CUE-101, a Novel HPV16 E7:pMHC:IL-2:Fc Fusion Protein, Enhances Tumor Antigen Specific T Cell Activation for the Treatment of HPV16-Driven Malignancies

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Background

- Oncogenic human papilloma virus (HPV) is the causative agent for many cervical and anal cancers and HNSCC (Trottier 2006; Forman 2012)
- Approximately 70-80% of HPV-driven oropharyngeal cancers in the US are HPV16 / 18 driven, and their incidence continues to rise (Chaturvedi 2011; Berman 2017). Innovative therapies are urgently needed for these malignancies, particularly in the largely incurable metastatic setting
- The E7 oncoprotein is constitutively expressed in HPV-associated cancers, is necessary for initiation and maintenance of malignant transformation, and is genetically conserved in cancer (Mirabello 2017)
- Clinical proof of concept for HPV-targeted T cell therapy includes demonstration of complete regression of metastatic cervical cancer upon adoptive transfer of tumor-infiltrating T cells (Stevanovic 2015; Stevanovic 2017)
- The E7 sequence, including that encoding the $E7_{11\text{-}20}$ peptide in CUE-101, is maintained in cancer and this epitope is immunodominant in humans (Ressing 1995)
- Immuno-STAT™ molecules are engineered to selectively modulate the activity of antigen-specific T cells in vivo

CUE-101

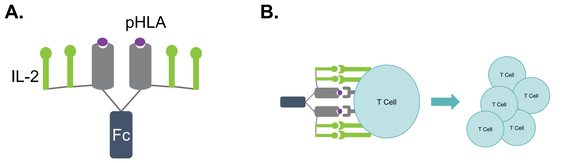

Figure 1 – Schematic of CUE-101 design and mechanism of action. (A) CUE-101, a novel human fusion protein, is comprised of a human leukocyte antigen (pHLA) complex, HLA-A*0201, with a peptide epitope derived from the HPV16 E7 protein (amino acid residues 11-20), a reduced affinity human interleukin-2 (IL-2) variant, and an effector attenuated human immunoglobulin G (IgG1) Fc domain. **(B)** CUE-101 is proposed to selectively bind and activate antigen-specific CD8⁺ T cells present in patients with HPV16-driven malignancies. Upon binding and activation, target CD8⁺ T cells are stimulated to proliferate and eradicate the tumor.

Attenuation of IL-2 binding & activity

A.

Construct	Human IL-2R	$K_D \pm SD$ (nM)
Wildtype IL-2-FLAG	IL-2Rα	10.7 ± 1.4
Mutated IL-2-FLAG	IL-2Rα	1180 ± 124.9
Wildtype IL-2-FLAG	IL-2Rβ	197.0 ± 1.6
Mutated IL-2-FLAG	IL-2Rβ	613.2 ± 31.0

B.

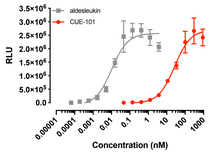

Figure 2 – Mutation of IL-2 components of CUE-101 reduce IL-2R binding and function. (A) Double H16A; F42A mutation of IL-2 reduces binding affinity to human IL-2Rα (~100-fold) and IL-2Rβ (~3-fold) subunits. **(B)** Relative light units (RLU) as a measure of proliferation of the IL-2-dependent cell line CTLL-2 upon culture with CUE-101 or recombinant IL-2, aldesleukin. While CUE-101 alone is sufficient to enable proliferation of CTLL-2 cells, the potency of CUE-101 is reduced >2,000-fold relative to wildtype aldesleukin.

CUE-101 selectively activates antigen-specific T cells

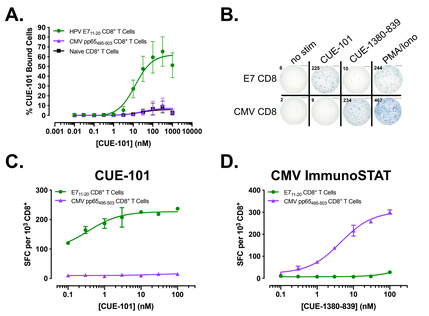

Figure 3 – CUE-101 selectively binds and activates antigen specific CD8⁺ T cells. (A) CUE-101 potently and selectively binds to E7-specific CD8⁺ T cells but not to CMV $pp65_{495\text{-}503}$-specific CD8⁺ T cells or primary naïve CD8⁺ T cells. CUE-101 binding induced phosphorylation of SLP76 and STAT5, demonstrating functional engagement of the TCR and IL-2R, respectively (not shown). **(B-D)** CUE-101 induced potent and concentration-dependent secretion of IFN-γ, a T cell effector cytokine, from primary purified human $E7_{11\text{-}20}$-specific T cells. In contrast, CUE-101 treatment did not activate CMV $pp65_{495\text{-}503}$-specific T cells **(C)**. Through substitution with the $pp65_{495\text{-}503}$ peptide, a CMV-specific Immuno-STAT (CUE-1380-839) was generated that specifically activated $pp65_{495\text{-}503}$-specific T cells but not $E7_{11\text{-}20}$-specific T cells **(D)**.

CUE-101 selectively expands HPV $E7_{11\text{-}20}$-specific CD8⁺ T cells from healthy human PBMCs

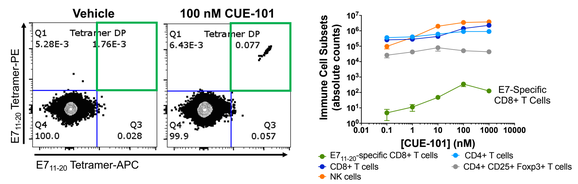

Figure 4 – CUE-101 selectively expands E7-specific CD8⁺ T cells from whole human PBMCs *in vitro*. (A) Primary human PBMCs were exposed to increasing concentrations of CUE-101 alone for 10 days. While $E7_{11\text{-}20}$-specific CD8⁺ T cells were undetectable at baseline (not shown) and after vehicle treatment, CUE-101 treatment elicited a population of $E7_{11\text{-}20}$-specific CD8⁺ T cells as measured by dual tetramer staining. **(B)** Expansion of E7-specific CD8⁺ T cells occurred in a concentration-dependent manner. Increasing expansion of total NK and total CD8⁺ cells was also observed in response to CUE-101 treatment.

CUE-101 expands polyfunctional $E7_{11\text{-}20}$-specific CD8⁺ T cells from human PBMCs

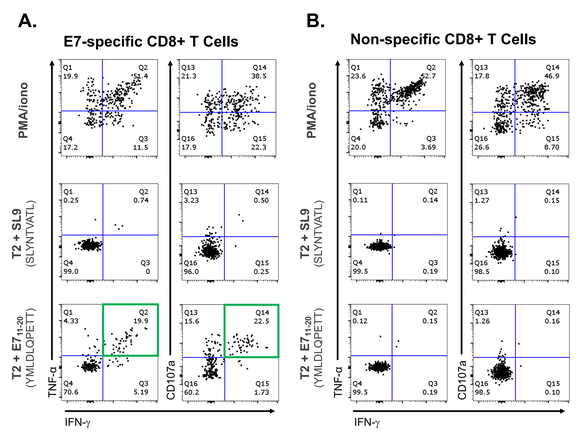

Figure 5 – Expanded E7-specific CD8⁺ T cells exhibit polyfunctional response to peptide presentation on target cells. PBMCs were cultured in the presence of CUE-101 for 10 days and CD8⁺ cells were then magnetically enriched and cultured with T2 target cells. **(A)** Representative dot plots demonstrating that CUE-101-expanded CD8⁺ T cells that bind $E7_{11\text{-}20}$-HLA-A*0201 tetramer produce IFN-γ, TNF-α, and CD107a in response to stimulation with $E7_{11\text{-}20}$ peptide presented by target T2 cells, confirming the specificity and functionality of these cells. Presentation of an irrelevant peptide (HIV SL9 peptide) does not result in activation of tetramer positive cells. **(B)** Amongst CD8⁺ cells that do not bind $E7_{11\text{-}20}$ tetramer there is no activation upon restimulation with either $E7_{11\text{-}20}$ or HIV SL9 peptides.

CUE-101 expands an oligoclonal TCR repertoire that recognizes both $E7_{11\text{-}20}$ and $E7_{11\text{-}19}$ peptides

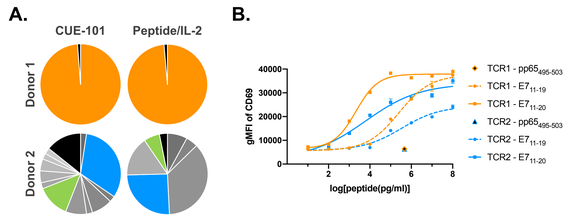

Figure 6 – CUE-101 expands an oligoclonal TCR repertoire. After CUE-101 expansion of PBMCs, tetramer-positive cells were single cell sorted and TCR sequenced. **(A)** Pie charts showing the relative proportion of individual TCR sequences amongst all those sequenced post CUE-101 treatment. Each color represents a single TCR clone, with identical colors identifying sequences that are shared between samples. Shades of grey indicate unique TCR clones, black represents all TCRs that were only identified once. **(B)** Geometric median fluorescence intensity (gMFI) of CD69 expression on SKW3 cells expressing the two most dominant TCRs identified in **(A)**. Cells were stimulated by T2 cells loaded with $E7_{11\text{-}20}$ or $E7_{11\text{-}19}$ peptide.

CUE-101 selectively expands HPV $E7_{11\text{-}20}$-specific CD8⁺ T cells in HLA-A2 transgenic mice

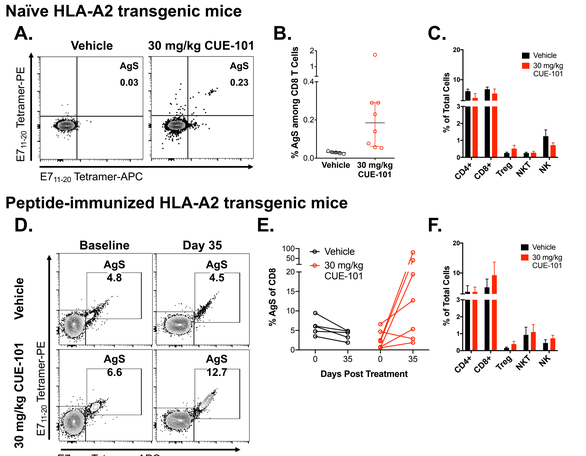

Figure 7 – CUE-101 expands $E7_{11\text{-}20}$-specific CD8⁺ T cells in HLA-A2 mice. (A-C) Naïve HLA-A2 transgenic mice were dosed intravenously (IV) once weekly for 5 weeks with CUE-101 and the frequency of $E7_{11\text{-}20}$-specific CD8⁺ T cells was assessed in peripheral blood. Ag-specific CD8⁺ T cells expanded in response to CUE-101 treatment **(A & B)** without broadly affecting other immune lineages **(C)**. **(D-F)** HLA-A2 transgenic mice were immunized with $E7_{11\text{-}20}$ peptide and rested to allow Ag-specific T cells to contract prior to IV dosing of CUE-101 on Days 1, 8, and 29. Preexisting Ag-specific CD8⁺ T cells expanded in response to CUE-101 treatment **(D & E)** and repeated CUE-101 treatment did not broadly affect the peripheral immunophenotype **(F)**.

E7-specific cells expanded in vivo by CUE-101 are functional and capable of killing target cells

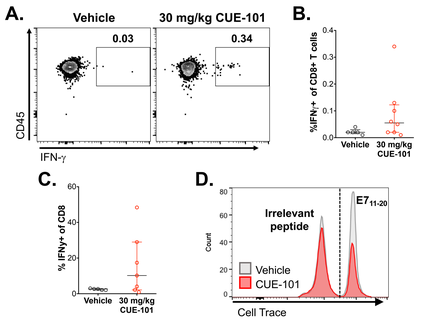

Figure 8 – CTL function of $E7_{11\text{-}20}$-specific CD8⁺ T cells expanded by CUE-101 in vivo. Treatment of naïve **(A & B)** or peptide-immunized **(C)** HLA-A2 transgenic mice with CUE-101 increases frequencies of CD8⁺ T cells in the spleen that produce IFN-γ in response to $E7_{11\text{-}20}$ peptide, showing that CUE-101 expands functional CD8⁺ T cells in vivo. **(D)** Naïve HLA-A2 mice treated with CUE-101 show in vivo antigen-specific killing of splenocytes pulsed with $E7_{11\text{-}20}$ vs. an irrelevant peptide, as shown by the reduced epitope-specific peak in CUE-101 treated mice.

Treatment with mCUE-101 results in functional CD8⁺ T cell-dependent immunological memory

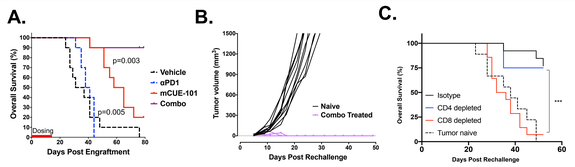

Figure 9 – Surrogate mCUE-101 inhibits TC-1 syngeneic tumor growth alone and in combination with αPD-1 blockade. (A) Kaplan-Meier analysis confirms single agent mCUE-101 significantly extends survival in this model, with significant further extension of survival upon combination treatment with αPD-1. **(B)** Mice remaining tumor-free after combination treatment were rechallenged with TC-1 tumors. While naïve mice formed tumors, previously treated animals rejected tumor formation, demonstrating functional immunologic memory. **(C)** Mice remaining tumor-free after combination treatment were depleted of CD8⁺ or CD4⁺ T cells prior to rechallenge with TC-1 tumors. CD8 depletion significantly reduced survival following rechallenge compared to animals that were not CD8 depleted.

mCUE-101 expands functional antigen-specific CD8⁺ T cells in the tumor and the periphery that express PD-1

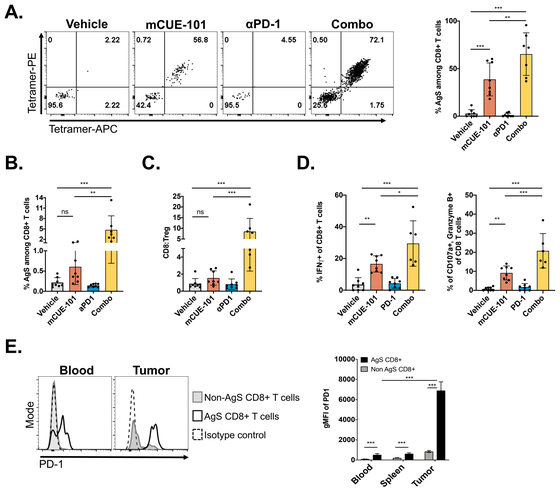

Figure 10 – Surrogate mCUE-101 expands tumor-specific CD8⁺ T cells in the tumor and periphery that functionally respond to cognate peptide. (A-B) Mice bearing established TC-1 tumors were treated with mCUE-101 alone or in combination with αPD-1. Expansion of Ag-specific cells was assessed one week after the last dose of mCUE-101. The frequency of tetramer-positive CD8⁺ T cells is increased in the tumors **(A)** and spleens **(B)** of mCUE-101 treated animals. **(C)** CD8:Treg ratio is significantly increased amongst tumor-infiltrating lymphocytes (TIL). **(D)** mCUE-101 increased the frequency of CD8⁺ T cells that produce IFN-γ and Granzyme B and upregulate CD107a in response to E7 peptide restimulation of TILs. **(E)** Flow histograms display PD-1 expression on E7-specific CD8⁺ T cells (black) vs non-E7-specific CD8⁺ T cells (gray), or isotype control (dashed line) in peripheral blood and tumor. mCUE-101 treatment significantly increased ($p<0.001$) PD-1 expression levels (gMFI) on E7-specific CD8⁺ T cells present within the TC-1 tumors, providing mechanistic rationale for combination therapy.

NCT03978689 Trial Design

A Phase 1, First-in-Human, Open-Label, Dose Escalation and Expansion Study of CUE-101 Monotherapy in Patients with HPV+ Recurrent/ Metastatic Head and Neck Squamous Cell Carcinoma (HNSCC)

Key Eligibility Criteria
- HLA-A*0201 genotype
- Tumor(s) positive for HPV16 and P16INK4A
- Recurrent/metastatic HNSCC that has progressed following at least 1 prior systemic therapy
- ECOG ≤1

Part A: Escalation
- 3+3 dose escalation
- CUE-101 Q3W IV

Part B: Expansion
- Up to n=20 patients at MTD or biologically effective dose

Primary Endpoints
- Safety & serum PK
- Biologically effective dose

Secondary Endpoints
- ORR, DOR, PFS, and OS
- Immunologic response (E7-specific CD8⁺ T cells)

Exploratory Endpoints
- Circulating HPV DNA, immunophenotyping, serum cytokines

Conclusions

- CUE-101 demonstrates selective binding, receptor signaling, effector T cell cytokine secretion, and expansion of functional HPV16 $E7_{11\text{-}20}$ specific primary human CD8⁺ T cells
- A murine surrogate of CUE-101 inhibits the growth of E7-expressing TC-1 syngeneic tumors, selectively expands antigen-specific CD8⁺ T cells in the tumor and periphery, and generates CD8⁺-dependent immunologic memory against TC-1 tumor cells
- Increased expression of PD-1 was observed in tumor-infiltrating antigen-specific T cells after Immuno-STAT treatment, and combination therapy with αPD-1 blockade further enhanced anti-tumor activity in the TC-1 model
- The novel mechanism of action of CUE-101, namely targeted activation of tumor-antigen-specific CD8⁺ T cells via delivery of reduced affinity mutant IL-2, supports its potential for anti-cancer efficacy in an ongoing Phase 1 clinical trial in relapsed/metastatic HNSCC (NCT03978689)

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