DISCOVERY AND ENGINEERING OF ANTI-CD3 HEAVY CHAIN-ONLY ANTIBODIES FOR USE IN T-CELL ENGAGING THERAPEUTICS

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ADIMAB

INTRODUCTION TO T CELL ENGAGERS

T cell engagers (TCEs) utilizing CD3 are an increasingly validated class of multispecific antibodies that have shown promise in treating oncologic conditions. Through pairing an arm targeting CD3 on T cells with an arm targeting a tumor associated antigen, the TCE forces the T cell and tumor cell into close proximity, triggering T cell activation leading to tumor cell killing[1].

- Eight TCEs are currently FDA approved- six since 2022[1]
- TCEs can be engineered in multiple bi- and multispecific formats. Activation level can be influenced by format[1]

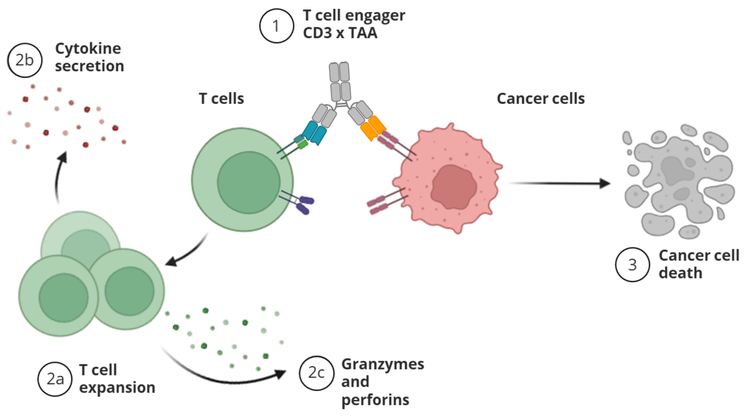

Figure 1. Summary of T cell engager mechanism of action.

ADIMAB a-CD3 CONVENTIONAL IgG LINEAGE: HUMAN x CYNO CROSS-REACTIVTY AND TUNABLE POTENCY

In vivo murine discovery followed by humanization and yeast-based optimization generated a broad affinity a-CD3 panel with excellent developability properties.

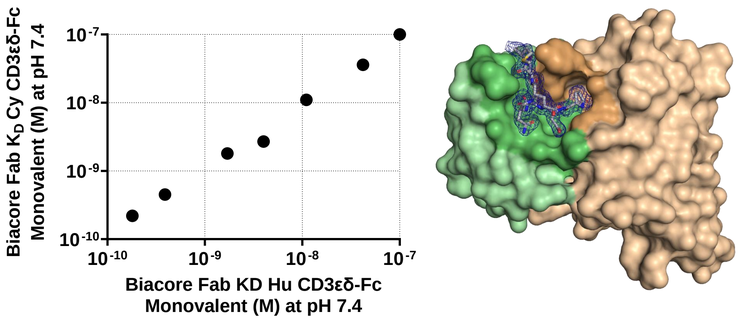

Figure 2. Adimab's a-CD3 antibody lineage has human-cyno cross-reactivity across a broad range of affinities and binds to the N-terminal portion of CD3ε[2].

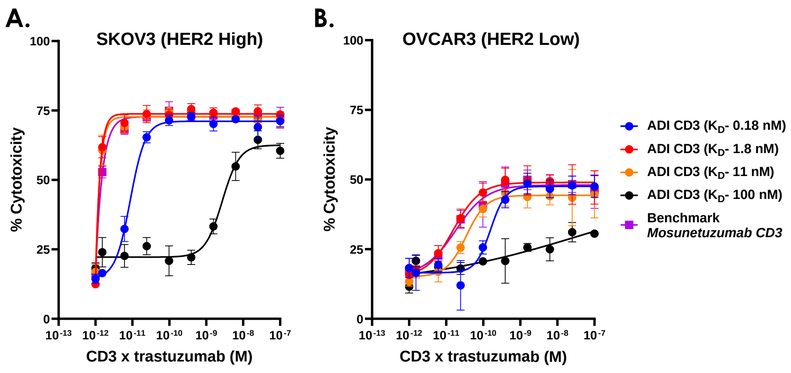

Figure 3. CD3 x HER2 bsAbs support potent Redirected T Cell Cytotoxicity (RTCC) on A. SKOV3 (HER2 high) and B. OVCAR3 (HER2 low) cell lines. The monovalent K_D for CD3 for each bispecific IgG is indicated in the legend. a-HER2 Fv is trastuzumab. Effector:target (E:T) 5:1, 24-hour time point shown.

EXPANDING ADIMAB'S CD3 BINDERS: HCAB DISCOVERY

Camelids naturally produce two classes of antibody: conventional (HC+LC) and HCAbs (HC-only)[3]. Absence of a light chain can simplify bi- or multispecific development[4]. Adimab's yeast-based immune library platform facilitates discovery of llama-derived HCAbs.

Figure 4. Comparison of conventional IgG to HCAb

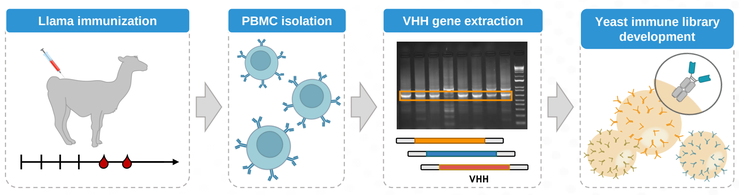

Figure 5. Building of a-CD3 immune libraries using Adimab platform

IMMUNE LIBRARY SELECTIONS DISCOVERED HUNDREDS OF a-CD3 HCABS

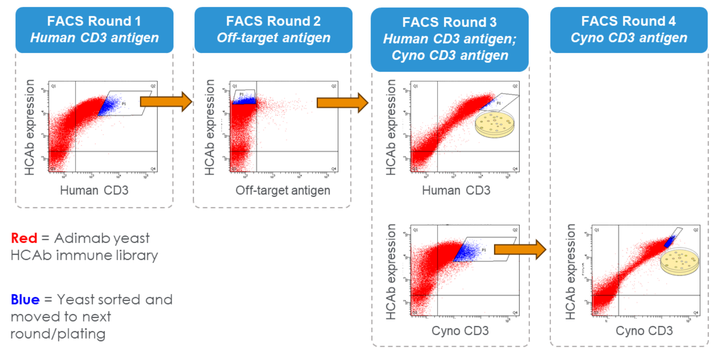

Figure 6. Multiple rounds of selections were performed on the Adimab yeast immune libraries to isolate a-CD3 HCAbs with desirable properties. Selections were performed under conditions to push for cyno cross-reactivity, wide affinity range, and favorable developability. *Note: figure displays only one representative selection*.

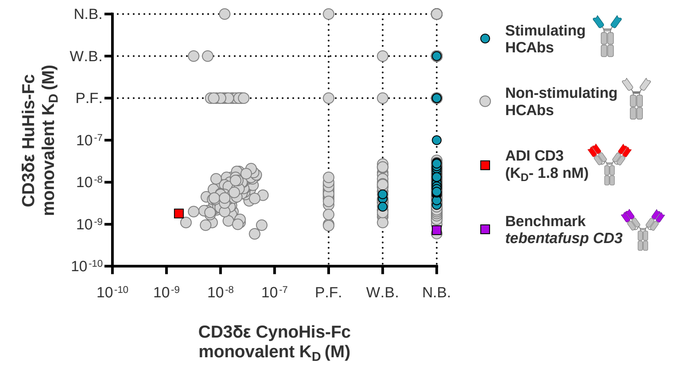

Figure 7. Isolated HCAbs screened for affinity and activity in CD69 upregulation T cell stimulation assay. While many human x cyno a-CD3 binding HCAbs were isolated, stimulating HCAbs exhibited only human-specific binding. P.F. = poor fit; W.B. = weak binding; N.B. = no binding.

a-CD3 T-CELL ENGAGERS SHOW ACTIVITY IN FUNCTIONAL ASSAYS AND DISPLAY FAVORABLE DEVELOPABILIBTY

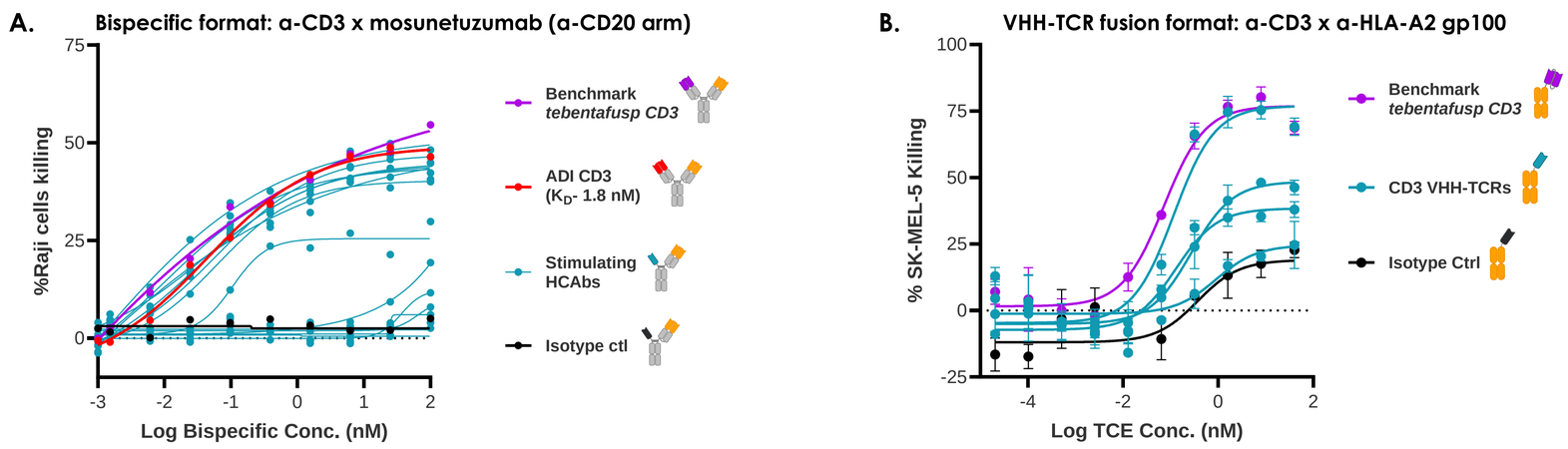

Figure 8. Stimulating HCAbs were downselected, reformatted in Chain Exchange vectors, and produced in two different TCE formats: as a-CD3 HCAb x a-CD20 bispecific antibodies (bsAbs) and as VHH-T-cell receptor fusions (VHH-TCRs). a-CD3 x a-CD20 bsABs evaluated in RTCC assays resulted in cytotoxicity against target Raji (CD20+) cells (A.). VHH-TCRs evaluated in RTCC assays resulted in cytotoxicity against melanoma (SK-MEL-5) cells (B.).

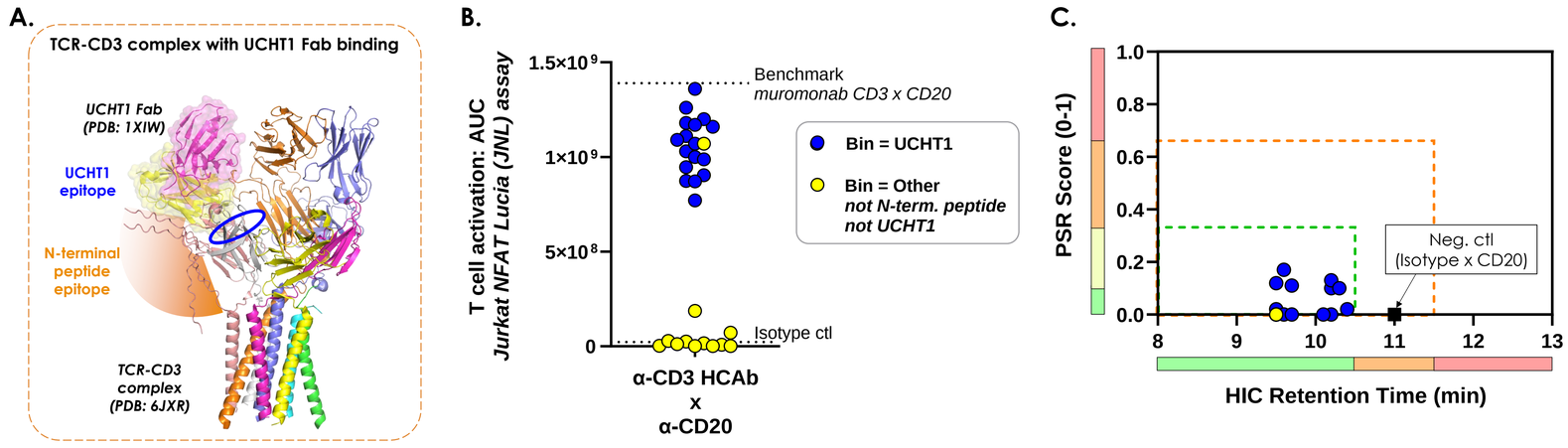

Figure 9. A. TCR-CD3 complex shown with an overlay of UCHT1 (basis for the CD3 arm of tebentafusp); schematic of N-terminal peptide also shown (target of the CD3 arm of mosunetuzumab). B. The majority of Adimab-discovered functional HCAbs competed with UCHT1, and none bound the N-terminal peptide. C. All clones with functional activity in the JNL and RTCC assays displayed favorable developability with low PSR and HIC retention times[5].

HUMANIZED a-CD3 HCABS MAINTAIN ABILITY TO STIMULATE T CELLS

Six functional HCAbs, spanning six lineages and two bins, were selected for humanization. A panel of humanized constructs were designed for each parent, retaining the llama CDRs and select amino acids within the frameworks.

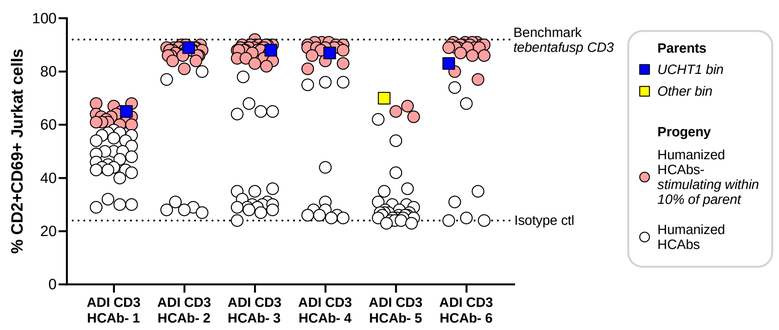

Figure 10. Humanized HCAbs were screened in a CD69 upregulation T cell stimulation assay. All parents had progeny that stimulated within 10% of parent.

SUMMARY

Heavy chain-only antibodies (HCAbs) offer promise in reducing the complexity of multispecific therapeutics. Here, we describe the discovery and engineering of a panel of high-affinity, CD3-specific HCAbs from immunized llamas, and their validation for use in a T cell engaging bispecific format. Further validation and optimization are in progress. The resulting set of HCAbs, and future improvements to the panel, can be accessed as part of Adimab's non-exclusive TCE offering.

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