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

Steven N. Quayle1, Natasha Giris1, Dharma Raj Thapa1, Zohra Merazaga2, Melissa Kemp3, Alex Histed4, Fan Zhao5, Miguel Moreta6, Paige Ruthardt7, Sandrine Hulot8, Alyssa Nelson9, Lauren D. Kramer10, Dominick R. Beal3, Mark Haydock3, Luke Witt3, Jessica Ryabin3, Jonathan Soriano3, Emily Spaulling3, John F. Ross3, Rodolfo Chaparro11, Ronald Seidt11, Sasu Cemerski12, Kenneth J. Pianta13, Mary C. Simon14

1Cue Biopharma, Cambridge, Massachusetts; 2The James Buchanan Brady Urological Institute and the Department of Urology, Johns Hopkins School of Medicine, Baltimore, Maryland

Background

- Oncogenic human papillomavirus (HPV) is the causative agent for many cervical and anal cancers and HNSCC (Trottier 2008; Forrest 2013).
- Approximately, 70% of HPV-driven neoplasms in the US are HPV16, 18 driven, and their incidence continues to rise (Chaturvedi 2011; Bereman 2017). Innovative therapies are urgently needed for these malignancies, particularly in the largely intractable 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 (S presently 2011).
- Clinical proof of concept for HPV-targeted T cell therapy includes demonstration of complete regression of metastatic cervical cancer caused by tumor-infiltrating T cells (Serebrjakov 2019, Steinov 2017).
- The E7 sequence is included in the mRNA of CUE-101, is maintained in cancer and is immunodominant in humans (Rasool 1995).
- TCRs are inducible to selectively modulate the antigen-specific T cells in vivo.

CUE-101 selectively activates HPV E7-specific CD8+ T cells from healthy human PBMCs

Naive HLA-A2 transgenic mice

- CTL function of E7-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 expression of CD8+ T cells in the system that produce IFN-γ in response to E7 T-cell peptide, showing that CUE-101 expands functional E7-specific CD8+ T cells in vivo.
  - Native HLA-A2 mice treated with CUE-101 shows in vivo antitumor activity of oligoclonal populations of activated T cells with an irrelevant peptide, as shown by the reduced epitope-specific peak, in CUE-101 treated mice.

CUE-101 expands oligoclonal TCR repertoire that recognises both E7p and E7m peptides

- Mice treated with CUE-101 show a functional antitumor response.
  - The novel mechanism of action of CUE-101, namely targeted activation of tumor-antigen-specific CD8+ T cells, could serve as a delivery of reduced toxicity motif (Liu 2017).
- A major surrogate of CUE-101 is the inhibition of the expression of T-cell 1 cytokines, selectively expanding antigen specific CD8+ T cells in the tumor and peripheral, and generates CD8+ dependent immunologic memory against TC1-tumor cells.
- Increased expression of PD-1 was observed in tumor-infiltrating antigenic-specific T cells after Immuno-STAT treatment, and combination therapy with PD-1 blockade further enhanced antitumor activity in the TC1 model.1

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

Figure 3 - Schematic of CUE-101 design and mechanism of action. (A) CUE-101, a novel human tumor vaccine, is composed of: (1) HPV16 E7 oncoprotein antigen (HPV16 E7), complexed with HLA-A2 (E7p), with a peptide epitope derived from the HPV16 E7 protein (pyrroloidine 1-10;20), a reduced affinity human interleukin-2 (IL-2) variant, and an efficacious attenuated human immunophospholipid (Gp[51]i) 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 secrete the tumor.

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 E7m-specific CD8+ T cells were undetectable at baseline (not shown) and after vehicle treatment, CUE-101 treatment elicited a population of E7p-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 exposure of total T and total CD8+ T cells was also observed.

Figure 5 – Expanded E7-specific CD8+ T cells exhibit polyfunctional phenotype to recognize target cells. PBMCs were cultured in the presence of CUE-101 for 10 days and CD8+ T cells were isolated and cocultured with E7 tetramer labeled target cells. (A) Representative dot plots demonstrating that CUE-101 expanded E7p-specific T cells bind E7m, E7/pMHC tetramer, labeled T2 target cells. (B) T2 target cells were pretreated with 50μM E7m peptide to reduce the background of E7m-specific T cells. (C) E7m-specific T cells in response to E7-pMHC stimulation with E7m peptide and specific E7m epitope, and then allowed to allow Ag-specific T cells to contract prior to FACS staining of CUE-101 on Day 8. TCR reporter expression was measured by intracellular tetramer expression in response to CUE-101 Treatment (A-E) and expanded CUE-101 treatment did not affect the peripheral immunophenotype (F).

Figure 6 – CUE-101 expands an oligoclonal TCR repertoire that recognises both E7p and E7m peptides. (A) E7-matched peptide and specific E7m CD8+ T cells but not CUE-101 expanded peptide-specific CD8+ T cells. CUE-101 binding induced phenotypic changes at IL-8 and STAT5 and demonstrated functional expression of the TCR and IL-2, respectively. (B) CUE-101-induced patient and concentration-dependent secretion of IL-12, IL-2 and IL-15 cytokines, and CUE-101 treatment did not activate CUE-101 specific peptide specific CD8+ T cells. (C) CUE-101 induced patient and concentration-dependent secretion of IFN-γ, IL-2, and IFN-γ as cytokine effector cytokines, from primary enriched human E7-specific CD8+ T cells. In contrast, CUE-101 treatment did not activate CUE-101 specific peptide specific CD8+ T cells. (D) CUE-101 induced patient and concentration-dependent secretion of IL-12, IL-15 cytokines, and CUE-101 binding induced phenotypic changes at IL-8 and STAT5 and demonstrated functional expression of the TCR and IL-2, respectively. (E) CUE-101-induced patient and concentration-dependent secretion of IFN-γ, IL-2, and IFN-γ as cytokine effector cytokines, from primary enriched human E7-specific CD8+ T cells.