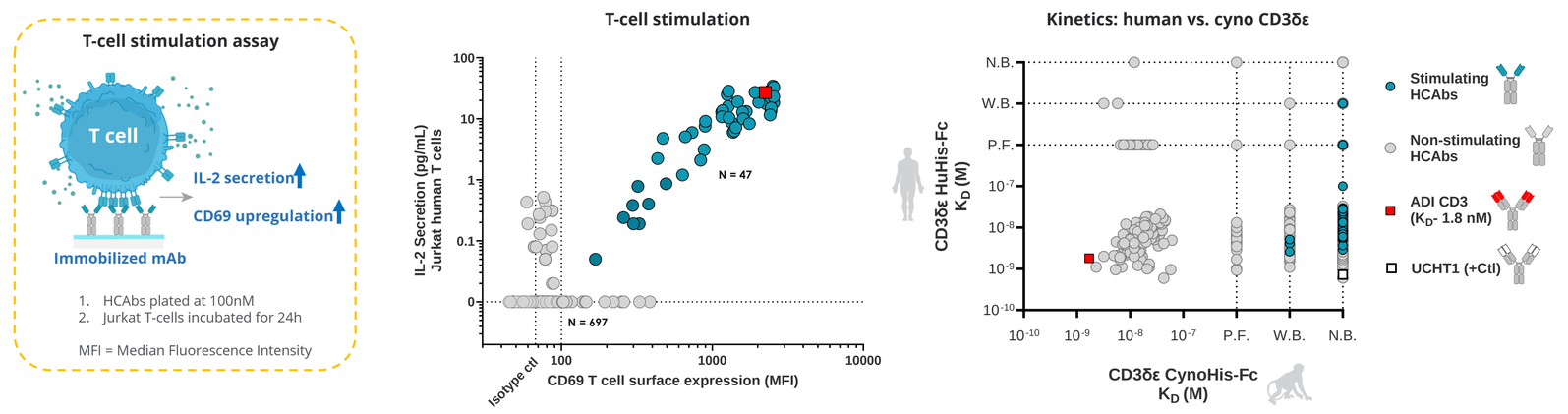


**Figure 6. Isolated HCAbs screened for activity in IL-2 secretion/CD69 upregulation T cell stimulation assay and for affinity. While many human x cyno anti-CD3 binding HCAbs were isolated, stimulating HCAbs showed only human-specific binding. P.F. = poor fit; W.B.= weak binding; N.B. = no binding.**

## LLAMA ANTI-CD3s SHOW POTENT ACTIVITY IN BISPECIFIC FORMAT

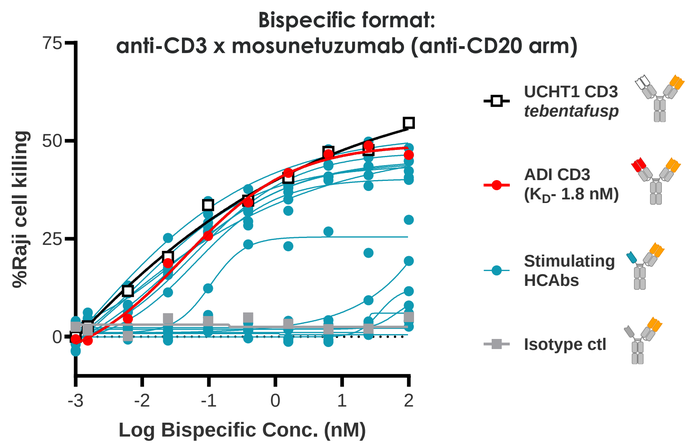


**Figure 7. Stimulating HCAbs were downselected, reformatted in Chain Exchange vectors, and produced as anti-CD3 HCAb x anti-CD20 bispecific antibodies (bsAbs). anti-CD3 x anti-CD20 bsAbs were evaluated in TDCC assays resulting in cytotoxicity against target Raji (CD20+) cells.**

## HUMANIZED CLONES MAINTAIN ABILITY TO STIMULATE T CELLS

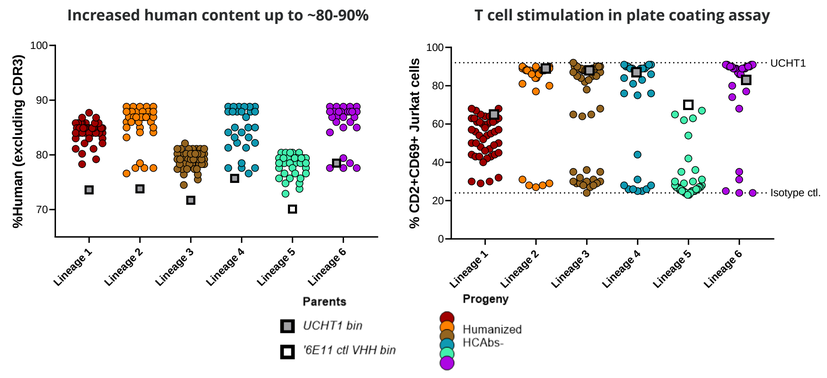


**Figure 8. One anti-CD3 llama-derived HCAb lead was selected for humanization from each of the six lineages of HCAbs that elicited killing in anti-CD3 x anti-CD20 bispecific format in the TDCC assay. Five of the leads competed with UCHT1 (basis for the CD3 arm of tebentafusp) and one of the leads competed with T0170PMP060E11 ('6E11). The leads underwent a humanization process, with ~35 humanized progeny designed and cloned for each parent. Humanized antibodies retained the ability to stimulate T-cells and cause upregulation of CD2 and CD69.**

## HUMANIZED ANTI-CD3 HCAB LEADS EXHIBIT EXCELLENT DEVELOPABILITY PROFILES WHILE SHOWING DIVERSE PHENOTYPIC BEHAVIORS

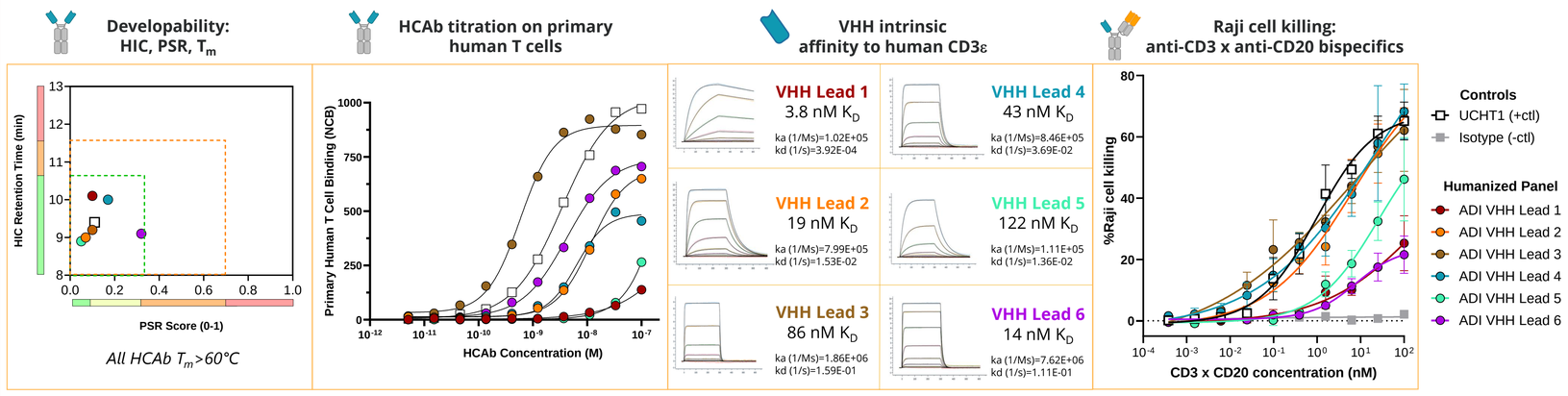


**Figure 9. Top humanized progeny from each of the six lineages were selected to generate an anti-CD3 HCAb panel. These leads exhibited favorable developability properties—low binding to polyspecificity reagent (PSR)[4], low HIC retention times (hydrophobicity assessment), and melting temperatures ($T_m$) above 60°C. Leads demonstrated a broad spectrum of cell-binding characteristics and affinity kinetics. In bispecific anti-CD3 x anti-CD20 format, all leads showed cancer cell killing activity, with some lineages comparable to UCHT1 x anti-CD20 bsAb. NCB = Normalized Cell Binding; $K_D$ = Dissociation constant; ka = Association rate constant; kd = Dissociation rate constant.**

## TOP ADI VHHs SHOW TEBENTAFUSP-LIKE POTENCY IN TDCC ASSAY; WHICH REVEALS ANTI-CD3 ARM AND GEOMETRY SENSITIVITY IN pHLA-TARGETING TCEs

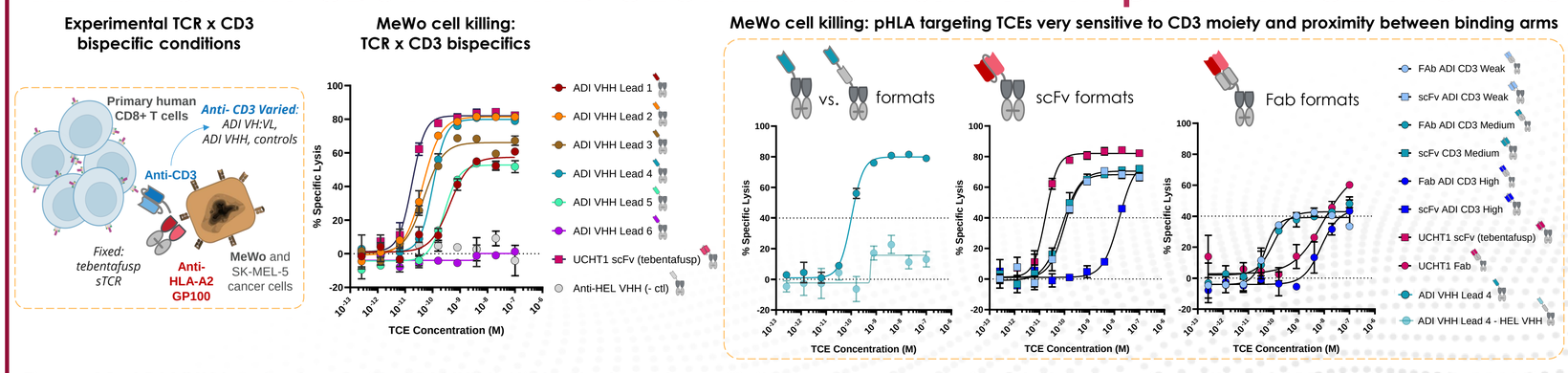


**Figure 10. ADI VHH leads along with ADI VH:VL High, Medium, Low affinity panel and a set of controls were reformatted in TCR (Anti-HLA-A2 GP100) x anti-CD3 bispecific fusion molecules, produced in transient CHO cells, and evaluated in TDCC assay. Five of the leads in TCR x VHH format showed MeWo and SK-MEL-5 cancer cells (not shown) killing activity, with some lineages demonstrating tebentafusp-like potency. A significant loss in potency was observed when separating away anti-CD3 and pHLA binding arms as highlighted by comparing TCR x scFv versus TCR x Fab formats with the same VH:VL CD3 binding moiety and by comparing TCR x VHH Lead 4 versus TCR x VHH Lead 4 – HEL VHH constructs.**

## SUMMARY

Currently, all approved TCE multispecifics rely on anti-CD3 antibodies built on traditional IgG architectures containing both heavy and light chains[5]. HCAbs offer promise in reducing the complexity of multispecific therapeutics. Here, we describe the discovery and engineering of a panel of novel humanized CD3-specific HCAbs which demonstrate T cell cytotoxicity comparable to clinically validated TCEs when paired with IgG or TCR modalities. This work introduces a flexible new tool for enabling this important class of biologics. The resulting set of HCAbs, and future improvements to the panel, can be accessed as part of Adimab's non-exclusive TCE offering.

## REFERENCES

1. Liu, CY, et al. 2023. Structure-based engineering of a novel CD3ε-targeting antibody for reduced polyreactivity. MABs 15(1): 2189974. PMID: 36991534.
2. Wesolowski, J. et al. (2009). Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. Medical microbiology and immunology, 198, 157-174. PMID: 19529959
3. Hamers-Casterman, C. T. S. G. et al. (1993). Naturally occurring antibodies devoid of light chains. Nature, 363(6428), 446-448. PMID: 8502296.
4. Jain, et al. (2017). Biophysical properties of the clinical-stage antibody landscape. Proceedings of the National Academy of Sciences, 114(5), 944-949. PMID: 28096333
5. Center for Drug Evaluation and Research. Bispecific Antibodies: An Area of Research and Clinical Applications, FDA, 14 Feb. 2024, www.fda.gov/drugs/news-events-human-drugs/bispecific-antibodies-area-research-and-clinical-applications.