Rapid Engineering of Soluble T Cell Receptors for Enhanced Affinity via a High-Throughput Yeast-Based Platform

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ADIMAB

BACKGROUND

- Peptide-HLA (pHLA) complex recognition by T cells facilitates targeting of intracellular proteins.
- Soluble T cell receptors (TCRs) and TCR-mimic (TCRm) antibodies can co-opt T cell function via pHLA recognition as anti-CD3 bispecifics.
- Engineered TCRs have shown greater peptide specificities relative to TCRm antibodies, and therefore may have reduced potential for off-target autoreactivity.
- Native TCRs have low affinities for their target pHLAs (1 - 200 μM), requiring substantial affinity maturation for use in clinically-validated CD3 bispecific formats.
- We have developed and validated a yeast-based high-throughput platform for soluble TCR expression and engineering, facilitating the development of TCR-based therapeutics.

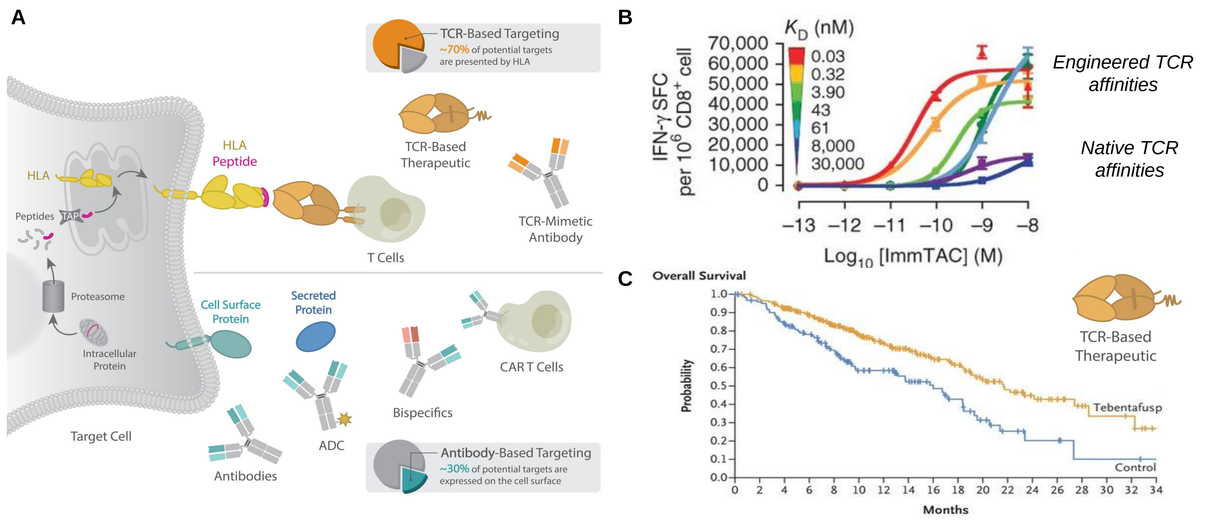

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Figure 1. Soluble TCRs and TCRm antibodies can co-opt T cell function
(A) pHLA recognition expands the targetable proteome to intracellular proteins. (B) Native TCR affinities are insufficient for use in soluble therapeutics. (C) The high-affinity soluble TCR-based CD3 bispecific tebentafusp shows efficacy against uveal melanoma.

SOLUBLE TCR PLATFORM: DESIGN AND VALIDATION

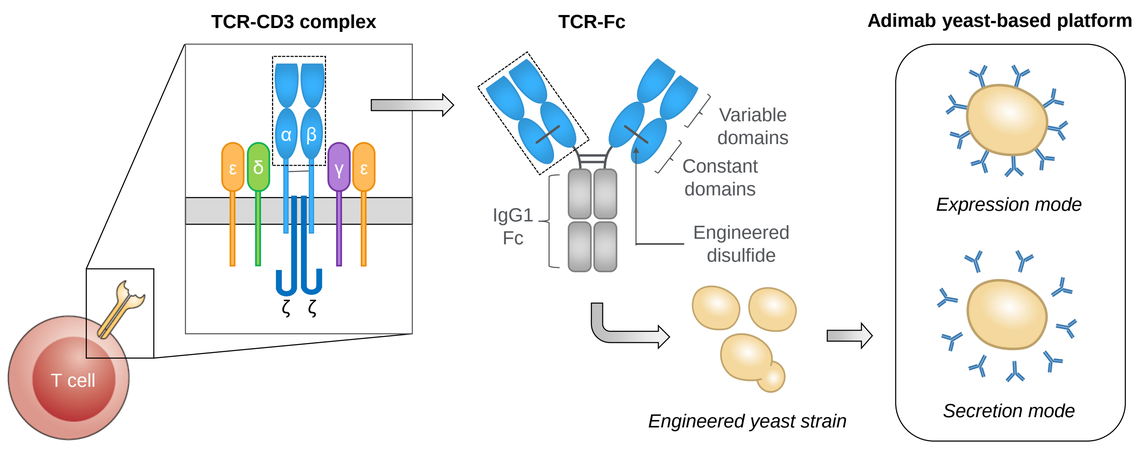

Figure 2. Yeast-based platform for TCR expression and engineering
Engineered soluble TCR, expressed in a bivalent IgG-like format on the surface of Adimab's engineered yeast, or secreted for purification.

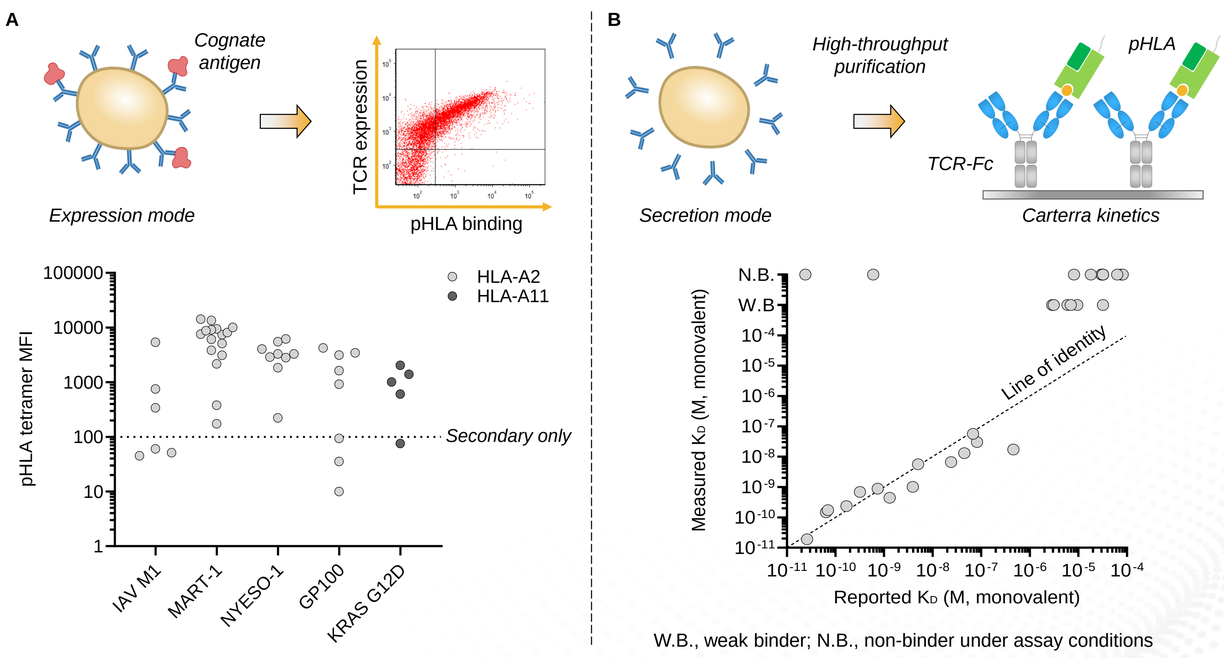

Figure 3. Yeast-expressed soluble TCRs bind their cognate antigens
(A) Mean fluorescent intensities (MFIs) for on-yeast pHLA tetramer staining of 44 literature controls in TCR-Fc format. (B) The measured monovalent pHLA affinities for secreted TCR-Fc proteins match their literature-reported affinities.

CASE STUDY: AFFINITY MATURATION OF 1G4 TCR

A

Clone	Target	K_D (M)	CDR1α	CDR2α	CDR3α	CDR1β	CDR2β	CDR3β
1G4	HLA-A2 NY-ESO-1	3.2E-05	DSAIYN	IQSSQRE	AVRPTSGGSYIPT	MNHEY	SVGAGI	ASSYVGNTGELF

Chen, J.L. et al., (2000) J Immunol;
Zhao, Y. et al., (2007) J Immunol

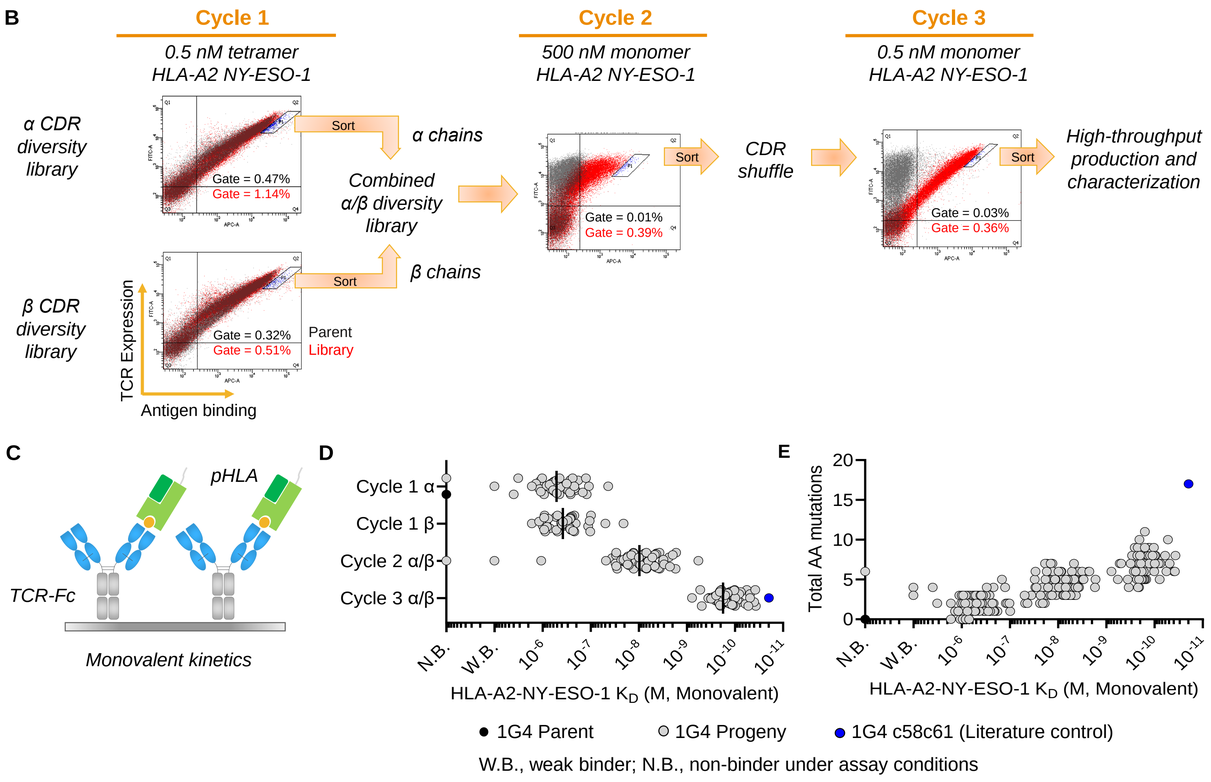

Figure 4. Overview of 1G4 TCR affinity maturation and validation of improved pHLA affinities
(A) Complementarity determining region (CDR) sequences and antigen-binding properties of the literature control TCR 1G4, selected for affinity maturation. (B) Overview of library generation and three cycle affinity maturation process to select for enriched pHLA binding. (C) Carterra kinetics assay used for measuring monovalent kinetics of soluble TCR-Fc protein binding to target pHLA. (D-E) Monovalent affinities of outputs to target HLA-A2 NY-ESO-1 monomers arranged by (D) affinity maturation cycle or (E) total protein mutations relative to parent.

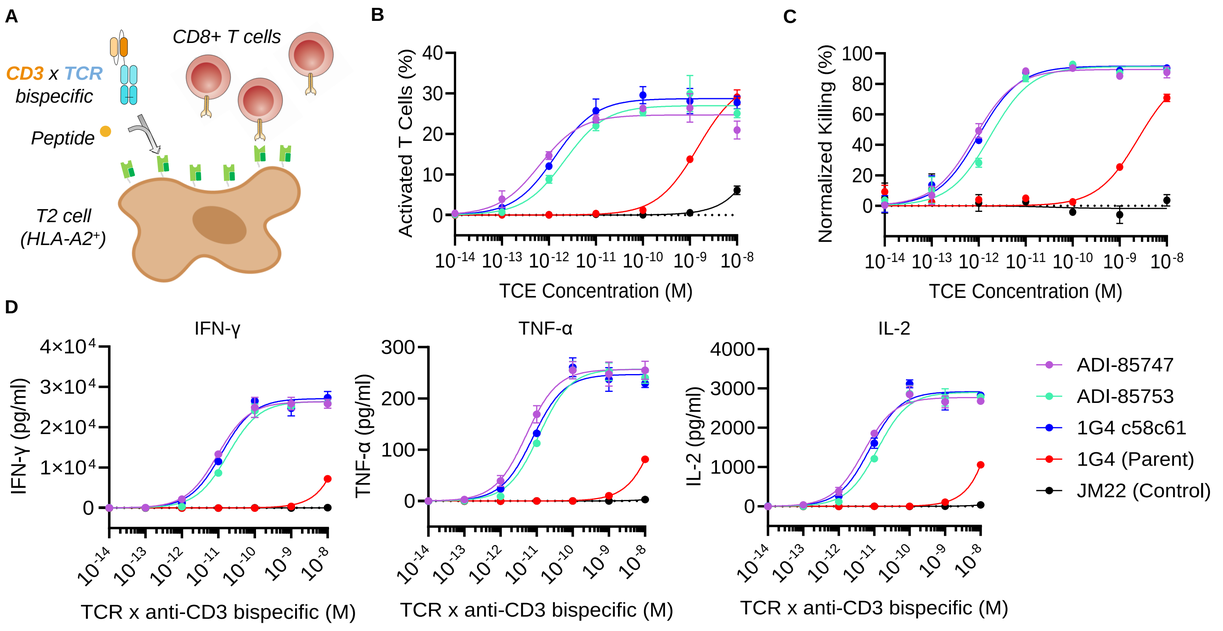

Figure 5. Functional assessment of optimized TCR progeny-based CD3 bispecifics
(A) Schematic overview of T cell functional assays wherein CD8+ T cells are incubated with target peptide-loaded antigen-presenting cells and TCR-based CD3 bispecific molecules. (B) T cell activation, (C) target cell killing, and (D) polyfunctional cytokine secretion elicited by CD3 bispecifics containing either parental 1G4 TCR, affinity-optimized 1G4 TCR variants, or negative control JM22 TCR.

CONCLUSIONS

- Adimab's extensively-engineered yeast express soluble TCR-Fc proteins that faithfully recapitulate the binding properties of natural and engineered TCRs.
- Three cycles of affinity maturation for a literature control TCR yielded TCR progeny with up to **300,000**-fold improved pHLA affinities.
- Adimab-optimized TCRs induced potent and polyfunctional T cell responses as CD3 bispecifics, with over **1,000**-fold improvement in potency relative to parent TCRs.

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