Pre-clinical development of a best-in-class bispecific T cell engager targeting a highly abundant peptide for the treatment of melanoma

T-Cypher Bio

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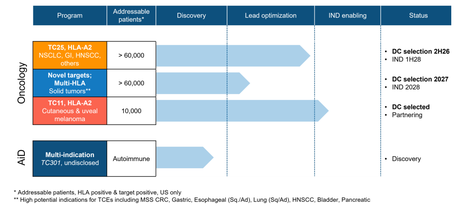
INTRODUCTION

Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape for melanoma; however, **40-50% of patients fail to achieve durable clinical benefit** due to primary resistance. Many more patients develop resistance subsequently. Following disease progression treatment options remain limited, highlighting a significant unmet need for novel immunotherapies capable of overcoming checkpoint resistance.

TCR-based T cell engagers (TCR-TCEs) represent a promising alternative approach, exemplified by the clinical success of tebentafusp in uveal melanoma [1]. However, the activity of current TCR-TCEs may be limited by the relatively low abundance of their target peptide-HLA complexes on tumor cells.

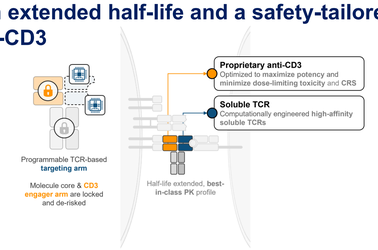
To address this challenge, we identified **TC11**, an exceptionally abundant HLA-A*02:01-presented melanoma peptide with broad tumor expression. These attributes provide the foundation for **T-Synapse-TC11**, a next-generation TCR-TCE designed to drive potent anti-tumor activity and expand treatment opportunities for patients with metastatic melanoma.

T-Cypher's immune engager pipeline



* 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

T-Synapse™: A programmable TCE format with extended half-life and a safety-tailored anti-CD3



Optimized anti-CD3 confers exceptionally wide therapeutic window

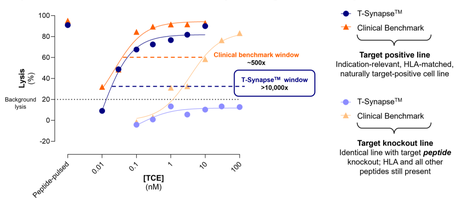


Figure 1: TC11 positive and peptide knockout lines were co-cultured with TCEs and PBMCs at an E:T ratio of 10:1. TCEs are T-Synapse-TC11, and a TC11 TCE with a clinically validated anti-CD3 in place of the proprietary T-Synapse™ anti-CD3. Cytotoxicity was quantified by imaging after 48 h

RESULTS

TC11 off-the-shelf biologic targets cell-therapy-like response rates in melanoma via exceptional peptide-HLA abundance

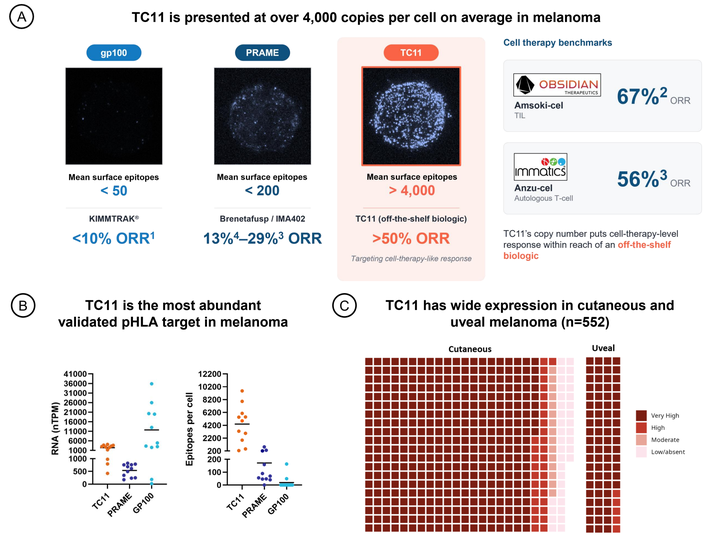


Figure 2: (A) TC11, PRAME and gp100 peptides were quantified in melanoma samples using fluorescently labelled pM affinity soluble TCRs by confocal microscopy. **(B)** Matched RNA-seq and cell surface peptide quantification by flow cytometry demonstrate a mismatch between RNA and peptide. **(C)** TC11 RNA expression in cutaneous and uveal melanoma in TCGA tumor samples, with very high, homogenous expression seen in the vast majority of patients.

A TCE targeting TC11 results in greater tumor cell lysis in heavily pre-treated patients compared to clinical PRAME and gp100 TCEs

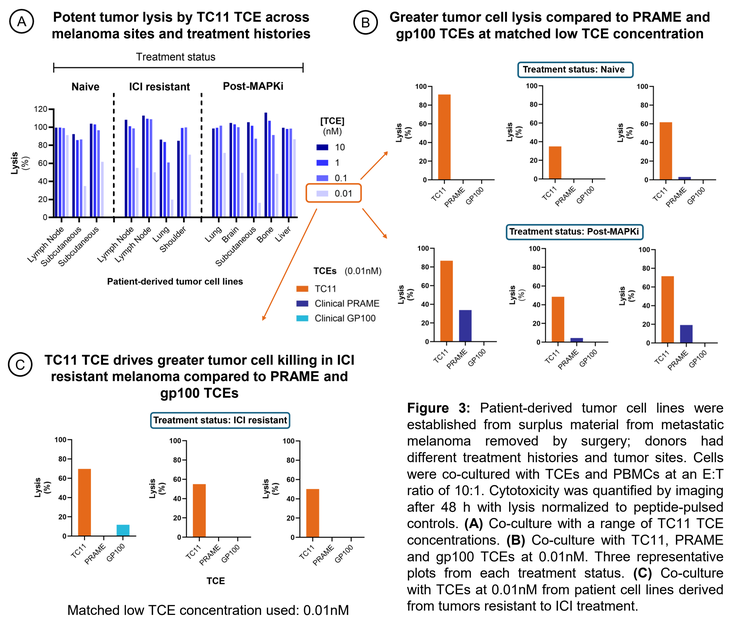
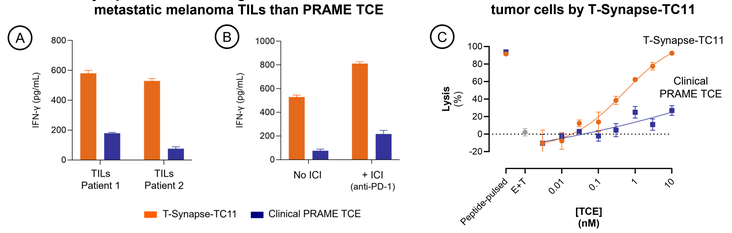
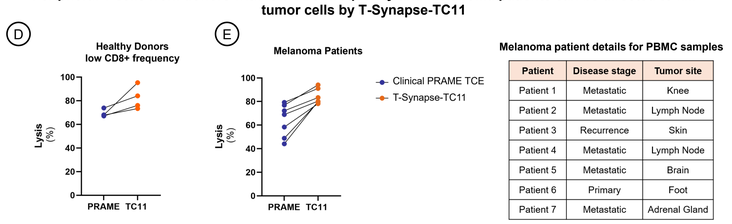


Figure 3: Patient-derived tumor cell lines were established from surplus material from metastatic melanoma removed by surgery; donors had different treatment histories and tumor sites. Cells were co-cultured with TCEs and PBMCs at an E:T ratio of 10:1. Cytotoxicity was quantified by imaging after 48 h with lysis normalized to peptide-pulsed controls. **(A)** Co-culture with a range of TC11 TCE concentrations. **(B)** Co-culture with TC11, PRAME and gp100 TCEs at 0.01nM. Three representative plots from each treatment status. **(C)** Co-culture with TCEs at 0.01nM from patient cell lines derived from tumors resistant to ICI treatment.

A TCE targeting TC11 drives superior T cell activation and tumor cell lysis in challenging late-stage disease settings *in vitro*





Melanoma patient details for PBMC samples

Patient	Disease stage	Tumor site
Patient 1	Metastatic	Knee
Patient 2	Metastatic	Lymph Node
Patient 3	Recurrence	Skin
Patient 4	Metastatic	Lymph Node
Patient 5	Metastatic	Brain
Patient 6	Primary	Foot
Patient 7	Metastatic	Adrenal Gland

Figure 4: (A) Tumor infiltrating lymphocytes (TILs) were isolated from metastatic melanoma and co-cultured with a patient cell line and T-Synapse-TC11 or a PRAME TCE (clinical benchmark). **(B)** TILs were co-cultured with a patient cell line and TCE -/+ anti-PD-1. T cell activation was assessed at 24 h by measuring IFN-γ. **(C)** *In vitro* exhausted T cells (repeated anti-CD3/CD28 stimulation) were co-cultured with SK-MEL-5 and TCE. Lysis was measured after 48 h. **(D)** PBMC from healthy donors with low CD8+ frequency and **(E)** PBMC from melanoma patients were co-cultured with a patient cell line and TCE. Cytotoxicity was quantified by imaging after 48 h with lysis normalized to peptide-pulsed controls.

T-Synapse-TC11 is highly target specific, shows no detectable alloreactivity, and exhibits minimal cytokine release in whole blood

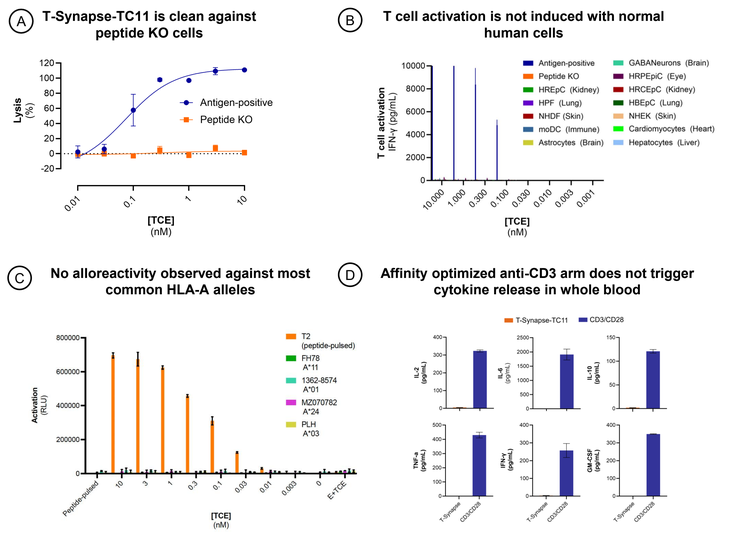
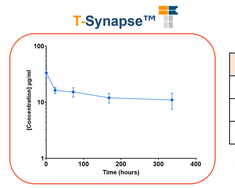


Figure 5: (A) TC11-positive (blue) and TC11 peptide-knockout (orange) were co-cultured with T-Synapse-TC11 and PBMCs at an E:T ratio of 10:1. Cytotoxicity was quantified by imaging after 48 h, with lysis normalized to peptide-pulsed controls. **(B)** T cell activation was assessed after 24 h by measuring supernatant IFN-γ. Normal cell lines from a range of organ sites were tested. **(C)** HLA-A alloreactivity panel showing no T-cell activation against the non-HLA-A*02:01 alleles tested. **(D)** Whole blood was incubated for 48 h with 100 nM T-Synapse-TC11 or plate-bound anti-CD3/CD28. Cytokine release was quantified by multiplex immunoassay. Data are representative of one of four donors tested.

T-Synapse-TC11 has a half life of 21 days in humanized mice



TCE	Half-life	Mouse strain
T-Synapse-TC11	21 days	Tg32
TCER®	6 days	Tg32
ImmTAC	<24 hours	NSG

All TCEs were dosed at 2mg/kg bolus

T-Synapse-TC11 demonstrates robust biophysical and process developability

Production

Transient CHO Expression, 100ml scale
Product titer 2.21 g/L
Product yield 1.05 g/L

Thermal Stability (DSF)

Tm1 (scTv/scFv) 64.0°C
Tm2 (Fc) 80.1°C

Single-transition scTv/scFv unfolding well separated from Fc; no low-Tm liability observed

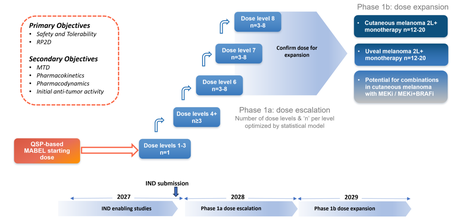
Stress testing

Stress condition	SEC-HPLC Monomer	High molecular weight
0 hr (baseline)	99.6%	0.4%
3 hr - pH3.5 30 mg/ml	99.5%	0.5%
1 week - 25°C 40 mg/ml	99.0%	1.0%
3 week - 25°C 10 mg/ml	100.0%	0.0%
1 week - 40°C	99.3%	0.7%
5 freeze-thaw cycles	99.2%	0.8%

No visible particulates or turbidity change across 5 freeze/thaw cycles (-196°C ↔ RT)

Molecule has good thermal stability and tolerates the intended low-pH viral-inactivation hold and routine freeze-thaw handling with no material quality loss – supports a conventional Protein A / low-pH platform process.

T-Synapse-TC11 FIH clinical trial design



References

1. Nathan et al., N Engl J Med 2021;385:1196-206.
2. Obsidian Therapeutics Corporate Presentation, Aug '26
3. Immatics Corporate Presentation, Aug '26
4. PRAME Phase 1 study of Brenetafusp (IMCF106C) in cutaneous melanoma, May 2024

Ethical Approval

Work using human blood derived cells was approved by the South Central - Berkshire Research Ethics Committee; approval number 22/SC/0025. Patient samples - Research samples were obtained from the Manchester Cancer Research Centre (MCRC) Biobank, UK. The MCRC Biobank holds a generic ethics approval which can confer this approval to users of banked samples via the MCRC Biobank Access Policy.

CONCLUSIONS

TC11 is a highly abundant, broadly expressed HLA-A*02:01-presented melanoma target and is detectable across diverse melanoma cohorts, including tumors exposed to checkpoint inhibitor therapy.

T-Synapse-TC11 converts the advantages of TC11 target abundance into potent T cell activation and tumor cell killing while maintaining a favorable preclinical safety profile.

Collectively, these data support T-Synapse-TC11 as a promising next-generation TCR-TCE for patients with advanced and checkpoint inhibitor-resistant melanoma.