CUE-102 Selectively Activates and Expands WT1-Specific T Cells for the Treatment of Patients with WT1+ Malignancies

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Background

- Immuno-STATs™ (ISTs) are rationally engineered biologics comprised of a bivalent peptide-MHC complex and multivalent co-stimulatory molecules built on an Fc framework to enable stability, valency, favorable PK and manufacturability
- CUE-100 series ISTs are designed to selectively deliver attenuated interleukin-2 (IL-2) to tumor-specific CD8⁺ T cells (Quayle 2020; Seidel 2021)
- Wilms' Tumor 1 (WT1) was previously ranked as the highest priority antigen for therapeutic targeting in an effort by the National Cancer Institute (Cheever 2009)
- Development of novel modalities targeting WT1 provide a significant opportunity to address high unmet medical need in WT1-positive malignancies, including AML, ovarian, endometrial, breast, lung, gastric, colorectal and pancreatic cancer
- CUE-102 is being developed as a novel therapeutic fusion protein to selectively activate tumor antigen-specific T cells to treat WT1-expressing cancers

CUE-100 Series Immuno-STATs

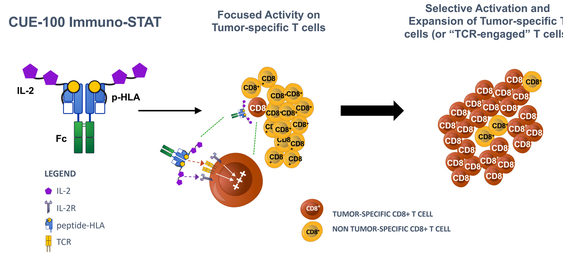
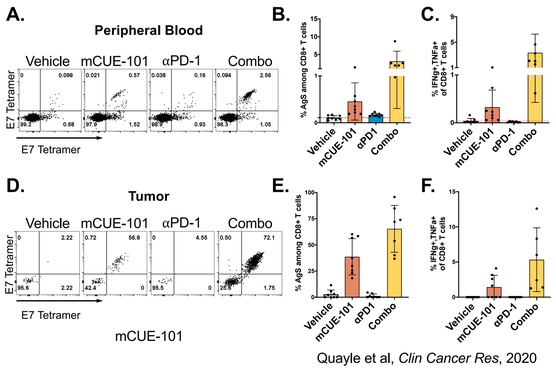


Figure 1 – The rationally engineered and modular biologics of the Immuno-STAT platform incorporate natural biological signals ("cues") for selective engagement and modulation of disease-relevant T cells. The CUE-100 series framework is designed to selectively deliver modified IL-2 to tumor-specific T cells and drive their expansion.

CUE-100 IST Murine Surrogate (mCUE-101) Expands Antigen-Specific CD8⁺ T Cells in Blood and Tumor



Quayle et al, *Clin Cancer Res*, 2020

Figure 2 – 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 antigen-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 antigen-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 peptide restimulation of splenocytes **(C)** and tumor-infiltrating lymphocytes **(F)**.

CUE-102

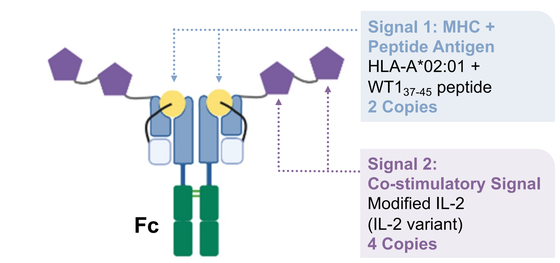


Figure 3 – Schematic of CUE-102 molecule. CUE-102 is comprised of a human leukocyte antigen (HLA) complex, HLA-A*0201, a peptide epitope derived from the WT1 protein, and 4 molecules of a reduced affinity human IL-2. CUE-102 is designed to bind and activate WT1-specific T cells for eradication of WT1-positive cancers.

CUE-102 Selectively Binds and Activates WT1$_{37-45}$-Specific T Cells

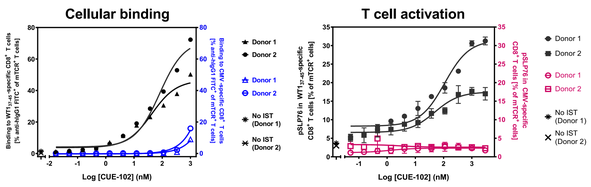


Figure 4 – CUE-102 selectively binds to primary CD8⁺ T cells transduced with a WT1$_{37-45}$-specific TCR, but not to CD8⁺ T cells transduced with a CMV pp65$_{495-503}$-specific TCR. In primary CD8⁺ T cells transduced with WT1$_{37-45}$-specific TCR, CUE-102 selectively activated the TCR in target cells as measured by increased phosphorylation of SLP76 (pSLP76), but not in CD8⁺ T cells transduced with CMV pp65$_{495-503}$-specific TCR.

Attenuation of IL-2 Activity in CUE-102

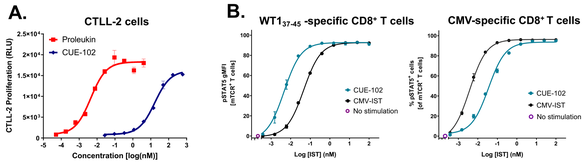


Figure 5 – (A) Human IL-2 molecules on CUE-102 are functionally attenuated and much less potent than recombinant IL-2 (Proleukin®) in a CTLL-2 cell proliferation assay. **(B)** The pHLA specificity of CUE-102 facilitates selective phosphorylation of STAT5 (pSTAT5) immediately downstream of IL-2R on target cells. CUE-102 induces pSTAT5 with greater potency in WT1$_{37-45}$ specific CD8⁺ T cells than a CMV-directed IST. A CMV-directed IST induces pSTAT5 with greater potency in CMV pp65$_{495-503}$-specific CD8⁺ T cells than does CUE-102.

CUE-102 Selectively Expands WT1$_{37-45}$-Specific CD8⁺ T Cells from Human PBMCs

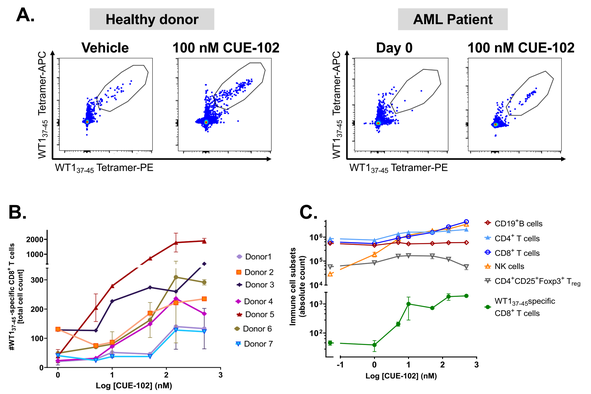


Figure 6 – CUE-102 selectively expands WT1$_{37-45}$-specific CD8⁺ T cells from whole human PBMCs *in vitro*. **(A)** Primary human PBMCs of healthy donors or acute myeloid leukemia (AML) patients were exposed to 100 nM CUE-102 for 10 days. CUE-102 expanded a population of WT1$_{37-45}$-specific CD8⁺ T cells as measured by double tetramer staining, while vehicle treatment did not. **(B)** CUE-102 induces expansion of WT1$_{37-45}$ specific CD8⁺ T cells in PBMCs of multiple donors in a dose-dependent manner. **(C)** Expansion of total NK cells was also observed in response to CUE-102 treatment. Other immune cells in donor PBMCs were not expanded, including CD4⁺ T$_{regs}$.

CUE-102 Selectively Expands Tumor Relevant T Cells

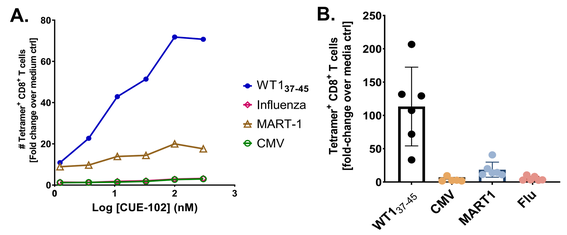


Figure 7 – CUE-102 selectively expands tumor-relevant WT1$_{37-45}$ -specific CD8⁺ T cells but not CD8⁺ T cells specific to other antigens (CMV, MART1 or Influenza). **(A)** Representative dose-dependent and antigen specific expansion of CD8⁺ T cells from a PBMC donor. **(B)** Antigen-selective expansion following exposure of multiple donor PBMCs to 100 nM CUE-102. Only donors that are reactive for all antigens are included here.

CUE-102 Expands Polyfunctional and Cytolytic WT1$_{37-45}$-Specific CD8⁺ T Cells from Human PBMCs

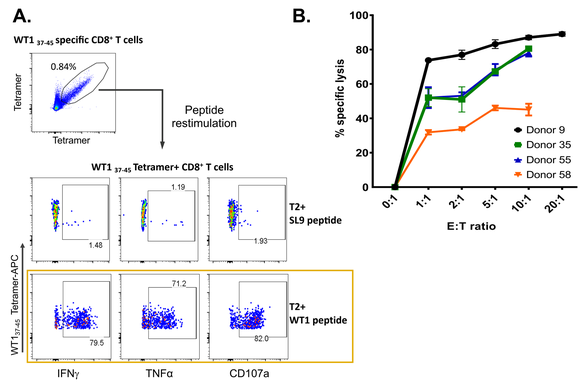
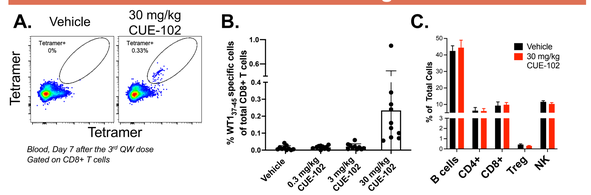


Figure 8 – (A) CUE-102 expanded WT1$_{37-45}$-specific CD8⁺ T cells produce intracellular IFN-γ, TNF-α and surface CD107a after restimulation with T2 cells loaded with WT1$_{37-45}$ peptide, but not T2 cells loaded with negative control HIV SL9 peptide. **(B)** CUE-102 expanded WT1$_{37-45}$-specific CD8⁺ T cells induce specific lysis of target T2 cells loaded with WT1$_{37-45}$ peptide relative to T2 cells loaded with irrelevant peptide. Percentage of specific lysis is plotted against effector: target (E:T) ratio of 1:1 - 20:1. Mean ±standard deviation from triplicate wells is shown for each E:T ratio.

CUE-102 Selectively Expands WT1$_{37-45}$-Specific CD8⁺ T Cells in naïve HLA-A2 Transgenic Mice



Blood, Day 7 after the 3rd QW dose
Gated on CD8+ T cells

Figure 9 – Treatment of naïve HLA-A2 transgenic mice leads to selective, dose-dependent expansion of WT1$_{37-45}$ specific CD8⁺ T cells. Naïve HLA-A2 transgenic mice were given 3 once weekly intravenous (IV) doses of CUE-102 at the indicated dose level. The frequency of WT1$_{37-45}$ specific CD8⁺ T cells **(A & B)** and of major immune lineages **(C)** was assessed in peripheral blood 7 days after the last dose. Treatment with CUE-102 led to dose-dependent expansion of WT1$_{37-45}$ specific CD8⁺ T cells **(A & B)** without broadly affecting other immune lineages **(C)**.

CUE-102 Selectively Expands Tumor Relevant T cells in immunized HLA-A2 Transgenic Mice

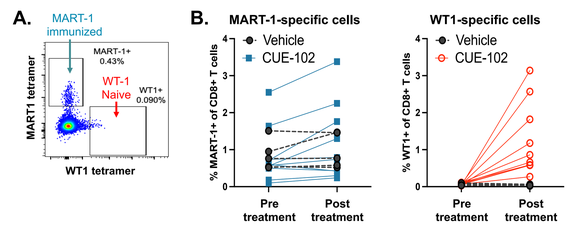


Figure 10 – CUE-102 selectively expands WT1$_{37-45}$-specific CD8⁺ T cells, but not CD8⁺ T cells of other specificities *in vivo*. **(A)** Naïve HLA-A2 transgenic mice were immunized with MART-1 peptide. MART-1 specific CD8⁺ T cells were detected 7 days after immunization. **(B)** CUE-102 treatment of these immunized mice resulted in statistically significant increases in frequencies of WT1$_{37-45}$-specific CD8⁺ T cells, but frequencies of MART-1-specific cells did not change.

Repeated Treatment with CUE-102 Results in Cumulative Expansion of WT1-Specific T Cells

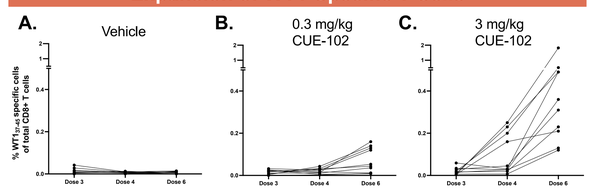


Figure 11 – Repeated treatment with CUE-102 expands WT1-specific CD8+ T cells. Graphs display frequencies of WT1$_{37-45}$-specific cells among total CD8⁺ T cells in PBMCs from mice that received 3, 4, or 6 total doses of **(A)** Vehicle, **(B)** 0.3 mg/kg CUE-102, or **(C)** 3 mg/kg CUE-102. WT1$_{37-45}$-specific cells become detectable in blood at both the 3 mg/kg and 0.3 mg/kg dose levels as the number of doses increases.

CUE-102 Treatment Expands Functional WT1$_{37-45}$-Specific Effector and Long-Term Memory CD8⁺ T cells In Vivo

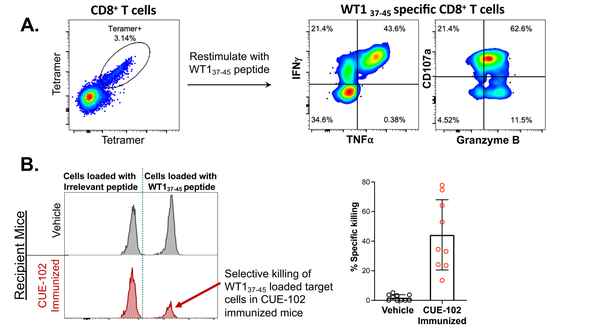


Figure 12 (A) Immunization of HLA-A2 transgenic with 4 doses of CUE-102 expands highly functional WT1$_{37-45}$-specific CD8⁺ T cells that produce IFNγ, TNFα, CD107a and Granzyme B in response to ex vivo restimulation. **(B)** HLA-A2 mice immunized with CUE-102 show antigen-specific in vivo killing of 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 transferred into CUE-102 immunized HLA-A2 transgenic mice (red), but not into naïve mice (black). **(C)** CUE-102 treatment induces long-term memory cells that are detectable >180 days following immunization with CUE-102 and remain highly functional and capable of producing multiple cytokines.

Less Frequent CUE-102 Treatment Increases Expansion of Highly Functional WT1-Specific CD8⁺ T Cells

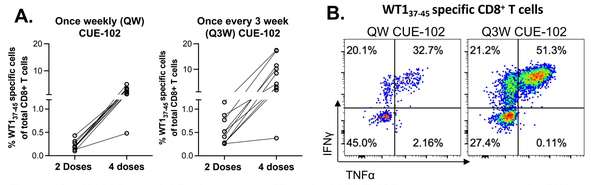


Figure 13 – (A) Naïve HLA-A2 mice received 30 mg/kg IV CUE-102 on either a once weekly (QW) or once every 3 week (Q3W) dosing schedule. Q3W dosing led to greater expansion of antigen specific cells detected in the blood by tetramer staining. **(B)** Q3W dosing also led to increased cytokine production by antigen-specific cells expanded by CUE-102. Thus, Q3W CUE-102 dosing leads to both greater expansion of antigen –specific cells and greater functionality of the expanded cells.

CUE-102 First-in-Human Clinical Trial

CUE-102-01 (NCT05360680) is a Phase 1, FIH study to characterize the safety, tolerability, PK, PD, immunogenicity, and preliminary antitumor activity of CUE-102 in subjects who are HLA-A*0201-positive, have WT1-positive, recurrent/metastatic cancers, and have failed conventional therapies.

Part A: Monotherapy Dose Escalation *(Q3W, 3 + 3 design)* → **Part B: Monotherapy Expansion** *(n=28 disease-specific patients)*

Eligibility:
- WT1+ Expressing Cancers
  - **Part A: Dose Escalation** Colorectal, Gastric/GEJ, Pancreatic and Ovarian cancer
  - **Part B: Dose Expansion** Colorectal cancers only
- HLA-A*0201 genotype
- Measurable disease by RECIST 1.1
- Life expectancy ≥ 12 weeks

Objectives:
- *Primary:* Safety and tolerability
- *Secondary:* PK/PD, Anti-tumor activity (RECIST 1.1)

Biomarkers: (Pre/Post CUE-102 dose)
- WT1-specific CD8+ T cell counts and functionality
- Immunophenotyping, serum cytokines, and TCR sequencing

For further details on the CUE-102-01 trial, see **Poster #636** Friday November 11th, 2022

Conclusions

- CUE-102 is a novel fusion protein designed to selectively deliver attenuated IL-2 to tumor-specific CD8⁺ T cells
- CUE-102 demonstrates selective binding, activation, and expansion of polyfunctional and cytolytic WT1$_{37-45}$ specific primary human CD8⁺ T cells from healthy and cancer patient samples
- Treatment of naïve HLA-A2 transgenic mice with CUE-102 elicits and selectively expands WT1$_{37-45}$ specific effector and long-term memory CD8⁺ T cells that are polyfunctional and cytotoxic in vivo
- The novel mechanism of action of CUE-102, namely targeted activation of tumor-antigen-specific CD8⁺ T cells via delivery of reduced affinity mutant IL-2, supports its potential for anti-cancer efficacy in a Phase 1 clinical trial in WT1+ relapsed/metastatic cancers

ACKNOWLEDGEMENTS:
This study is sponsored by Cue Biopharma Inc. and conducted in collaboration with LG Chem, a subsidiary of LG Corp., Seoul, South Korea.