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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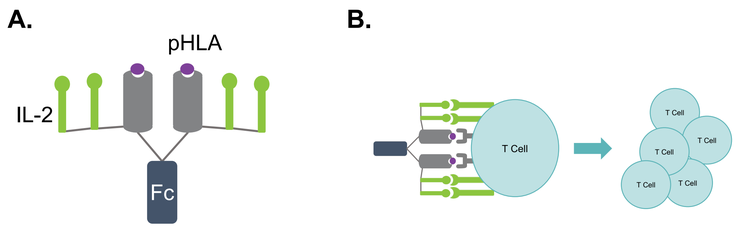
Background

- Human papilloma virus (HPV) is responsible for 72% of oropharyngeal, 90% 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 $E7_{11\text{-}20}$ 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 situ*

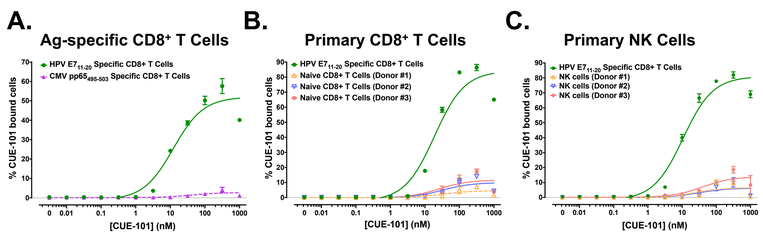
CUE-101



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.

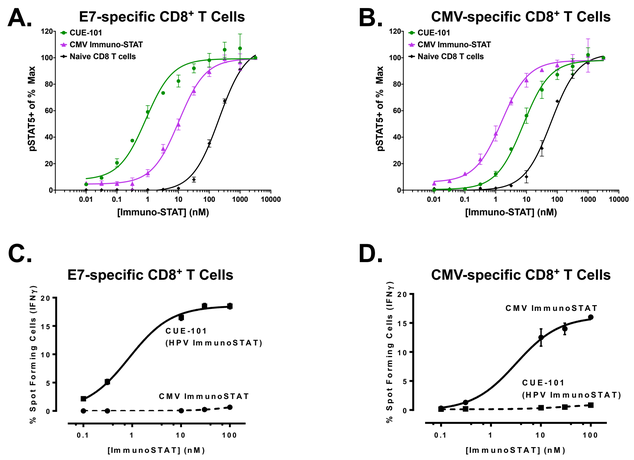
CUE-101 selectively binds antigen-specific T cells and elicits effector cytokine production

CUE-101 selectively binds antigen (Ag)-specific CD8⁺ T cells



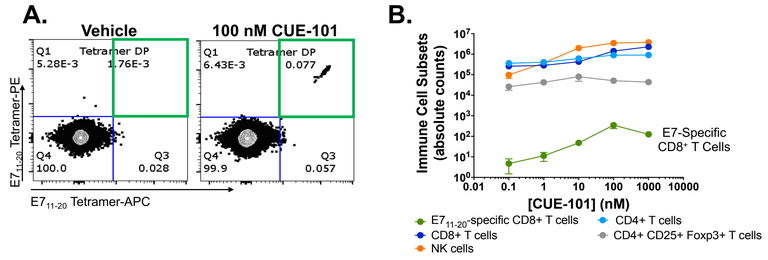
CUE-101 potently and selectively binds to E7-specific T cells but not to CMV $pp65_{495\text{-}503}$-specific CD8+ T cells (**A**), primary naïve CD8⁺ T cells (**B**), or primary NK cells (**C**) that also express IL-2 receptor (IL-2R).

CUE-101 selectively induces signaling and effector cytokine production in antigen-specific CD8⁺ T cells



(**A-B**) The pHLA specificity of CUE-101 enables selective stimulation of phosphorylation of STAT5 (pSTAT5) immediately downstream of IL-2R on target T cells. (**A**) CUE-101 (HPV-directed) induces pSTAT5 with greater potency in $E7_{11\text{-}20}$-specific CD8⁺ T cells than does a CMV-directed Immuno-STAT. (**B**) A CMV-directed Immuno-STAT induces pSTAT5 with greater potency in CMV $pp65_{495\text{-}503}$-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. (**C**) CUE-101 treatment of E7-specific CD8⁺ T cells induces dose-dependent secretion of IFN-γ as assessed by ELISpot. In contrast, treatment of E7-specific CD8⁺ T cells with a CMV-directed Immuno-STAT does not elicit IFN-γ secretion, supporting that the pHLA complex of CUE-101 drives selectivity. (**D**) In CMV-specific CD8⁺ T cells, only treatment with a CMV-directed Immuno-STAT elicits IFN-γ secretion while CUE-101 does not.

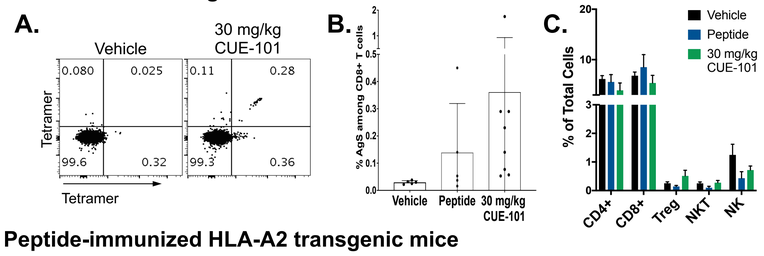
CUE-101 selectively expands $E7_{11\text{-}20}$-specific CD8⁺ T cells from healthy human PBMCs



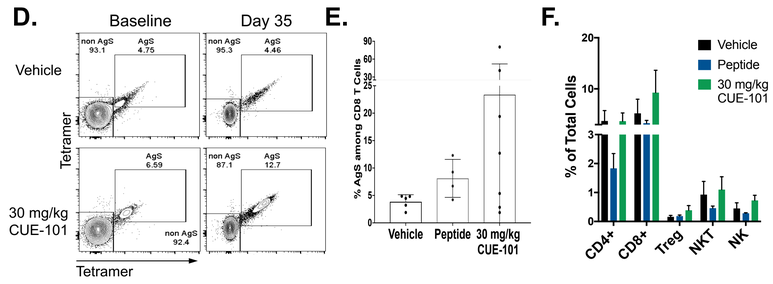
CUE-101 selectively expands $E7_{11\text{-}20}$-specific CD8⁺ T cells from whole human PBMCs *in vitro*. (**A**) Primary human PBMCs were exposed to increasing concentrations of CUE-101 for 10 days. CUE-101 elicited a population of $E7_{11\text{-}20}$-specific CD8⁺ T cells measured by tetramer staining, while vehicle treatment did not. (**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 functional HPV $E7_{11\text{-}20}$-specific CD8⁺ T cells in HLA-A2 transgenic mice

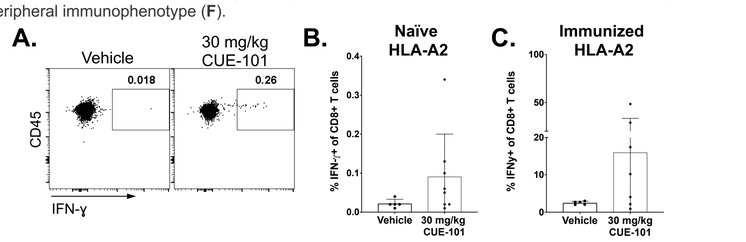
Naïve HLA-A2 transgenic mice



Peptide-immunized HLA-A2 transgenic mice

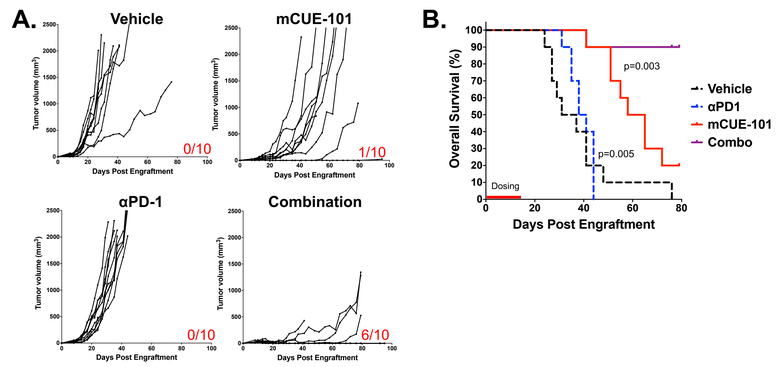


CUE-101 expands $E7_{11\text{-}20}$-specific CD8⁺ T cells in HLA-A2 mice. (A-C) Naïve HLA-A2 transgenic mice were dosed intravenously (IV) once weekly for 5 weeks with CUE-101 and the frequency of $E7_{11\text{-}20}$-specific CD8⁺ T cells was assessed in peripheral blood. Ag-specific CD8+ T cells expanded 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**).



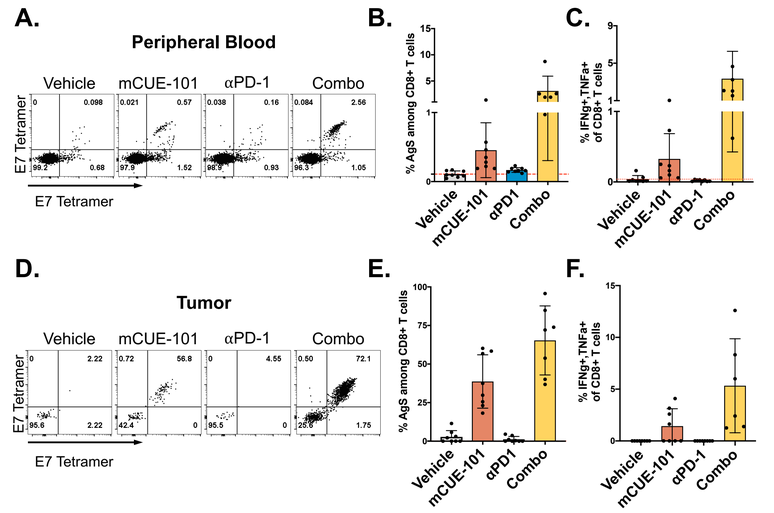
Cytokine production in $E7_{11\text{-}20}$-specific CD8⁺ T cells expanded by CUE-101. Treatment of naïve (**A & B**) or peptide -immunized (**C**) HLA-A2 transgenic mice with CUE-101 as described above increases frequencies of CD8+ T cells in the spleen that produce IFN-γ in response to $E7_{11\text{-}20}$ peptide, demonstrating that CUE-101 expands functional CD8+ T cells in vivo.

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



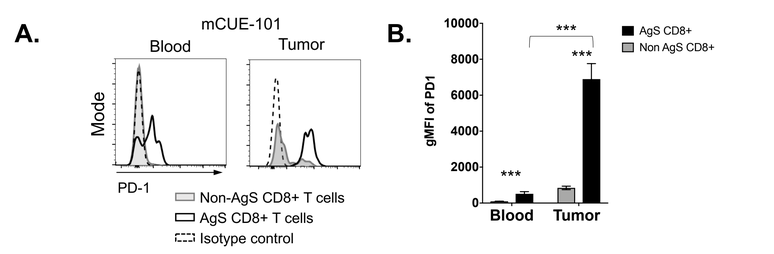
mCUE-101 inhibits TC-1 syngeneic tumor growth alone and in combination with αPD-1 blockade. (**A**) Spider plots of individual tumor volumes following treatment with the indicated agents. The frequency of tumor-free mice at Day 90 post-injection is indicated. (**B**) Kaplan-Meier survival analysis confirms single agent mCUE-101 significantly extends overall survival in this model, with significant further survival upon combination treatment with αPD-1.

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



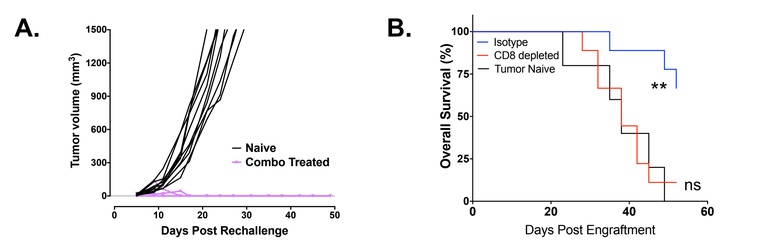
Mice bearing established TC-1 tumors were treated with 15 mg/kg mCUE-101 alone or in combination with αPD-1. Expansion of Ag-specific cells was assessed one week after the last dose of mCUE-101. Representative flow plots show the frequency of tetramer-positive CD8⁺ T cells in the blood (**A & B**) and tumor (**D & E**). Only animals treated with mCUE-101 exhibited increased frequency of Ag-specific T cells, which was greatly increased in the tumor vs blood. mCUE-101 increased the frequency of CD8⁺ T cells that produced IFNγ and TNFα in response to $E7_{49\text{-}57}$ peptide restimulation of splenocytes **(C)** and tumor-infiltrating lymphocytes **(F)**.

Surrogate mCUE-101 upregulates PD-1 expression on tumor-infiltrating E7-specific CD8⁺ T cells



(A) 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. **(B)** 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 rationale for combo therapy.

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



(A) 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, demonstrating functional immunologic memory. **(B)** Mice remaining tumor free after combination treatment were depleted of CD8⁺ T cells or treated with an isotype control antibody prior to rechallenge with TC-1 tumors. CD8 depletion of previously treated animals significantly reduced survival following rechallenge compared to animals that were not CD8 depleted, demonstrating that control of tumor growth following rechallenge is dependent on CD8⁺ T cell memory.

Conclusions

- CUE-101 demonstrates selective binding, receptor signaling, effector T cell cytokine secretion, and expansion of HPV16 $E7_{11\text{-}20}$ specific primary human CD8⁺ T cells.
- CUE-101 expands functional $E7_{11\text{-}20}$-specific CD8+ T cells in both naïve and peptide-immunized HLA-A2 transgenic mice, demonstrating that CUE-101 is able to expand functional $E7_{11\text{-}20}$-specific CD8+ T cells from the naïve repertoire or from a pre-existing population of antigen-specific cells.
- A murine surrogate of CUE-101 inhibits the growth of E7-expressing TC-1 syngeneic tumors, selectively expands functional antigen-specific CD8⁺ T cells in the tumor and periphery, and generates CD8+ T cell-dependent immunologic memory against TC-1 tumor cells.
- Treatment with mCUE-101 was associated with increased expression of PD-1 on tumor antigen-specific CD8⁺ T cells resident in the tumor microenvironment, providing a rationale for combination therapy with PD-1 blockade.
- 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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