ENGINEERING A PANEL OF POTENT AND DEVELOPABLE ANTI-CD3 ANTIBODIES FOR CONDITIONAL ACTIVATION IN THE TUMOR MICROENVIRONMENT

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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 cancer indications. By pairing an arm targeting CD3 on T cells with an arm targeting a tumor associated antigen (TAA), the TCE coordinates formation of an MHC-independent immunological synapse, 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 bi- and multispecific formats. Activation level can be influenced by format[1]

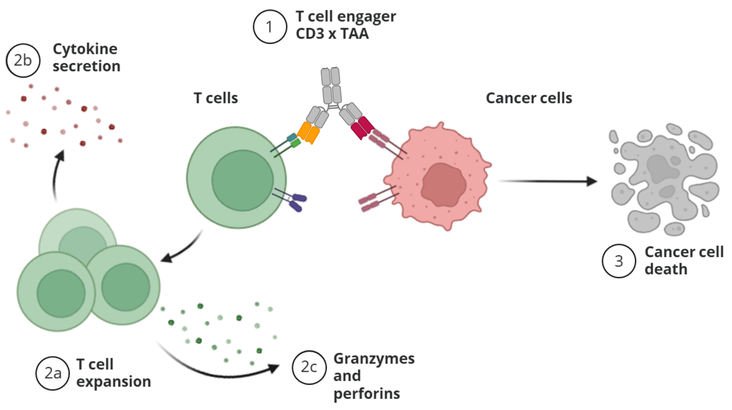


Figure 1. Summary of T cell engager mechanism of action.

ADIMAB a-CD3 IgG PANEL: 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 (Fig. 5).

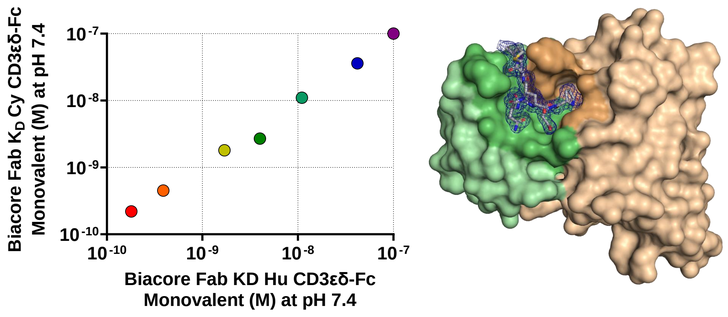


Figure 2. Adimab's a-CD3 antibody panel has human-cyno cross-reactivity across a broad range of affinities and binds to the N-terminal portion of CD3ε.[2]

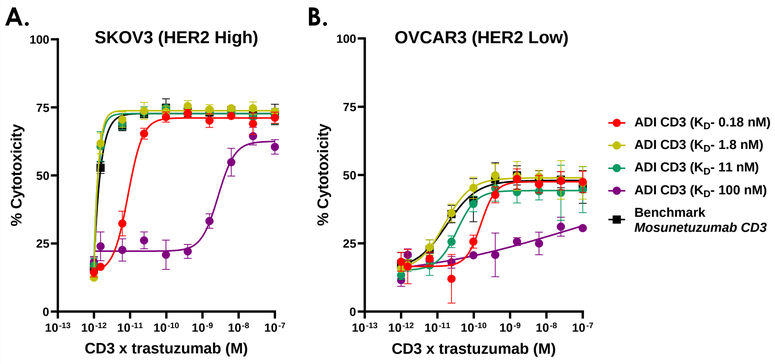


Figure 3. CD3 x HER2 bsAbs support potent Redirected T Cell Cytotoxicity (RTCC) on A. SKOV3 (HER2 high) and B. OVCAR3 (HER2 low) cell lines. The monovalent K_D for CD3 for each bispecific IgG is indicated in the legend. a-HER2 Fv is trastuzumab. Effector:target (E:T) 5:1, 24-hour time point shown.

ENGINEERING ADIMAB'S CD3 PANEL FOR CONDITIONAL ACTIVATION

Few cell surface markers exist which are specifically expressed in the cancer state. This has necessitated alternative strategies to improve therapeutic index by minimizing T cell activation away from the tumor site.

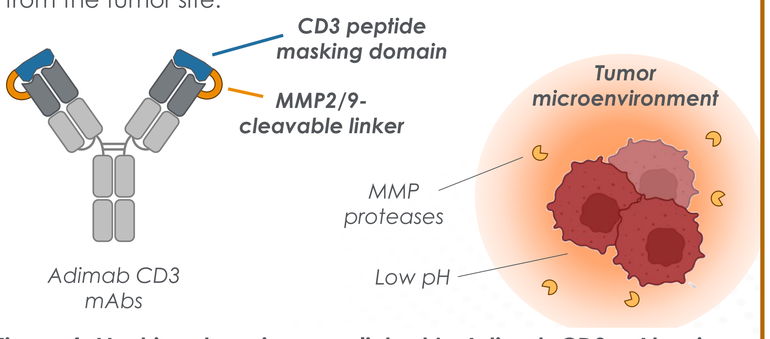


Figure 4. Masking domains were linked to Adimab CD3 mAbs via a matrix metalloprotease (MMP) recognition site. MMPs are enriched in the microenvironment of many solid tumors.[3] Protease cleavage of the linker facilitates mAb activation through mask dissociation.

EXCELLENT DEVELOPABILITY PROFILE OF MASKED a-CD3 IgGs

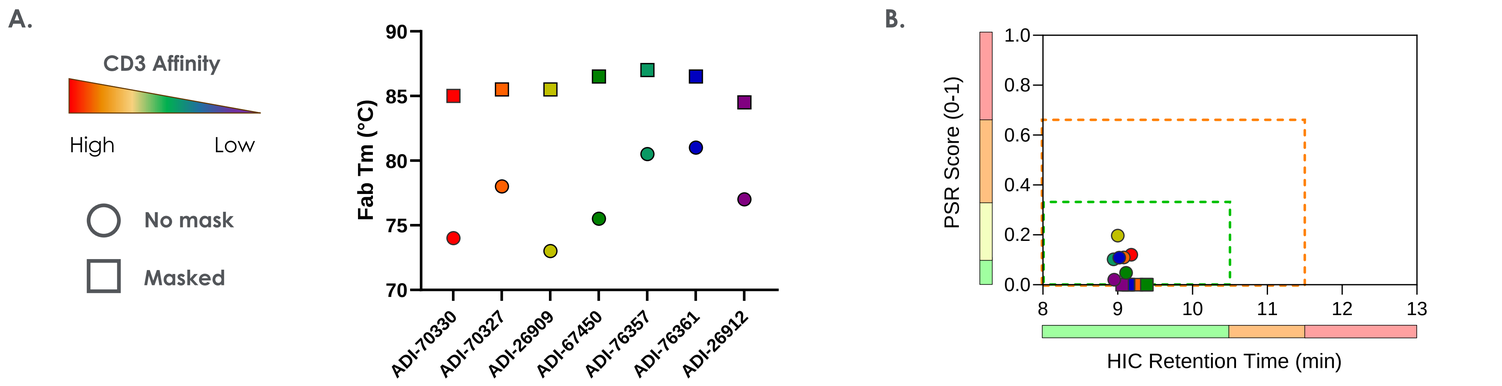
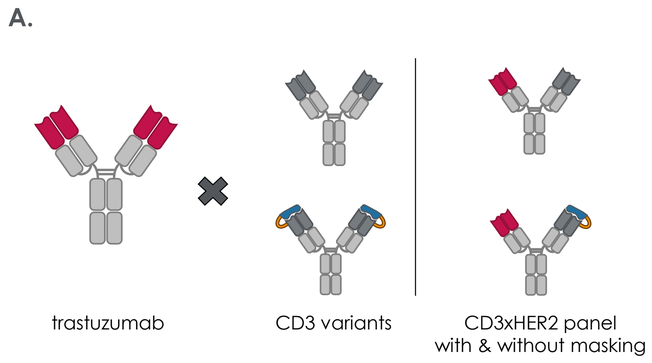


Figure 5. Masking increases thermal stability and decreases polyreactivity in Adimab's a-CD3 antibody panel. A. Differential scanning fluorimetry shows Fab stabilization up to ~85°C when masked, consistent with the CD3 peptide binding across both heavy and light chains. B. Low PSR reagent binding in parental molecules is eliminated in masked IgGs. HIC retention is unaffected and remains low.[4]

GENERATION OF MASKED CD3xHER2 BISPECIFIC PRODRUGS



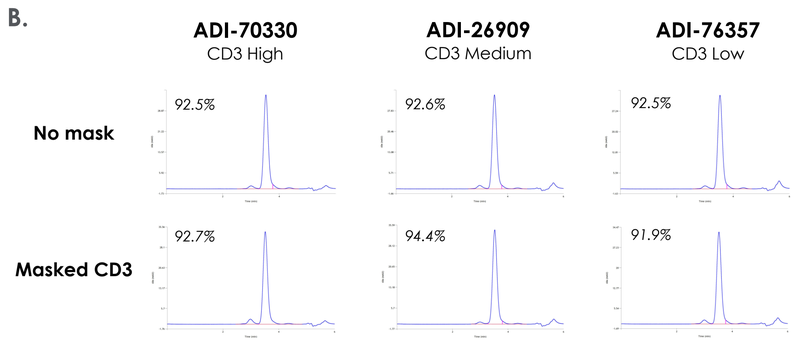


Figure 6 (top). Production of a masked CD3xHER2 bsAb panel. A. Chain exchange methods were used to pair Adimab's CD3 panel, with and without masking, to the HER2-targeting arm of trastuzumab. B. Size-exclusion chromatography shows that addition of peptide masks does not impact sample quality. Representative chromatograms shown for 3 of 7 a-CD3 mAbs.

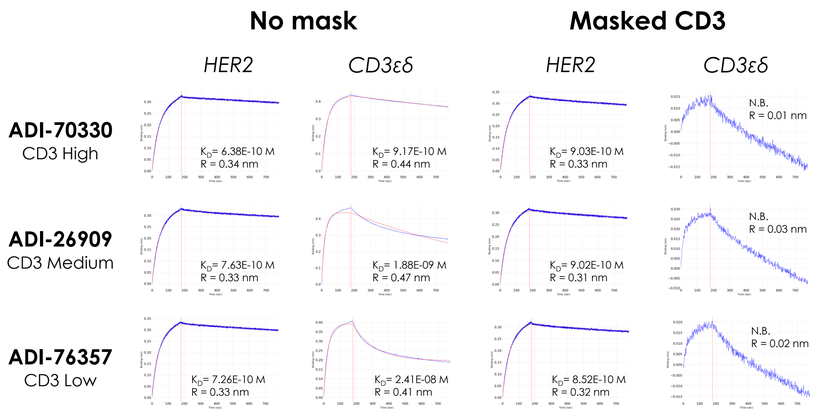


Figure 7 (right). Masking blocks association of bsAbs with recombinant CD3εδ antigen. Biolayer interferometry (BLI) shows attenuation of binding, regardless of CD3 affinity in the parental molecule without masking. HER2 binding is unaffected by masking on the CD3 arm. Representative sensorgrams shown for 3 of 7 a-CD3 mAbs.

Tumor cell proteases activate masked CD3xHER2 bsAbs in cell culture

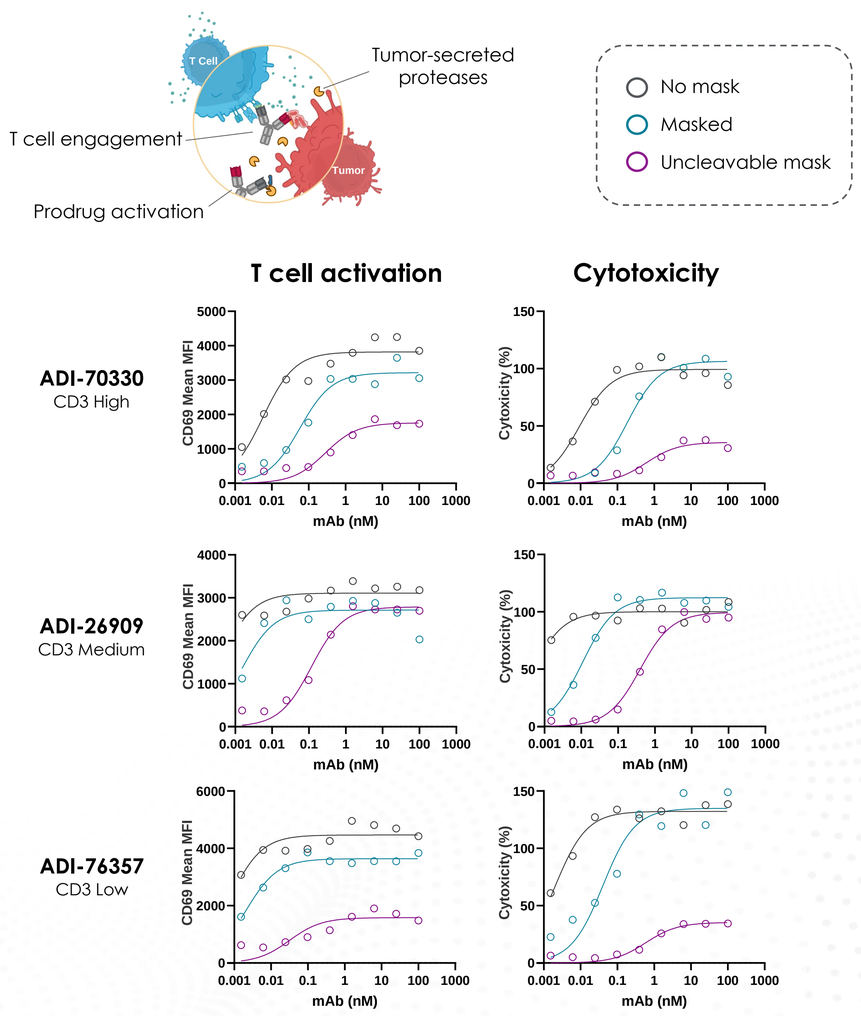


Figure 8. Masked bsAbs were evaluated in Redirected T Cell Cytotoxicity (RTCC) assays for T cell stimulation (left) and cytotoxicity towards SKOV3 HER2-positive target cells (right). 48-hour timepoint shown.

SUMMARY

Tissue-specific activation is a promising approach to harness the potency of bispecific T cell engagers while minimizing toxicity away from the tumor site.[5] Here, we describe a panel of anti-CD3 mAbs, currently in clinical testing, which have been reformatted for activation in the tumor microenvironment by MMP protease digestion. RTCC assays demonstrate potent T cell stimulation and target cell killing after prodrug activation in culture with protease-secreting HER2+ tumor cells.[6] The resulting set of anti-CD3 prodrugs, and future improvements to the panel, can be accessed as part of Adimab's non-exclusive TCE offering.

REFERENCES

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