Leveraging Immuno-STAT stimulation via TCR and IL-2 signaling to specifically control CAR T cell expansion and persistence *in vivo*

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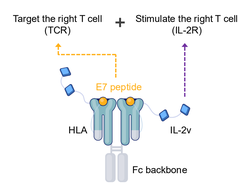
Abstract: CAR-T cell therapy (CAR-T) has remarkable success in hematologic malignancies, but limited persistence remains a challenge contributing to disease relapse. IL-2 signaling through a CAR-independent circuit uniquely eradicated solid tumor models, but the clinical utility of non-specific cytokine CAR-T support (e.g. IL2; aldesleukin) is limited by toxicity. Immuno-STATs™ are designed to activate and expand antigen-specific CD8⁺ T-cells via co-delivery of peptide/HLA-based TCR stimulation and an IL-2 variant. The first clinical-stage Immuno-STAT, CUE-101, selectively activates and expands HPV-specific CD8⁺ T-cells and is associated with durable anti-tumor responses in HPV⁺ recurrent/metastatic head and neck cancers, with the objective response rate more than doubled in combination with pembrolizumab vs historic control of pembrolizumab alone (NCT03978689). While IL-2 signaling pathway remains a major component of T-cell persistence, combining natural TCR signaling could lead to optimal expansion and persistence of CAR-T cells. Here we report pre-clinical proof-of-concept for genetic engineering of combined exogenous-TCR co-expression plus a CAR (*E*xogenous-*T*CR; E.T. or E.T.CAR) paired with TCR and IL-2 signaling from an Immuno-STAT to enable direct regulation of E.T.CAR T cell expansion and persistence *in vivo*.

1 CUE-101 is a selective engager of HPV16-specific T cells

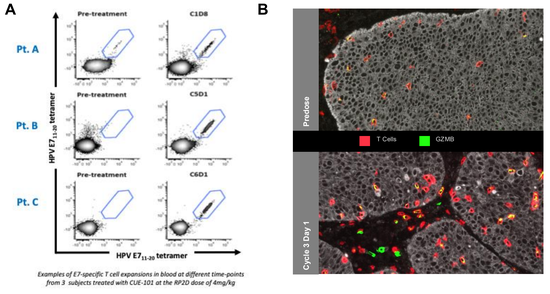


CUE-101 is a modular T cell engager:

- Two HLA-A*02:01 complexes
- An immunodominant peptide epitope derived from the HPV16 E7 viral oncoprotein
- 4 molecules of reduced affinity Interleukin-2 variant (IL-2v)

Quayle *et al*, *Cancer Res*, 2020

2 Tumor-specific T cell expansion and infiltration



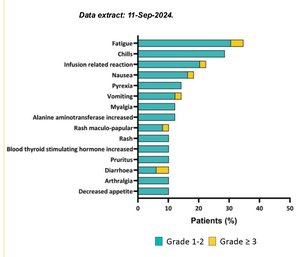
Examples of E7-specific T cell expansions in blood at different time-points from 3 subjects treated with CUE-101 at the RP2D dose of 4mg/kg

(A) Selective expansion of HPV16 E7-specific CD8 T cells in blood of relapsed/metastatic HPV16⁺ Head and Neck Squamous Cell Carcinoma (R/M-HNSCC) patients treated with CUE-101 monotherapy in study CUE-101-01 (NCT03978689). RP2D, recommended Phase 2 dose.

(B) Pre- and post-treatment biopsies from a patient exhibited an increase in tumor-infiltrating, Granzyme positive T cells. GZMB, Granzyme B.

Chung *et al*, ASCO, 2022

3 CUE-101 is safe and tolerable in patients



CUE-101 monotherapy (N=49; All doses)

>90% AEs are CTCAE Grade 1-2

No capillary leak syndrome

No CRS >Grade 1

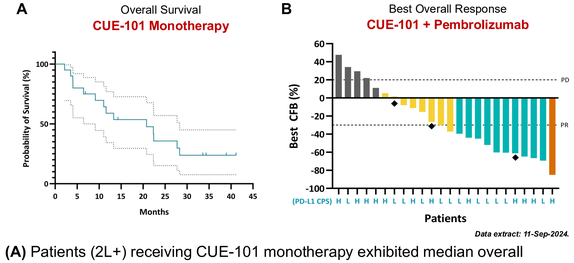
Most common AEs include fatigue, chills, IRR, nausea, and vomiting

AEs readily managed with standard of care treatment

AEs: Adverse Events
CTCAEs: Common Terminology Criteria for Adverse Events
CRS: Cytokine release syndrome
IRR: Infusion related reaction

Chung *et al*, *Journal for ImmunoTherapy of Cancer*, SITC 2024

4 Favorable evidence of CUE-101 clinical benefit in R/M HNSCC



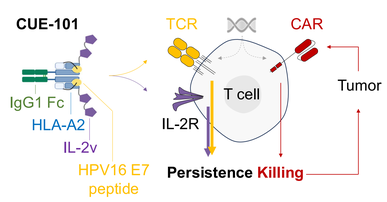
(A) Patients (2L+) receiving CUE-101 monotherapy exhibited median overall survival of 20.8 months.

(B) CUE-101 + Pembrolizumab combination therapy in treatment naïve patients (1L) resulted in a confirmed objective response rate of 46%.

CFB, Change from baseline. PD, Progressive Disease. PR, Partial Response. CPS, Combined Positive Score.

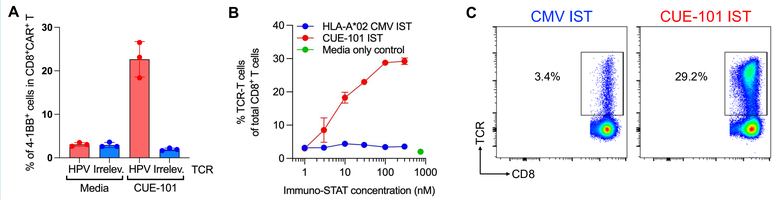
Chung *et al*, *Journal for ImmunoTherapy of Cancer*, SITC 2024

5 Boosting CAR-T cells with CUE-101



We propose to implement a novel orthogonal system where CAR T cells co-express an exogenous TCR (E.T.CAR) responsive to CUE-101. A unique combination of survival and proliferation signals (TCR + IL-2) will then be selectively delivered decoupled from CAR signaling.

6 CUE-101 specifically activates E.T.CAR T cells *in vitro*

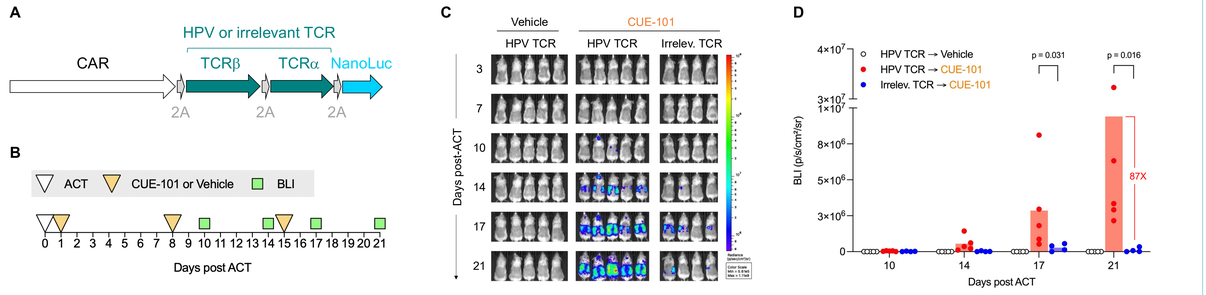


(A) HPV or irrelevant TCR E.T.CAR T cells were treated with CUE-101 (300 nM) or media only. After 24 hours, 4-1BB induction was quantified by Flow cytometry in CD8⁺ transduced cells.

(B) HPV E.T.CAR CD8⁺ T cells were mixed with PBMCs at a 1:9 ratio and treated with increasing concentrations of on-target (CUE-101) or off-target (CMV) Immuno-STATs (IST). After one week in culture, the percentage of TCR⁺ cells among live CD8⁺ T cells was quantified by Flow cytometry.

(C) Representative Flow cytometry analysis at 300 nM dose. Gated on live CD8⁺ T cells.

7 *In vivo* CUE-101 treatment induces substantial and selective E.T.CAR T cell expansion in tumor-free mice



(A) Schematic of multi-cistronic viral E.T.CAR vectors including NanoLuc reporter for *in vivo* bioluminescence imaging (BLI).

(B) Experimental timeline for adoptive cellular transfer (ACT) of E.T.CAR T cells (0.65×10^6, IV), CUE-101 treatment (30 mg/kg, IV, 3 weekly doses) or vehicle, and BLI analysis.

(C) Representative BLI images showing NanoLuc signal as a readout of E.T.CAR T cell expansion.

(D) Quantification of BLI signal over time.

Conclusions

CAR-T cells can be engineered to co-express an exogenous HPV-specific TCR (E.T.CAR T cells) and become responsive to CUE-101.

CUE-101 boosts E.T.CAR T cell expansion *in vivo* in a selective manner, independently of CAR-mediated activation.

This novel orthogonal system could prevent cancer relapse resulting from CAR-T cell exhaustion/contraction in both liquid and solid tumors.

High applicability using already **clinically derisked** CUE-101 and **flexibility** to any CAR specificity.