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CUE-100 series Immuno-STATs from concept to the clinic: Leveraging protein engineering to stimulate and selectively deliver affinity-attenuated IL-2 to antigen-specific T cells

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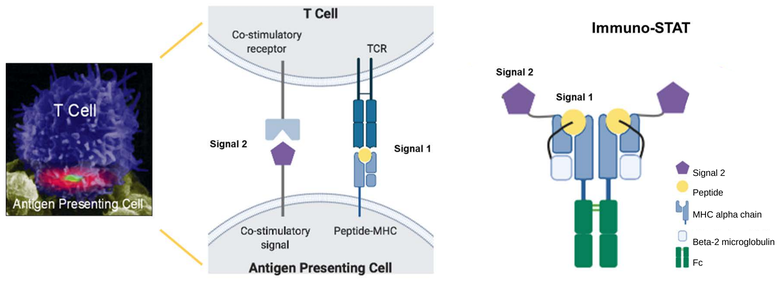
Background

Activation of T cells requires a specific peptide/HLA (human leukocyte antigen) signal presented by an interacting immune or target cell along with engagement of co-stimulatory molecules or cytokine receptors. Cue Biopharma has developed a proprietary biologics platform, termed Immuno-STAT™ (*Selective Targeting and Alteration of T cells*), wherein a singular protein framework incorporates peptide/HLA complexes and co-stimulatory, co-inhibitory or cytokine molecules. The CUE-100 series Immuno-STATs selectively deliver rationally engineered IL-2 molecules (IL-2 variants) to antigen-specific T cells. The IL-2 variants in the CUE-100 series Immuno-STATs contain mutations that attenuate binding to IL-2 receptors alpha and beta, which minimizes activation of regulatory T cells (Tregs) and the irrelevant non-antigen-specific T cell repertoire. We have demonstrated that CUE-100 series Immuno-STATs specific for different antigenic peptides (from HPV16, WT1, MART-1, CMV, FLU virus, and HIV) induce expansion of functional, oligoclonal, antigen-specific repertoires from human PBMCs. The lead clinical candidate CUE-101, presenting the $E7_{11-20}$ peptide from HPV-16 in the context of HLA-A*02:01, is currently being tested in a Phase 1 clinical trial in recurrent/metastatic head and neck cancer patients with evidence of dose-proportional PK, early pharmacodynamic effects and signals of clinical activity.

Cue Biopharma's Immuno-STAT Platform



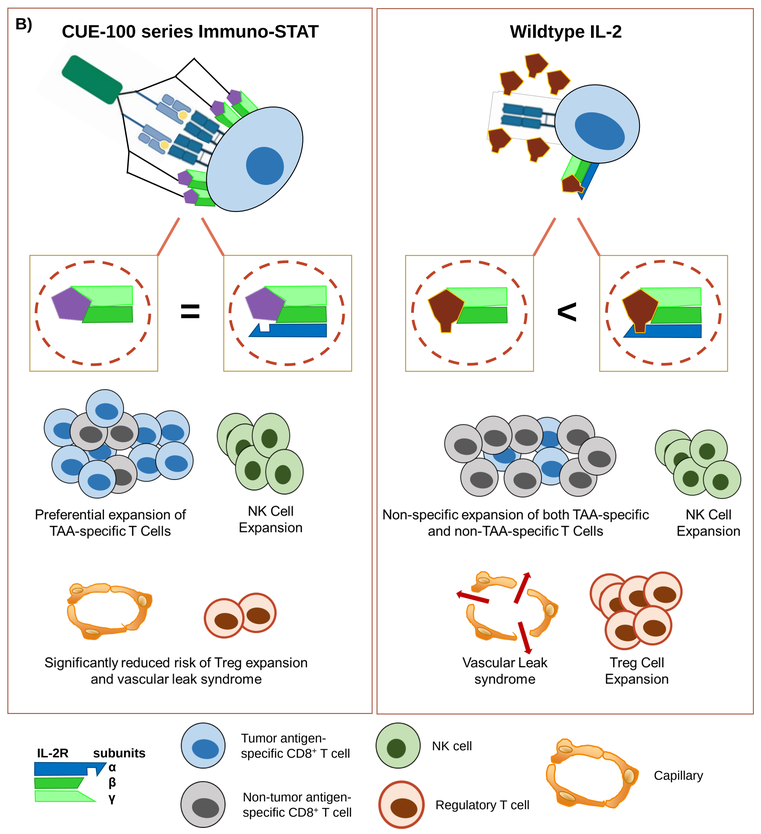
The intimate interactions between the T cells and APCs occur within a molecular interface known as the immunological or immune synapse. The immune synapse allows controlled engagement and selective activation of T cells through the presentation of two key distinct signals: Signal 1, TCR engagement by the pMHC; and Signal 2, co-stimulatory, co-inhibitory or cytokine signals. Through rational protein engineering, we have developed a proprietary class of biologics termed **Immuno-STATs** that induce and modulate T cell activity via delivery of the distinct signals provided naturally to T cells within the immune synapse. We accomplish this by the co-engineering of a TCR targeting pMHC with co-stimulatory, co-inhibitory or cytokine signaling molecules in a singular biologic on an Fc framework.

CUE-100 Series Immuno-STATs

CUE-100 Series Signal 2: Affinity Attenuated IL-2 Variant

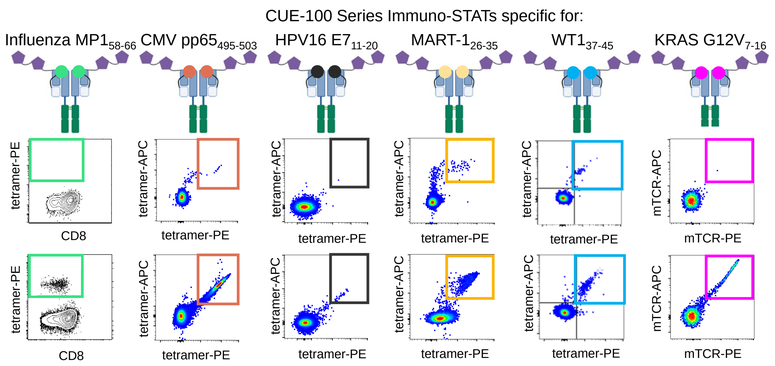
- Abrogated binding to IL-2Rα
- Reduced binding to IL-2Rβ
- Four affinity attenuated IL-2 units per Immuno-STAT

Maintains ability of IL-2 to stimulate antigen-specific T cells while reducing undesired effects of IL-2



Through structure-based protein engineering, CUE-100 series molecules selectively deliver IL-2 to tumor associated antigen (TAA)-specific T cells via binding of the pHLA complex to TAA-specific TCRs. To increase selectivity for target T cells and to minimize the potential for toxicity mediated through global IL-2-driven activation of IL-2 receptor (IL-2R) expressing cells, two point mutations (H16A and F42A) were introduced into the IL-2 sequences of CUE-100 series Immuno-STATs. These mutations were previously demonstrated to reduce IL-2 interaction with the IL-2Rα and IL-2Rβ chains, respectively. The binding affinity of a double mutant for human IL-2Rα and IL-2Rβ was decreased 110-fold and 3-fold, respectively, compared to wildtype IL-2 binding, predominantly due to a faster off-rate for each of these interactions. Functional attenuation of the mutant IL-2 components of a CUE-100 series Immuno-STAT (CUE-101) was also demonstrated in a CTLL-2 proliferation assay, where CUE-101-induced proliferation was reduced ~2,600-fold relative to recombinant human IL-2 (rhIL-2). As a reminder, a slightly modified rhIL-2 is commercially available as aldesleukin (Proleukin®).

CUE-100 Series Immuno-STATs Induce Expansion of Human Antigen-Specific T Cells



Healthy donor PBMCs were stimulated for 10 days with the CUE-100-Series Immuno-STATs specific for the indicated antigens (*bottom*). Each IST can expand relevant antigen-specific T cells from healthy donor PBMCs or TCR-transduced CD8+ T cells spiked into healthy donor PBMCs (in the case of G12V KRAS). Peptide-specific CD8+ T cells were detected by flow cytometry upon staining with antigen-specific tetramers or anti-mTCR antibodies to detect the transduced G12V KRAS TCR containing a murine constant region. Unstimulated cells were used as negative control (*top*).

CUE-101 and CUE-102/A02 for Patients with HPV16-Associated and WT1-Associated Malignancies: Design, Manufacturability Assessment and Biophysical Characterization

Peptide	11-20
Peptide Sequence	YMLDLQPETT
Titer (g/L)	1.1
% Monomer (post Pro A)	90
% Monomer (final)	99
Stability (1m @ 4°C / 25°C / 40°C) Monomer Recovery %	99 / 99 / 42
Freeze-thaw (0x / 1x / 3x) (% monomer by SEC)	99 / 99 / 99
T_{m1} (°C)	48
Intact mass (LC-MS)	Confirmed

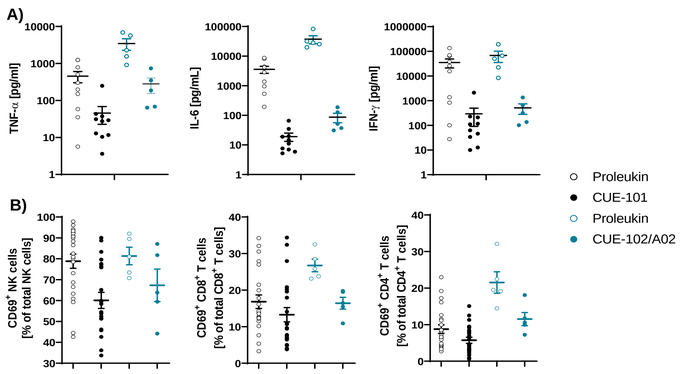
Clonal stable cell line
Optimized formulation

Peptide	37-45
Peptide Sequence	VLDFAPPGA
Titer (mg/L)	281
% Monomer (post Pro A)	61
% Monomer (final)	98
Stability (10d @ 4°C / 37°C / 42°C) Monomer Recovery %	100 / 88 / 24
Freeze-thaw (0x / 1x / 3x) (% monomer by SEC)	98 / 98 / 98
T_{m1} (°C)	51
Intact mass (LC-MS)	Confirmed

Transiently transfected cells
Non-optimized formulation

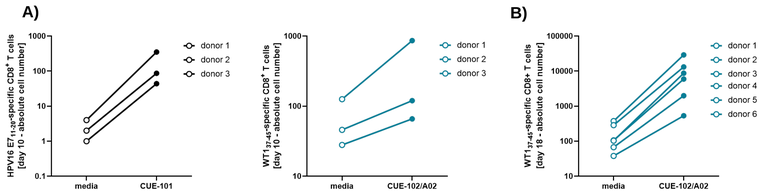
Diagrams of CUE-101 and CUE-102/A02: The chains are assembled (N-term to C-term) in the following order: Chain A, IL-2–HLA alpha chain–Fc; Chain B, peptide–beta-2-microglobulin.
Tables: Manufacturability assessment and biophysical characterization of CUE-101 and CUE-102/A02.

CUE-101 and CUE-102/A02 vs Wild-Type IL-2: CUE's Attenuated IL-2 Mitigates the Risk Associated with Systemic IL-2 Activation



Healthy donor PBMCs were stimulated with Proleukin® (100 nM or 125 nM), CUE-101 (100 nM or 125 nM) or CUE-102/A02 (100 nM) in ImmunoCult™ media for 18 hours. **A)** Upon stimulation, supernatants were harvested, and levels of TNF-α, IL-6 and IFN-γ were assessed by MSD. **B)** Upregulation of CD69 on NK cells, CD4+ T cells and CD8+ T cells was assessed by flow cytometry on cells from the same culture wells. Supernatants and cells from unstimulated wells were used as control and showed no/minimal cytokine production and CD69 expression.

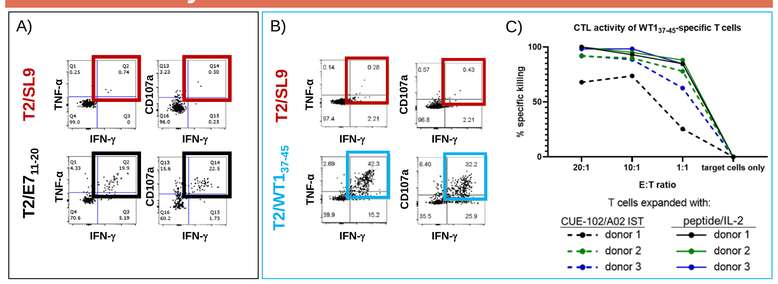
CUE-101 and CUE-102/A02 Selectively Expand Antigen-Specific CD8+ T Cells from Healthy Human PBMCs



A) Healthy donor PBMCs were stimulated for 10 days with CUE-101 or CUE-102/A02 Immuno-STAT in ImmunoCult™ media. Peptide-specific CD8+ T cells were detected by flow cytometry upon staining with HPV16 $E7_{11-20}$ and $WT1_{37-45}$-specific tetramers. Unstimulated cells were used as negative control.

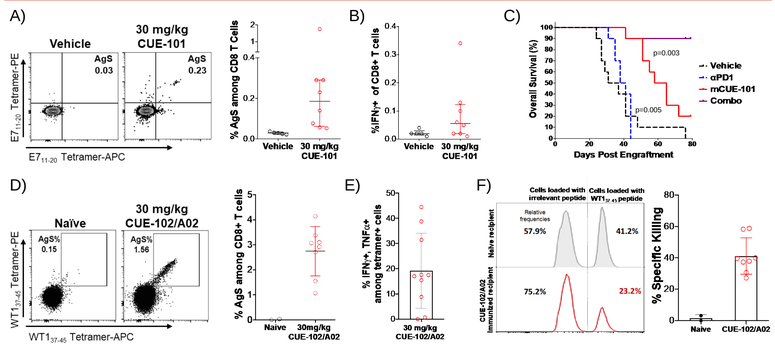
B) Healthy donor PBMCs were primed for 10 days with $WT1_{37-45}$ peptide in the presence of rhIL-2. CD8+ T cells were then enriched by magnetic separation and restimulated with the CUE-102/A02 Immuno-STAT in ImmunoCult™ media in the presence of mitomycin C-treated autologous PBMCs for 8 days. Peptide-specific CD8+ T cells were detected by flow cytometry upon staining with $WT1_{37-45}$-specific tetramers. Unstimulated cells were used as negative control.

CUE-101 and CUE-102/A02 Expand Antigen-Specific Polyfunctional Effector CD8+ T Cells



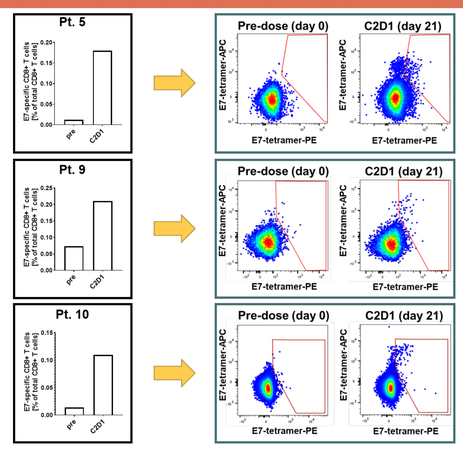
Healthy donor PBMCs were primed for 10 days with $E7_{11-20}$ peptide **(A)** and $WT1_{37-45}$ peptide **(B,C)** in the presence of rhIL-2 and then expanded for 8 days with CUE-101, CUE-102/A02 or $WT1_{37-45}$ peptide in ImmunoCult™ media in the presence of mitomycin C-treated autologous PBMCs. **(A,B)** CUE-101- and CUE102/A02-expanded antigen-specific T cells express effector cytokines IFN-γ and TNF-α, and upregulate the degranulation marker CD107a upon 4 hours of interaction with target T2 cells pulsed with the cognate $E7_{11-20}$ and $WT1_{37-45}$ peptides, but not when pulsed with an irrelevant peptide. **(C)** Expanded $WT1_{37-45}$-specific T cells, enriched using bead-based positive selection, kill cognate $WT1_{37-45}$ peptide-pulsed T2 cells but not control peptide-pulsed T2 cells in overnight cultures performed at different T cell effector:target cell ratios. Specific killing is assessed by flow cytometry comparing the ratio of viable T2 cells pulsed with cognate vs control peptide upon overnight culture.

CUE-101 and CUE-102/A02 Expand Antigen-Specific Polyfunctional Effector CD8+ T Cells *In Vivo*



CUE-101 and CUE-102/A02 expand antigen-specific CD8+ T cells in naïve HLA-A2 mice. **(A)** Naïve HLA-A2 transgenic mice were dosed intravenously (IV) once weekly for 5 weeks with CUE-101 and the frequency of $E7_{11-20}$-specific CD8+ T cells was assessed in peripheral blood. **(B)** CUE-101 treatment of naïve mice increases the frequency of CD8+ T cells producing IFN-γ in response to ex vivo $E7_{11-20}$ peptide stimulation. **(C)** Murine surrogate of CUE-101 (mCUE-101) significantly inhibits TC-1 syngeneic tumor growth as monotherapy and in combination with αPD-1 blockade. **(D)** Naïve HLA-A2 transgenic mice were dosed IV once weekly for 4 weeks with CUE-102/A02 and the frequency of $WT1_{37-45}$-specific CD8+ T cells was assessed in peripheral blood. **(E)** CUE-102/A02 treatment increases the frequency of CD8+tetramer+ T cells producing IFN-γ and TNF-α in response to ex vivo $WT1_{37-45}$ peptide stimulation. **(F)** HLA-A2 mice immunized with CUE-102/A02 show antigen-specific in vivo killing of HLA-A2+ target cells pulsed with $WT1_{37-45}$ peptide vs. an irrelevant peptide, as shown by the loss of $WT1_{37-45}$-labeled target cells in CUE-102/A02 immunized HLA-A2 transgenic mice (red), but not in naïve mice (black).

PD Biomarkers in CUE-101 Clinical Trial



CUE-101 is currently being tested in an open-label, 2-part clinical Phase I study to characterize the safety, tolerability, PK, PD, immunogenicity, and preliminary antitumor activity as monotherapy in HLA-A*0201-positive patients with HPV16+ recurrent/metastatic HNSCC tumors. Assessment of the potential to generate an antitumor immune response with CUE-101 treatment is an important secondary objective of the trial. Blood samples are collected prior to dosing and at several different time points following CUE-101 administration. Preliminary data from PBMC immunophenotyping and tetramer staining show early signals of expansion of HPV-16 $E7_{11-20}$-specific CD8+ T cells.

Conclusions

We have developed novel Immuno-STAT fusion proteins comprised of HLA class I molecules presenting peptide epitopes derived from tumor associated antigens of interest, four copies of affinity-attenuated human IL-2, and an effector attenuated human IgG1 Fc domain. CUE-101 Immuno-STAT and CUE-102/A02 Immuno-STAT (developed in collaboration with LG Chem, Korea) induce selective expansion of antigen-specific CD8+ T cells from both unprimed and peptide-primed PBMC. The repertoire of CUE-101 and CUE-102/A02-expanded CD8+ T cells, their polyfunctionality and ability to recognize and respond to cognate peptide-presenting target cells, and their ability to induce expansion of antigen-specific, polyfunctional CD8+ T cells in vivo, suggest that CUE-101 and CUE-102 Immuno-STATs have the potential to enhance anti-tumor immunity in patients with HPV16-positive and WT1-positive malignancies. As exemplary of the platform and the IL-2-based CUE-100 series, our first molecule CUE-101 is currently being evaluated in a Phase I trial in recurrent-metastatic HPV-driven HNSCC.

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