A versatile, yeast-based synthetic heavy chain-only antibody platform that facilitates the construction and functional screening of multispecific CD28 T cell engagers

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ADIMAB

BACKGROUND

Heavy chain-only antibodies (HCAbs) are emerging as key modalities in a variety of therapeutic contexts due to their unique features. Notably, since HCAbs do not require a light chain, they enable the efficient generation of bi- and multi-specific antibodies. We have generated a human, synthetic HCAb discovery platform in yeast, which has been utilized in efforts against multiple therapeutically relevant targets, including the isolation of CD28-binding HCAbs. The variable domains from our CD28 HCAbs have been subsequently engineered into IgG-like bispecific antibodies where one arm contains the CD28 single domain antibody, and the other arm contains a Fab against a tumor-associated antigen (TAA). These bispecific costimulatory molecules show enhancement of T-cell activation and cell killing when combined with CD3 x TAA bispecific molecules.

Adimab synthetic HCAb library design and platform overview

Figure 1: Library design principles

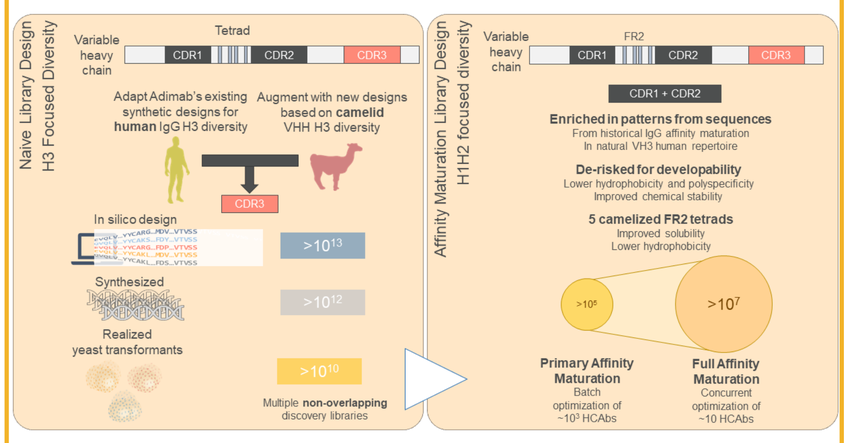
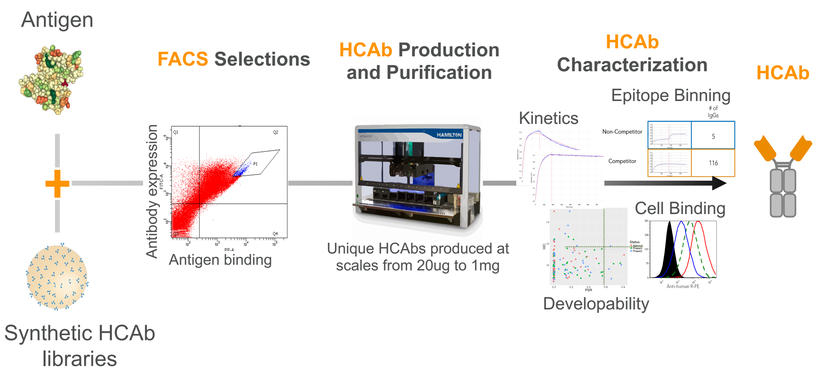


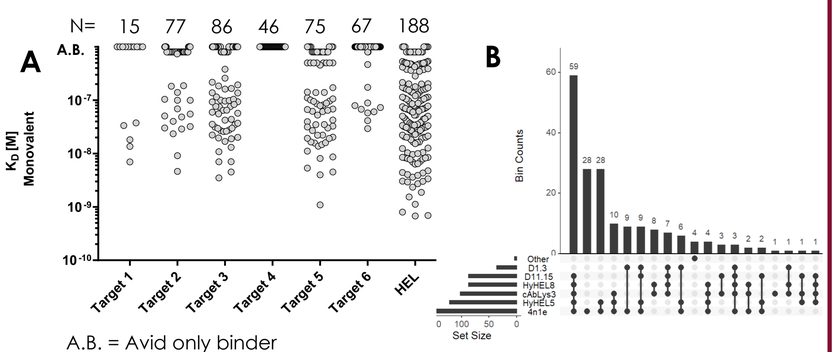
Figure 2: HCAb discovery project flow
Platform allows for delivery of panel of HCAbs with desired specificity profile within 4-5 months from selection start



RESULTS

MACS/FACS-based selections using yeast HCAb platform delivers robust HCAb outputs for a variety of therapeutic targets

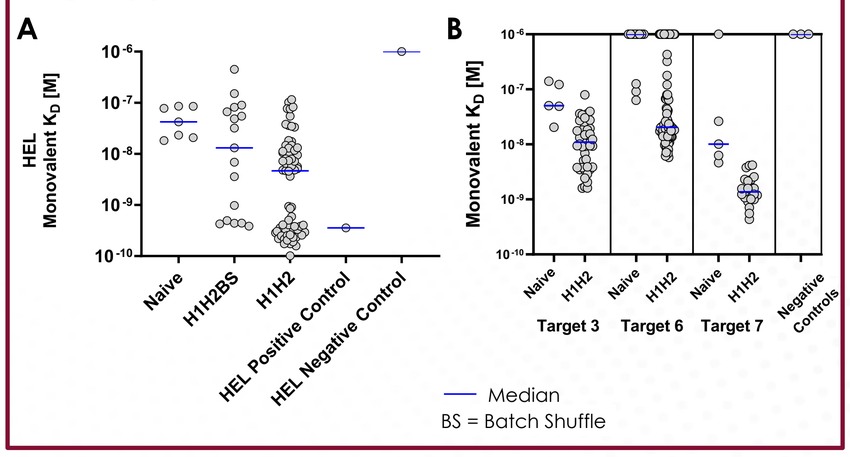
Figure 3: Binding affinity and epitope coverage
Platform delivers HCAbs with nanomolar binding affinity (A) and broad epitope coverage (B)



A.B. = Avid only binder

Bins are defined with respect to antibodies of known structure in complex with HEL

Figure 4: Affinity maturation yields large affinity improvements against Hen Egg Lysozyme (HEL) (A) and three undisclosed targets (B)

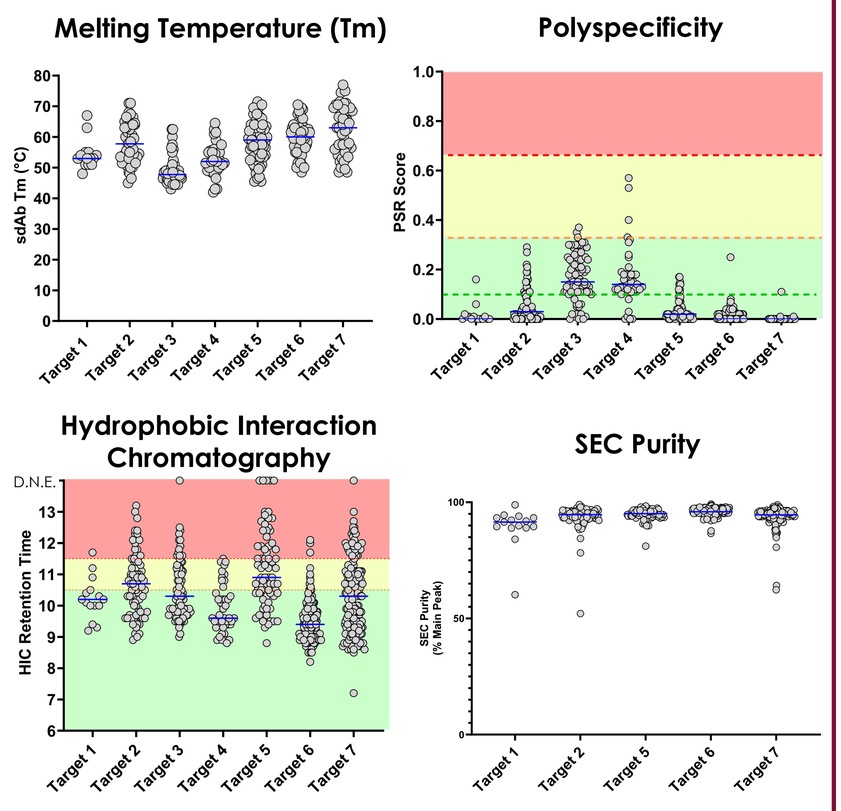


Median

BS = Batch Shuffle

Output HCAbs show favorable developability properties

Figure 5: Favorable developability for selected HCAbs across multiple Adimab campaigns



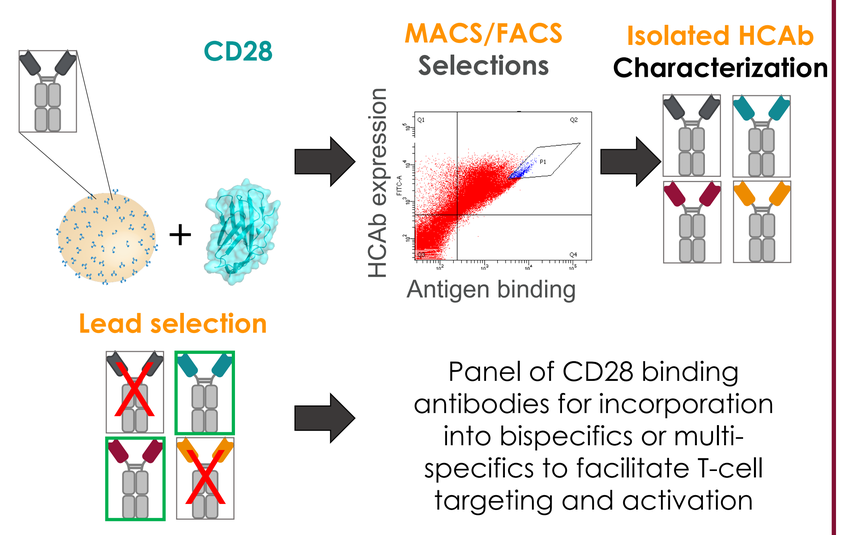
D.N.E. = Does not elute

Median

Clean | Medium | High — Clean, Medium, and High categories based on historical IgG data

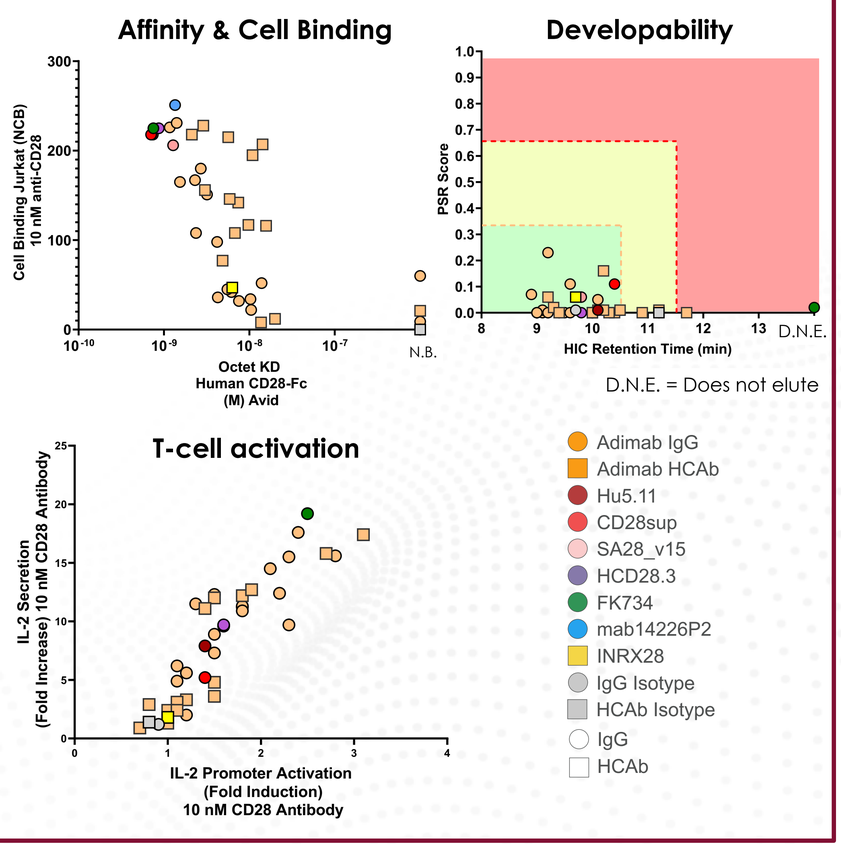
Isolation of CD28-binding HCAbs

Figure 6: Overview of the CD28 HCAb selection process
HCAb libraries were subjected to MACS and FACS selections using CD28 antigen; isolated antibodies were characterized; a panel of leads were selected as candidates for multi-specific antibodies



Characterization of CD28-binding HCAbs

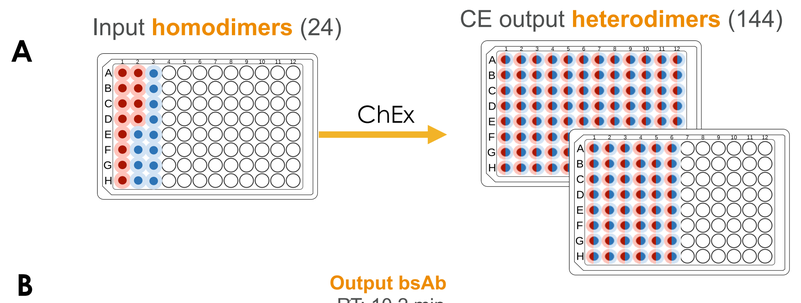
Figure 7: Affinity & cell binding, developability, and functional activity of CD28 HCAb outputs



D.N.E. = Does not elute

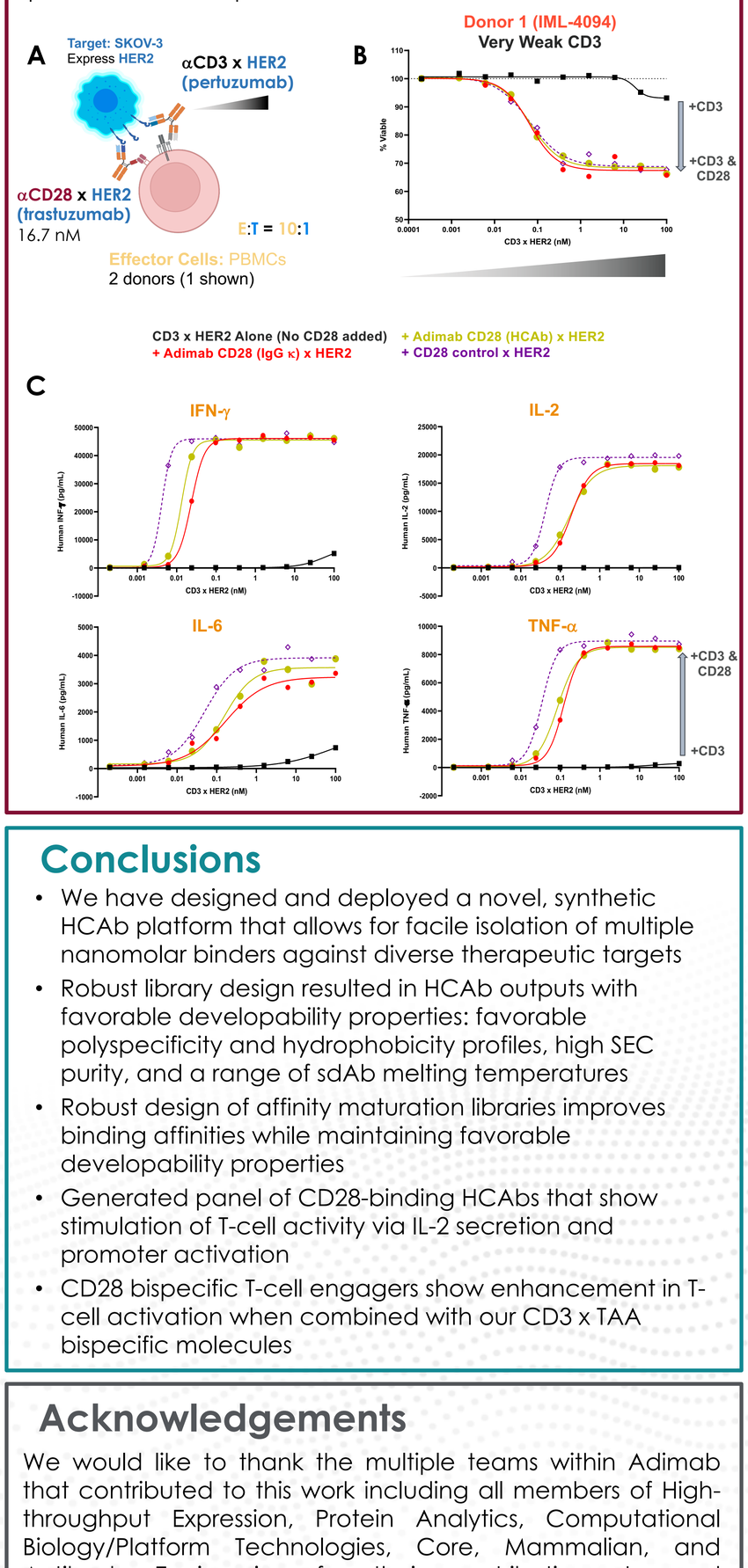
Efficient, high-throughput generation of multi-specific panels

Figure 8. Adimab Chain Exchange (ChEx) is applied to generate panels of multi-specifics.
ChEx generates panels of multi-specific antibodies for functional assays (A); analytical Ion Exchange (aIEX) output of CD28 x HER2 bispecific (B)



CD28 x HER2 bispecifics show costimulatory activity

Figure 9. Adimab αCD28 in bispecifics
Experimental set-up (A); Adimab αCD28 bispecifics enhance SKOV-3 cell killing (B) and increase cytokine release (C) when paired with αCD3 panel



Conclusions

- We have designed and deployed a novel, synthetic HCAb platform that allows for facile isolation of multiple nanomolar binders against diverse therapeutic targets
- Robust library design resulted in HCAb outputs with favorable developability properties: favorable polyspecificity and hydrophobicity profiles, high SEC purity, and a range of sdAb melting temperatures
- Robust design of affinity maturation libraries improves binding affinities while maintaining favorable developability properties
- Generated panel of CD28-binding HCAbs that show stimulation of T-cell activity via IL-2 secretion and promoter activation
- CD28 bispecific T-cell engagers show enhancement in T-cell activation when combined with our CD3 x TAA bispecific molecules

Acknowledgements

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