CUE-101, a novel Fc fusion protein for selective targeting and expansion of anti-tumor T cells for treatment of HPV-driven malignancies

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Background

- Human papilloma virus (HPV) is responsible for 72% of oropharyngeal, 70% of cervical, 90% of anal, and 71% of vulvar, vaginal, or penile cancers, causing significant morbidity and mortality worldwide. 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 $E7_{11-20}$ 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 situ*

CUE-101

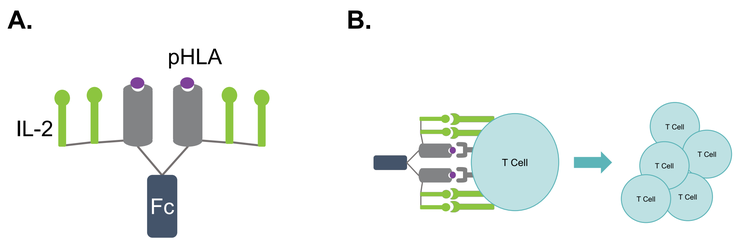

Figure 1 – Schematic of CUE-101 design and mechanism of action. (**A**) CUE-101, a novel human fusion protein, is comprised of a human leukocyte antigen (pHLA) complex, HLA-A*0201, with a peptide epitope derived from the HPV16 E7 protein (amino acid residues 11-20), a reduced affinity human interleukin-2 (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 endogenously present in patients with HPV16-driven malignancies. Upon binding and activation, target CD8+ T cells are stimulated to proliferate and eradicate the tumor.

Methods

- CUE-101 cellular binding, specificity, TCR- and IL-2 receptor (IL-2R)-induced signaling, and induction of activation and cytotoxic T lymphocyte markers, were measured using flow cytometry with human $E7_{11-20}$-specific CD8+ T cells (Astarte Biologics, Bothell, WA)
- Enzyme-Linked ImmunoSpot (ELISpot) assays were performed to measure peptide-specific secretion of interferon (IFNγ)
- Selective expansion of HPV16 $E7_{11-20}$-specific CD8+ T cells by CUE-101 was performed from primary human PBMCs *in vitro*, and in HLA-A2 transgenic mice *in vivo*
- Anti-tumor efficacy with a murine surrogate molecule (mCUE-101) was assessed in the TC-1 syngeneic tumor model, and antigen-specific T cell expansion *in vivo* was measured via tetramer staining

CUE-101 selectively binds antigen-specific T cells

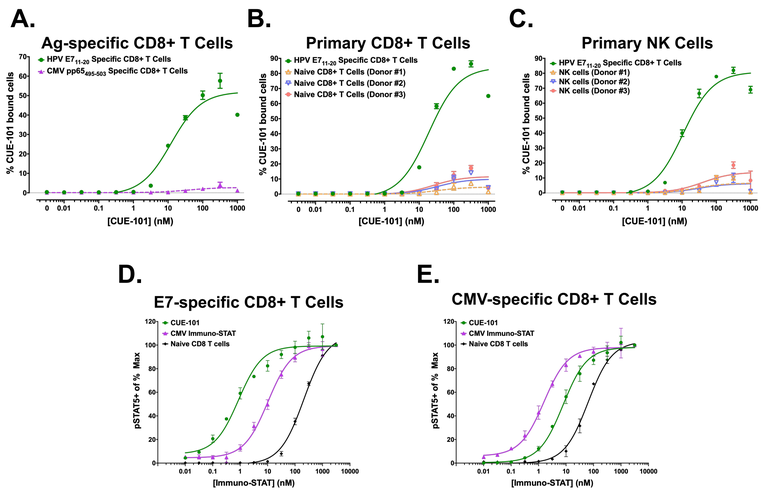

Figure 2 – CUE-101 selectively binds and stimulates signal transduction in antigen (Ag) - specific CD8+ T cells. (**A-C**) CUE-101 potently and selectively binds to E7-specific T cells but not to CMV $pp65_{495-503}$-specific T cells (**A**), primary naïve CD8+ T cells (**B**), or primary NK cells (**C**) that also express IL-2 receptor (IL-2R). (**D-E**) The pHLA specificity of CUE-101 enables potent and selective stimulation of phosphorylation of STAT5 (pSTAT5) immediately downstream of IL-2R engagement on target T cells. (**D**) CUE-101 (HPV-directed) induces pSTAT5 with greater potency in $E7_{11-20}$-specific CD8+ T cells than does a CMV-directed Immuno-STAT. (**E**) A CMV-directed Immuno-STAT induces pSTAT5 with greater potency in CMV $pp65_{495-503}$-specific CD8+ T cells than does CUE-101 (HPV-directed). Induction of pSTAT5 is further reduced in naïve CD8+ T cells relative to activated antigen-specific CD8+ T cells.

CUE-101 selectively elicits effector cytokine production

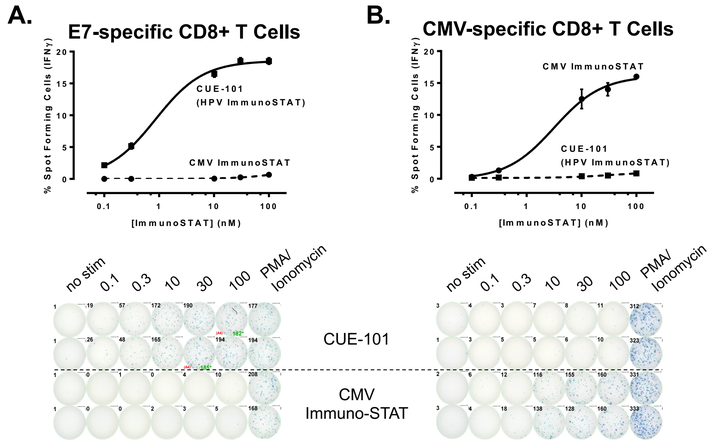

Figure 3 – CUE-101 selectively induces effector cytokine production from antigen-specific CD8+ T cells. (**A**) CUE-101 treatment of E7-specific CD8+ T cells induces dose-dependent secretion of IFNγ as assessed by ELISpot. In contrast, treatment of E7-specific CD8+ T cells with a CMV-directed Immuno-STAT does not elicit IFNγ secretion, supporting that the pHLA complex of CUE-101 drives selectivity. (**B**) In CMV-specific CD8+ T cells, only treatment with a CMV-directed Immuno-STAT elicits IFNγ secretion while CUE-101 does not.

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

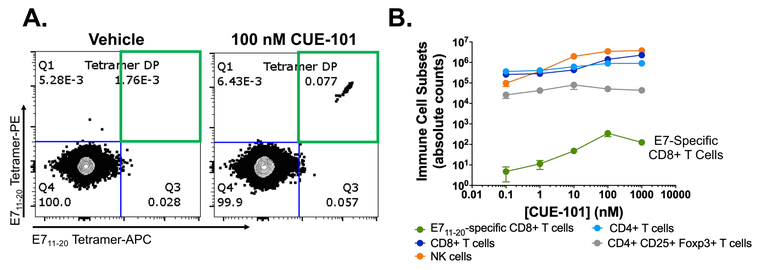

Figure 4 – CUE-101 selectively expands E7-specific CD8+ T cells from whole human PBMCs *in vitro*. (**A**) Primary human PBMCs were exposed to increasing concentrations of CUE-101 alone for 10 days. While $E7_{11-20}$-specific CD8+ T cells were undetectable at baseline (not shown) and after vehicle treatment,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

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

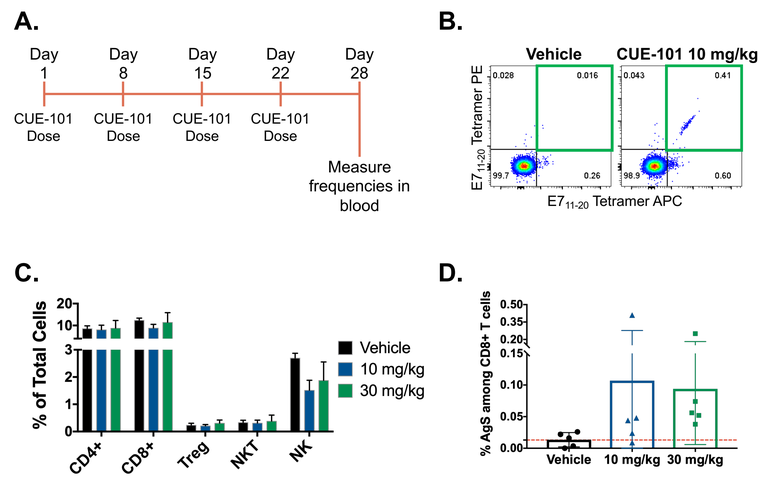

Figure 5 – CUE-101 selectively expands E7-specific CD8+ T cells after *in vivo* treatment of HLA-A2 transgenic mice. (**A**) Schematic of experimental design. CUE-101 was dosed intravenously (IV) once weekly and the frequency of antigen (Ag) – specific cells and other immune lineages was assessed in peripheral blood. (**B**) Tetramer staining identified expansion of a population of $E7_{11-20}$-specific CD8+ T cells in response to CUE-101 treatment. (**C-D**) Repeated CUE-101 treatment did not broadly affect the peripheral immunophenotype of mice (**C**) at *in vivo* exposures resulting in selective expansion of target T cells (**D**).

Murine CUE-101 (mCUE-101) surrogate inhibits tumor growth in the TC-1 syngeneic model

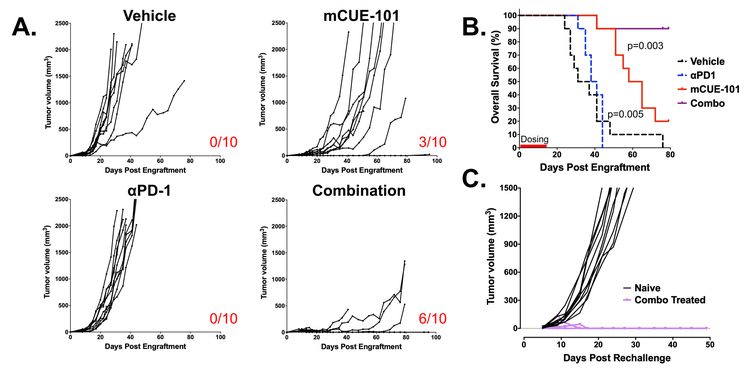

Figure 6 – A CUE-101 murine surrogate (mCUE-101) inhibits TC-1 syngeneic tumor growth alone and in combination with αPD-1 blockade, and generates immunologic memory. (**A**) Spider plots of individual tumor volume growth following treatment with the indicated agents. The frequency of tumor-free mice at Day 90 post-injection is indicated. (**B**) Kaplan-Meier survival analysis confirms single agent mCUE-101 significantly extends overall survival in this model, with significant further survival upon combination treatment with αPD-1. (**C**) Mice remaining tumor-free after combination treatment were rechallenged with TC-1 tumors 97 days post primary tumor challenge. While naïve mice all formed tumors, previously treated animals rejected tumor formation, thus demonstrating functional immunologic memory.

mCUE-101 expands antigen-specific CD8+ T cells in the tumor and the periphery

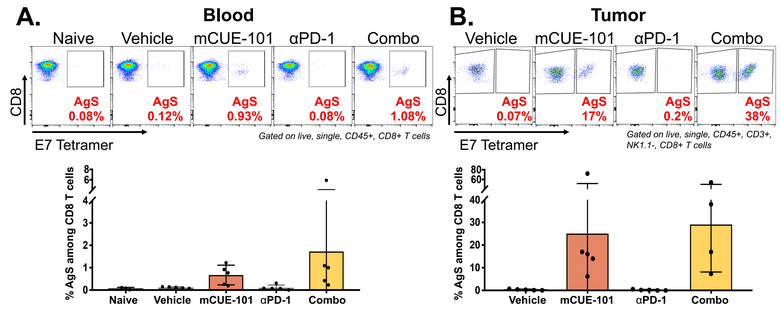

Figure 7 – Surrogate mCUE-101 increases the frequency of E7-specific CD8+ T cells in the tumor and in the periphery. Representative flow dot plots show the frequency of tetramer-positive CD8+ T cells in the blood (**A**) and tumor (**B**) one week after the last treatment. Only animals treated with mCUE-101 exhibited increased frequency of antigen-specific T cells, which was greatly increased within the tumor relative to the periphery.

Antigen-specific TILs upregulate PD-1 expression

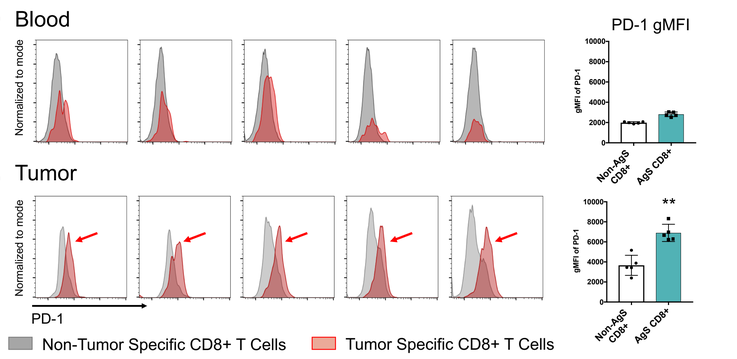

Figure 8 – E7-specific CD8+ tumor infiltrating lymphocytes (TILs) expanded by mCUE-101 treatment upregulate PD-1. Flow histograms are shown of PD-1 expression on E7-specific CD8+ T cells (red) vs non-E7-specific CD8+ T cells (gray) in the peripheral blood (**A**) and within the tumor (**B**) from individual animals treated with single agent mCUE-101. mCUE-101 treatment resulted in significantly (** p = 0.007) increased PD-1 expression levels (gMFI) on the E7-specific CD8+ T cells present within the TC-1 tumors.

Conclusions

- CUE-101 demonstrates selective binding, receptor signaling, effector T cell cytokine secretion, and expansion of HPV16 $E7_{11-20}$ 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 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 increased potential for anti-cancer efficacy and reduced toxicity relative to non-targeted forms of immunotherapy.

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