CUE-102 Immuno-STATs for selective targeting and expansion of WT1-specific T cells for the treatment of HLA-A02 and/or HLA-A24 cancer patients expressing WT1

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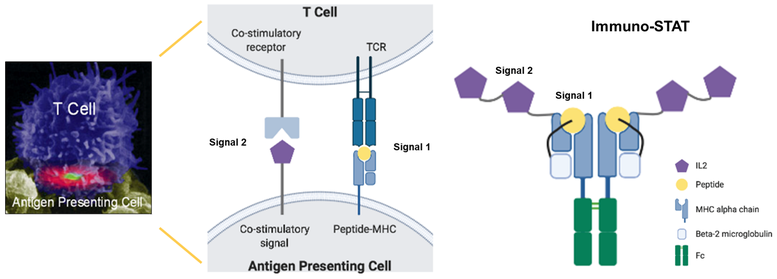
Background

WT1 has been ranked first amongst 75 tumor associated antigens in an effort by the National Cancer Institute to prioritize cancer antigens for therapeutic targeting. Development of novel modalities to target WT1 provide a significant opportunity to address high unmet medical need in WT1-positive malignancies, including AML, ovarian, endometrial, breast, lung, colorectal and pancreatic cancer.

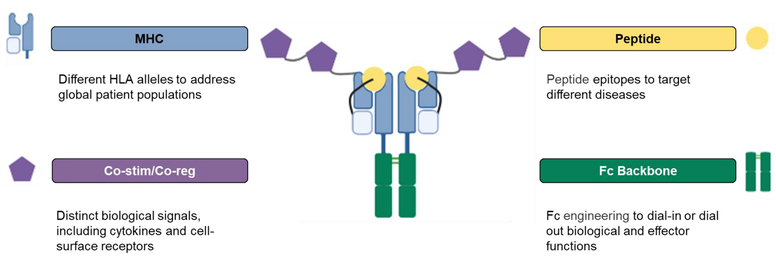
We have developed novel fusion proteins, termed Immuno-STATs™ *(Selective Targeting and Alteration of T cells)*, that are comprised of HLA-A*02 or HLA-A*24 molecules presenting peptide epitopes derived from WT1. Each CUE-102 Immuno-STAT molecule also contains four copies of affinity-attenuated human interleukin-2, and an effector attenuated human immunoglobulin G Fc domain. We present here the biochemical characterization of these molecules and their bioactivity across a variety of in vitro and ex vivo studies, demonstrating the potential for CUE-102 Immuno-STATs to enhance anti-tumor immunity in patients with WT1-positive malignancies.

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 or co-inhibitory 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 signaling molecules in a singular biologic on an Fc framework.

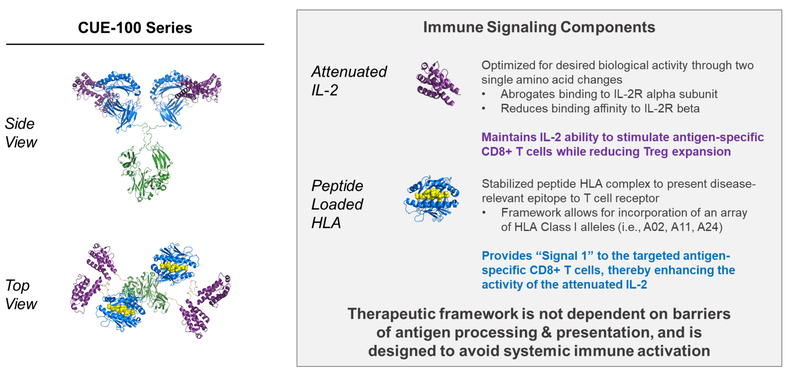
Modularity of the Immuno-STAT Platform



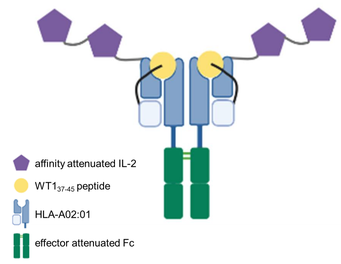
All Immuno-STAT components can be readily exchanged and tailored to appropriately target and regulate the activity of disease relevant T cells in the right patient population in the right disease indication. This modularity and versatility of the platform allows us to design Immuno-STATs that address particular T cell modulation objectives/criteria, including different co-stimulatory/co-inhibitory signals or different targeting peptides and HLA alleles.

- In the context of cancer and chronic infectious disease, Immuno-STATs are designed to selectively activate disease-specific T cells
- For the treatment of autoimmune disease, Immuno-STATs are designed to selectively inhibit pathogenic autoreactive T cells

CUE-100 Series Immuno-STATs Deliver Affinity Attenuated IL-2 to Targeted Antigen-Specific T Cells



CUE-102/A02: Design, Manufacturability Assessment and Biophysical Characterization



Peptide	37-45
Peptide Sequence	VLDFAPPGA
Titer (mg/L)	281
% Monomer (post Protein A)	61
% Monomer (post SEC)	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_m (°C)	51
	66
	81
Intact mass (LC-MS)	Confirmed

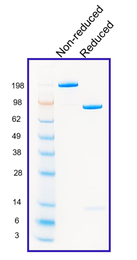
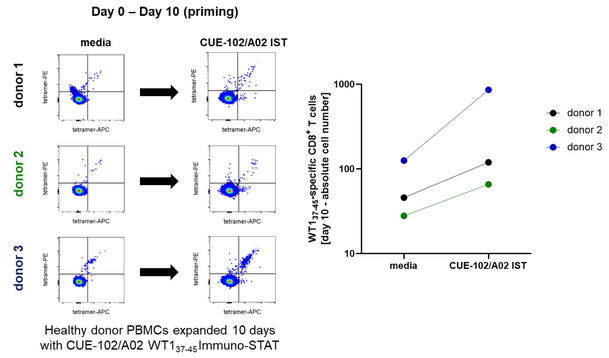


Diagram of CUE-102/A02. The chains are assembled (N-term to C-term) in the following order: Chain A, IL2–HLA alpha chain–Fc; Chain B, peptide–beta-2-microglobulin. Table: Manufacturability assessment and biophysical characterization of CUE-102/A02. Above: Reduced and non-reduced Coomassie-stained SDS-PAGE of the purified CUE-102/A02.

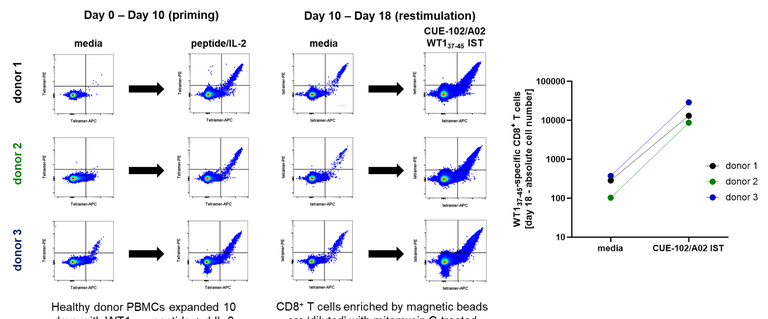
CUE-102/A02 WT1$_{37-45}$ IST Induces Expansion of WT1$_{37-45}$-Specific CD8$^+$ T Cells from Unprimed PBMCs



Healthy donor PBMCs expanded 10 days with CUE-102/A02 WT1$_{37-45}$ Immuno-STAT

Healthy donor PBMCs were stimulated for 10 days with the CUE-102/A02 WT1$_{37-45}$ Immuno-STAT in Immunocult™ media. Unstimulated cells were used as negative control. Peptide-specific CD8$^+$ T cells were detected by flow cytometry upon staining with WT1$_{37-45}$-specific tetramers.

CUE-102/A02 Immuno-STAT Induces Expansion of WT1$_{37-45}$-Specific CD8$^+$ T Cells from Primed PBMCs

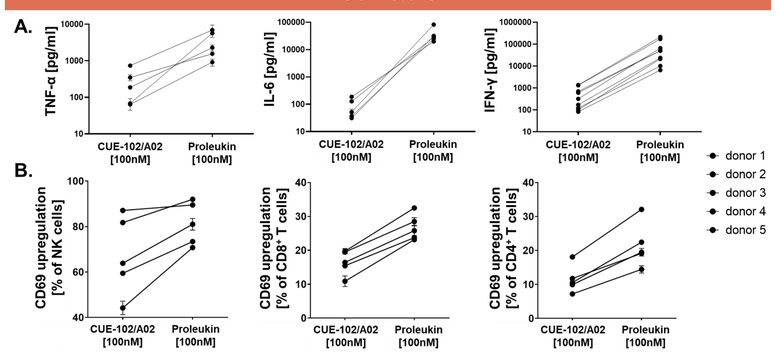


Healthy donor PBMCs expanded 10 days with WT1$_{37-45}$ peptide + rhIL-2

CD8$^+$ T cells enriched by magnetic beads are 'diluted' with mitomycin C-treated autologous PBMCS and restimulated for 8 days with CUE-102/A02 WT1$_{37-45}$ IST

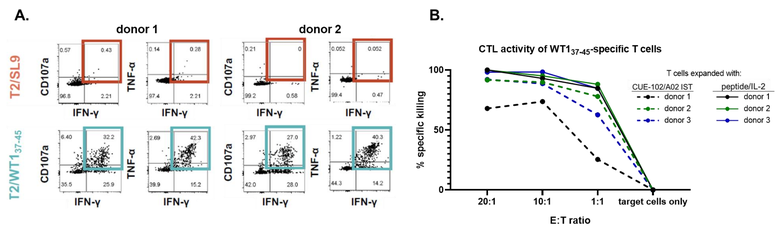
Healthy donor PBMCs were primed for 10 days with WT1$_{37-45}$ peptide in the presence of recombinant human IL-2. CD8$^+$ T cells were then enriched by magnetic separation and restimulated with the CUE-102/A02 Immuno-STAT in Immunocult™ media in the presence of mitomycin C-treated autologous PBMCs for 8 days. Unstimulated cells were used as negative control. Peptide-specific CD8$^+$ T cells were detected by flow cytometry upon staining with WT1$_{37-45}$-specific tetramers.

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



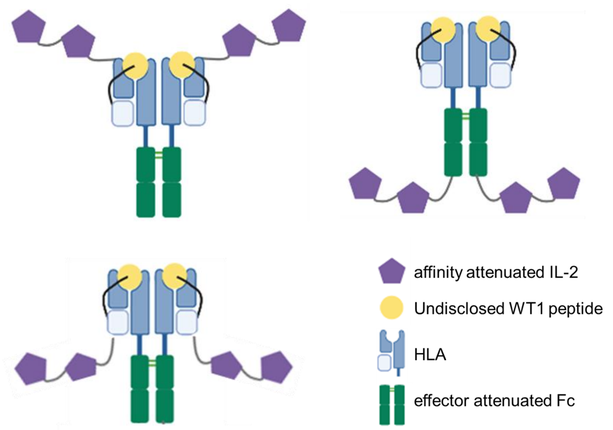
Five healthy donor PBMCs were stimulated with Proleukin® or CUE-102/A02 in Immunocult™ media for 18 hours. Upon stimulation, supernatants were harvested, and levels of TNF-α, IL-6 and IFN-γ were assessed by MSD (A). NK cell, CD4$^+$ T cell and CD8$^+$ T cell CD69 upregulation was assessed by flow cytometry on cells from the same culture wells (B). Unstimulated cells were used as negative control (not shown).

CUE-102/A02 Expanded T Cells are Polyfunctional CTLs that Recognize and Kill WT1$_{37-45}$ Peptide-Presenting Target Cells



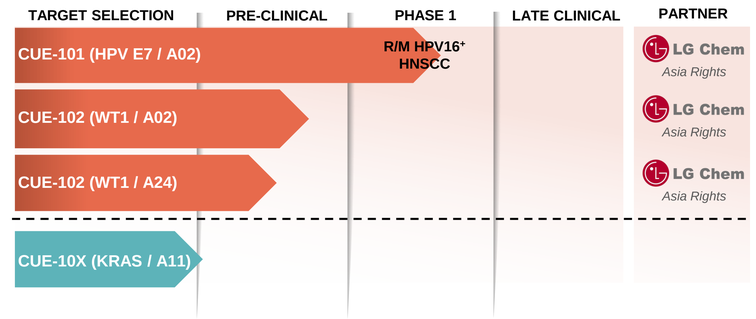
Healthy donor PBMCs were primed for 10 days with WT1$_{37-45}$ peptide in the presence of recombinant human IL-2 and expanded for 8 days with WT1$_{37-45}$ peptide or with the CUE-102/A02 WT1$_{37-45}$ Immuno-STAT in Immunocult™ media in the presence of mitomycin C-treated autologous PBMCs. WT1$_{37-45}$-specific CD8$^+$ T cells were enriched by magnetic bead-based separation using WT1$_{37-45}$-specific PE-labeled tetramers. (A) CUE102/A02 IST-expanded WT1$_{37-45}$-specific T cells express effector molecules IFN-γ, TNF-α, and upregulate the degranulation marker CD107a upon 4 hours of interaction with target T2 cells pulsed with the cognate WT1$_{37-45}$ peptide but not with an irrelevant peptide. (B) Expanded WT1$_{37-45}$-specific T cells 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 cell pulsed with cognate vs control upon overnight culture.

Harnessing platform modularity to develop Immuno-STATs targeting patients of different HLA haplotypes



Immuno-STATs targeting patients of different HLA haplotypes were designed and various Immuno-STAT frameworks, such as those shown above having different epitope candidates and different HLA molecules, were analyzed. Our analysis considered numerous factors specific to the combinations of the framework, HLA allele and potential epitopes, including manufacturability, protein stability and bioactivity.

Immuno-STAT modularity enables platform expansion to HLA-A11$^+$ and HLA-A24$^+$ patients across tumor indications associated with WT1 and KRAS



Conclusions

In partnership with LG Chem, we have developed novel Immuno-STAT fusion proteins that are comprised of HLA-A*02 or HLA-A*24 molecules presenting peptide epitopes derived from WT1, four copies of affinity-attenuated human IL-2, and an effector attenuated human IgG1 Fc domain. CUE-102 molecules induce expansion of WT1-specific CD8$^+$ T cells from both unprimed and peptide-primed PBMC. The repertoire of the CUE-102-expanded CD8$^+$ T cells, their polyfunctionality and ability to recognize and respond to WT1 peptide-presenting target cells suggest that CUE-102 Immuno-STATs have the potential to enhance anti-tumor immunity in patients with 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.

QR code

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