**Figure 1** - The rationally engineered and modular biology of the immuno-DNA platform incorporates natural biological signals (‘tagger’) for selective engagement and modulation of diseaserelated T cells. The CUE-102 tumor vaccine is designed to selectively deliver modified TCR to tumor-specific T cells and drive their expansion.

**Figure 2** - mCUE-101 expands functional antigen-specific CD8+ T cells in the tumor and the periphery. Mice bearing established DC-tumor burdens were treated with 15 mg/kg CUE-101 alone or in combination with ipilimumab. Expansion of antigen-specific cells was assessed once a week after the last dose of CUE-101. Representative data show the frequency of tumor-reactive CD8+ T cells in the blood and tumor (A & B) and tumor-draining lymph nodes (C & D). Only animals treated with mCUE-101 maintained increased frequency of antigen-specific cells, which was greatly increased in the tumor vs. blood. mCUE-101 increases the frequency of antigen-reactive T cells in the blood and tumor and induces expansion of T cells in response to peptide restimulation of splenocytes (B) and tumor-draining lymph nodes (D).

**CUE-102 Selectively Expands Tumor Relevant T Cells in Immune-DNA Tumor Transplant Models**

**Figure 3** – Schematic of CUE-102 molecules. CUE-102 is comprised of a human lactobacillus antigen (HLA) complex, HLA-A2/01, a peptide epitope derived from the WT1 protein, and 4 molecules of a reduced affinity humanized mAb. CUE-102 is designed to bind and activate WT1-specific T cells for eradication of WT1-positive cancers.

**Figure 4** – CUE-102 selectively binds to primary CD8+ T cells transduced with a WT1-specific TCR and CUE-102 T cells transfected with a CMV-pulsed plasmid. (A) mCUE-101 primary CD8+ T cells transduced with WT1-specific TCR. CUE-102 selectively activated the TCR in target cells as assessed by increased phosphorylation of pSTATs (n=3, siPMI), but not in CD8+ T cells transfected with CMV-pulsed plasmid.

**Figure 5** – (A) Human WT1-specific CD8+ T cells are functionally activated and most likely not redirected to 0 (0.7% of n=3). In a CUE-102, 101 proliferation assay. The WT1-specificity of CUE-102 facilitates selective phosphorylation of STATs (pSTATs) immediately downstream of IL-2R on target cells. CUE-102 expands WT1-specific CD8+ T cells with a CMV-directed ST. A CMV-directed ST induces pSTATs with greater potency in CMV-pulsed CD8+ specific CD8+ T cells than does CUE-102.

**Figure 6** – (A) CUE-102 expands WT1-specific CD8+ T cells transduced with WT1-specific TCR and CUE-102 T cells transfected with CMV-pulsed plasmid. (B) CUE-102-expanded WT1-specific CD8+ T cells induce cytotoxicity, upregulation of target T2, and proliferation in target T2. CUE-102 was generated by WT1 peptide presentation to CD8+ T cells loaded with irrelevant peptide. Percentages of specific lysis is plotted against effector:target (ET) ratios of 1:1 – 20. Mean unstandard deviation from triplicate wells is shown for each ET ratio.

**Figure 7** – CUE-102 selectively expands tumor-reactive WT1-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 RM1 donor. (B) Autologous expansion of CMV-specific CD8+ T cells following exposure of multiple donor RM1 to 100 μl CUE-102. Only donors that were reactive for all antigens are included here.

**Figure 8** – (A) CUE-102 expands WT1-specific CD8+ T cells transduced with WT1-specific TCR, CUE-102 selectively activated the TCR in target cells as assessed by increased phosphorylation of pSTATs (n=3, siPMI), but not in CD8+ T cells transfected with CMV-pulsed plasmid.

**Figure 9** – Treatment of naive HLA-A2 transgenic mice leads to selective, dose-dependent expansion of WT1-specific CD8+ T cells. Naive HLA-A2 transgenic mice were given 3 once weekly intravenous (IV) doses of CUE-102 at the indicated dose level. The frequency of WT1-specific CD8+ T cells was determined using WT1 peptide presentation to CD8+ T cells in vitro with WT1 peptide-loaded target cells transfected with CUE-102. CUE-102 transgenic mice (red), but not naïve mice (black), show increased WT1-specific T cell responses in lymph nodes that are detectable ~150 days following immunization with CUE-102 and remain highly functional and capable of killing WT1 tumor cells.

**Figure 10** – CUE-102 Selectively Expands WT1-specific CD8+ T Cells in naive HLA-A2 Transgenic Mice

**Figure 11** – CUE-102 Treatment Results in Cumulative Expansion of WT1-specific CD8+ T Cells

**Figure 12** – (A) Naive HLA-A2 mice received 30 mg/kg CUE-102 in either a once weekly (QW), or once every 3 weeks (Q3W) intravenous injection. QW 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 CD8+ T cells. (C) QW dosing leads to both greater expansion of antigen-specific cells and greater functionality of the expanded cells.

**Figure 13** – CUE-102 First-In-Human Clinical Trial

**Figure 14** – CUE-102 provides a novel fusion protein designed to selectively deliver IL-2 to tumor-specific CD8+ T cells.

**Figure 15** – CUE-102 doubles the number of viable T cells per ml and_kernel density estimates (KDE) of CD8+ T cell function in vitro. CUE-102 treatment results in increased functional capacity of WT1-specific CD8+ T cells, but frequencies of WT1-specific T cells did not change.

**Conclusions**

- **CUE-102** is a novel fusion protein designed to selectively deliver IL-2 to tumor-specific CD8+ T cells.
- **CUE-102** demonstrates selective binding, activation, and expansion of polyfunctional and cytotoxic WT1-specific primary human CD8+ T cells from healthy and cancer patient samples.
- Treatment of naive HLA-A2 transgenic mice with CUE-102 selectively and extensively WT1-specific effectors 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, is highly relevant for anti-cancer efficacy in Phase 1 clinical trials that will be performed in lymphopenic metastatic cancer patients.

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