Discovery and preclinical development of a half-life-extended TCR-based bispecific T cell engager targeting a novel cancer antigen presented across multiple solid tumor indications

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T-Cypher Bio

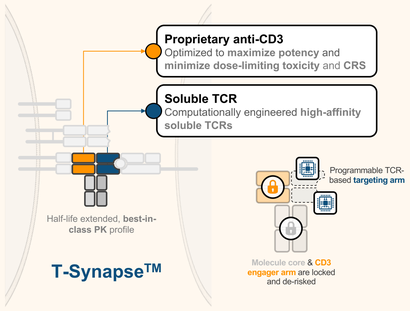
INTRODUCTION

T-cell engagers (TCEs) redirect polyclonal T cells against malignant cells and have demonstrated clinical activity in solid tumors[1]. TCR-based TCEs extend this approach to intracellular cancer antigens presented as peptide-HLA complexes which expands the targetable antigen repertoire. However, identifying targets that combine high tumor presentation while maintaining low normal tissue expression remains challenging.

TC25 is an HLA-A*02:01-restricted peptide expressed in multiple solid tumor indications. It has an estimated peptide copy number five-fold higher than PRAME and it is detected in up to 80% of HLA-A*02:01-positive patients in selected indications.

We developed a half-life extended TC25-specific TCE combining a picomolar affinity TCR domain, silenced Fc, and a low-affinity anti-CD3 domain designed to promote tumor cytotoxicity while limiting systemic cytokine release.

Bispecific platform supports development of half-life extended TCE therapeutics



TCR repertoire analysis across hundreds of donors was used to isolate TC25-specific receptors from CD8⁺ T cells. Selected TCRs underwent affinity engineering using a proprietary platform combining machine learning-guided mutagenesis with high-throughput functional selection. Engineered TCRs were formatted as single-chain TCR×CD3 bispecifics and screened for stability, potency and peptide-dependent activity in T cell-target cell co-cultures.

T-Cypher's immune engager pipeline

	Program	Addressable patients*	Discovery	Lead optimization	IND enabling	Status
Oncology	TC25, HLA-A2 NSCLC, GI, HNSCC, others	> 60,000				• DC selection 2H26 • IND 1H28
Oncology	Novel targets; Multi-HLA Solid tumors**	> 60,000				• DC selection 2027 • IND 2028
Oncology	TC11, HLA-A2 Cutaneous & uveal melanoma	10,000				• DC selected • Partnering
AID	Multi-indication TC30†, undisclosed	Autoimmune				• Discovery

* Addressable patients, HLA positive & target positive, US only
** High potential indications for TCEs including MSS CRC, Gastric, Esophageal (Sq./Ad), Lung (Sq/Ad), HNSCC, Bladder, Pancreatic

RESULTS

TC25 is validated as a multi-indication target

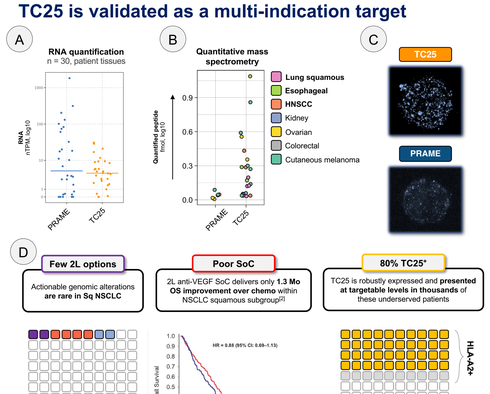


Figure 1. TC25 is a peptide presented in the context of HLA-A*02 in multiple indications. We collected patient tissues and performed **(A)** RNA-seq and **(B)** Parallel Reaction Monitoring to determine peptide copy number in matched patient tissues. We estimate TC25 to have a peptide copy number five-fold higher than PRAME. **(C)** Imaging of cancer cell line following staining with fluorophore-tagged TC25 and PRAME soluble TCRs. **(D)** Squamous NSCLC has few molecularly-defined second-line treatment options and a poor prognosis with current standard of care. TC25 is presented at targetable levels in approximately 80% of HLA-A*02:01 patients, providing broad coverage despite HLA restriction. *Immunopeptidomics performed at Biognosys.*

T-Cα™ discovery platform uncovers TCR specificity and potency on a single cell level from T cell repertoires

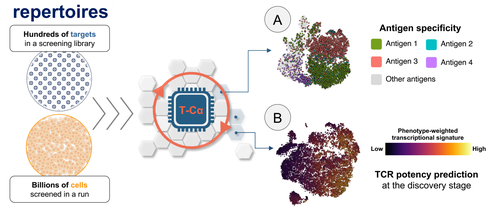


Figure 2. The T-Cα™ platform screens highly-multiplexed pHLA libraries against T cells from human blood donors, identifying thousands of unique target-specific T cell receptors in each sequencing library. Antigen-specific T cells are interrogated at single-cell resolution, linking TCR sequence and antigen specificity with functional state and cellular phenotype. **(A)** High-dimensional clustering analyses reveal the distribution of antigen-specific T cell populations and **(B)** phenotype-weighted transcriptional signature correlates with TCR potency, facilitating the identification of rare, therapeutically-relevant TCRs at the discovery stage.

T-Cα™ platform has been used to select lead TCRs against TC25

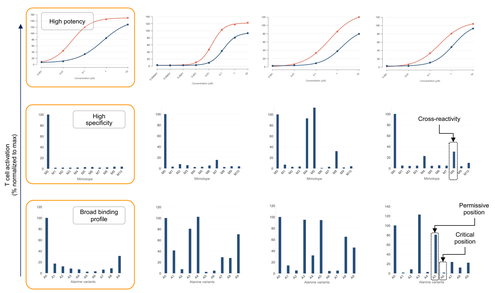


Figure 3. Functional screening of diverse TC25-specific TCRs discovered by the T-Cα™ platform. Activation of a T cell line was measured without (blue) or with (orange) the CD8 co-receptor. Specificity was assessed by mimotope-peptide screening and alanine scanning to identify cross-reactivity and key peptide positions (M0 and A0 = cognate peptide normalization controls). This process enables the identification of TCRs with desired functional and specificity profiles, and prioritization for engineering.

A computational method for rapidly engineering TCRs as soluble, developable, picomolar-affinity scTv proteins

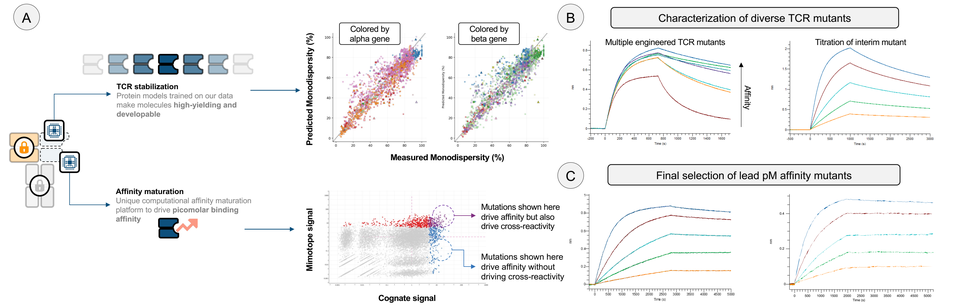


Figure 4. (A) Wild-type TCRs are naturally membrane-bound and low (μM) affinity. We use computational-led methods to rapidly solubilize and affinity mature wild-type TCRs into developable, soluble, high-affinity single-chain TCRs while maintaining the natural specificity and binding geometry of the human T cell receptor. **(B)** Screening binding of 7 different TCRs to target pHLA by Octet BLI identifies highest affinity mutants with example of affinity determination by titration. **(C)** Octet BLI affinity measurement of two pM affinity TC25 TCRs to biotinylated target pHLA.

T-Synapse-TC25 drives potent lysis across indication-relevant cell lines and disease models

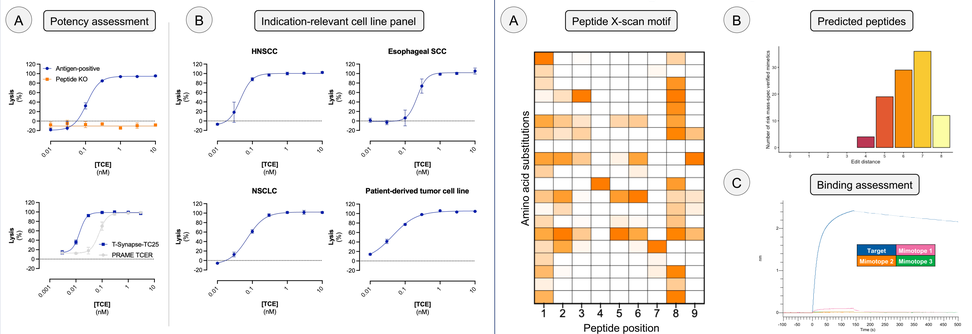


Figure 5. (A) TC25-positive cells (blue) and TC25 peptide-knockout controls (orange) were co-cultured with TCE and PBMCs at an effector-to-target (E:T) ratio of 10:1. Cytotoxicity was quantified by imaging after 48 h with lysis normalized to peptide-pulsed controls. T-Synapse-TC25 is 10-fold more potent than a PRAME-specific benchmark molecule. **(B)** T-Synapse-TC25 bispecific induced concentration-dependent lysis across tumor cell lines from multiple indications, including HNSCC, esophageal SCC, NSCLC and a patient-derived tumor cell line. **(C)** In a re-challenge assay, PBMC effectors were transferred after 36 h of co-culture onto fresh tumor cell line targets, re-dosed with TCE, and monitored up to 60 h to demonstrate sustained cytotoxicity following repeat target exposure.

T-Synapse-TC25 demonstrates extended half-life and potent anti-tumor activity *in vivo*

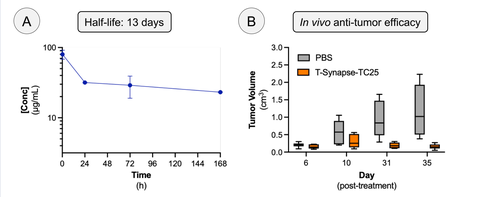


Figure 6. (A) T-Synapse-TC25 administered at 2 mg/kg IV demonstrates a favorable PK profile with a 13 day half-life. **(B)** T-Synapse-TC25 treatment of Hs695T tumors in PBMC-humanized NXG B2M KO mice. 87% tumor reduction observed at day 35 post-treatment with T-Synapse-TC25 (n = 6 mice per group, 3 PBMC donors).

T-Synapse-TC25 demonstrates high specificity with minimal off-target reactivity

Figure 7. (A) Peptide X-scan showing the effect of amino-acid substitutions at each position of the cognate peptide, revealing a highly restricted binding motif. **(B)** Predicted peptides from the human transcriptome grouped by edit distance from the cognate sequence; no peptides were identified at an edit distance <4. **(C)** Octet BLI analysis showing binding of the TC25-specific TCR to cognate pHLA, but not to selected mimotope or irrelevant pHLA complexes. **(D)** HLA-A alloreactivity panel showing no T-cell activation against the non-HLA-A*02:01 alleles tested. **(E)** TC25 TCE did not induce T-cell activation against bone-marrow mononuclear cells (BMMC) from two independent donors.

T-Synapse-TC25 affinity-optimized anti-CD3 arm does not trigger cytokine release in whole blood

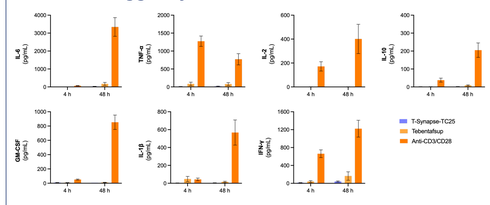


Figure 8. Whole blood assay was performed to measure cytokine release at 4 and 48 h to 100 nM T-Synapse-TC25. Tebentafusp and plate-bound anti-CD3/CD28 were included as controls. Data are representative of one of four donors tested (n = 4).

TC25 is expressed in tumor tissues and absent from healthy tissue

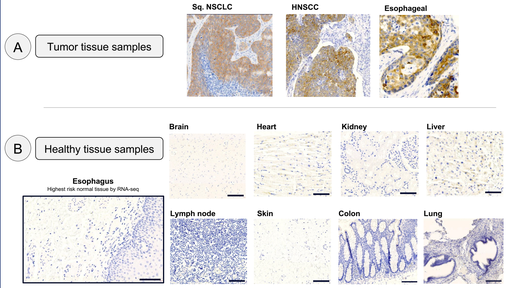
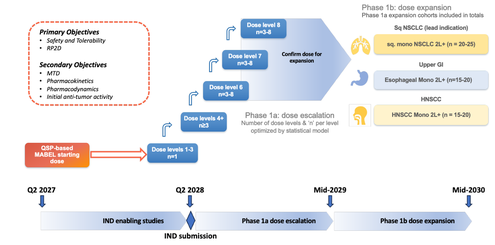


Figure 9. (A) Strong TC25 IHC staining observed in tumor tissue (TMAs) from multiple indications. **(B)** Negative TC25 IHC staining observed across healthy tissue blocks, n=6 donors for lung and esophagus, n=3 for other tissues. Scale Bar = 100 μm. *IHC performed at Concept Life Sciences.*

T-Synapse-TC25 FIH clinical trial design



REFERENCES

[1] Paz-Ares L, Champiat S, Lai WV, et al. Tarlatamab, a first-in-class DLL3-targeted bispecific T-cell engager, in recurrent small-cell lung cancer: an open-label, phase I study. J Clin Oncol. 2023;41:2893-2903.

[2] Garon EB, Ciuleanu TE, Arrieta O, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. Lancet. 2014;384:665-673.

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CONCLUSIONS

TC25 is a pHLA target with favorable tumor prevalence and high peptide presentation across multiple solid tumor indications. Using the T-Cα™ discovery platform and computationally-guided TCR engineering approaches, we generated a half-life extended T-Synapse™ bispecific targeting TC25 with picomolar affinity, potent tumor cell killing, and exquisite peptide specificity. These findings support further clinical development of T-Synapse-TC25 as a treatment for patients with TC25-positive solid tumors with high unmet need. IND-enabling studies are planned for 2027.