(793) Targeting engineered interleukin-2 (IL-2) to antigen specific T cells via novel biologic platforms

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CUE™ BIOPHARMA

Abstract

Background: A key challenge with IL-2 immunotherapy for cancers is lack of selectivity for anti-tumor immune cells and safety liabilities related to indiscriminate activation of immune cells. The CUE-100 series of Immuno-STATs™ (ISTs) are designed to selectively activate tumor-specific T cells while avoiding IL-2 toxicities due to systemic activation. CUE-100 series ISTs are rationally engineered Fc fusion proteins comprised of bivalent tumor-peptide-HLA (pHLA) complexes and four affinity-attenuated IL-2 molecules to preferentially engage and activate tumor-specific T cells directly in the patient. Emerging clinical data from our lead candidate CUE-101, which targets HPV-specific T cells in 2L+ R/M HNSCCC (Poster #438), provides PoC for the approach and builds confidence for broad applications in numerous cancers. Building on the CUE-100 series framework, our Neo-STAT™ (NST) platform contains HLA molecules manufactured with an "empty" peptide-binding pocket, into which diverse tumor-peptides can be chemically conjugated, hence addressing tumor heterogeneity in a cost- and time-efficient manner. Our RDI-STAT (Re-Directed Immuno-STAT) platform further expands the CUE-100 series by redirecting the pre-existing protective viral-specific T cell repertoire to target tumor cells via scFv moieties. RDI-STATs are designed to circumvent potential tumor escape mechanisms linked to HLA loss or defects in antigen-presenting pathways, and to harness a robust repertoire of protective anti-viral T cells to destroy cancers. We present here preclinical data supporting the mechanism of action of these platforms to enhance anti-tumor immune responses.

Methods: NSTs were engineered with "empty" HLA-A02 complexes, into which relevant antigenic peptides were conjugated, and assessed for capacity to expand T cells. RDI-STATs were engineered with TAA-specific scFv and viral-specific pHLA complexes and assessed for their capacity to induce redirected killing of tumor cells while avoiding systemic activation of all T cells.

Results: The NST platform demonstrated that different T cell epitopes can be efficiently conjugated into the engineered HLA-binding pocket, and that these molecules activate and expand antigen specific T cells *in vitro*. RDI-STATs were able to expand anti-viral T cell repertoires and drive anti-viral T cell redirected killing of TAA-expressing cells. In contrast to pan anti-CD3 bispecific molecules, RDI-STATs demonstrated significantly lower induction of pro-inflammatory cytokines.

Conclusions: The IST, NST, and RDI-STAT platforms provide novel opportunities for selective targeting of IL-2 to tumor-relevant T cells while avoiding global immune activation and cytokine release. The scalability and versatility of NSTs highlight the potential to efficiently target multiple TAA T cell responses, while RDI-STATs highlight a novel means to harness antiviral immunity against cancer, especially in cases where the tumor may escape immune detection due to loss of HLA.

Application of Engineered IL-2 on the Immuno-STAT Platform

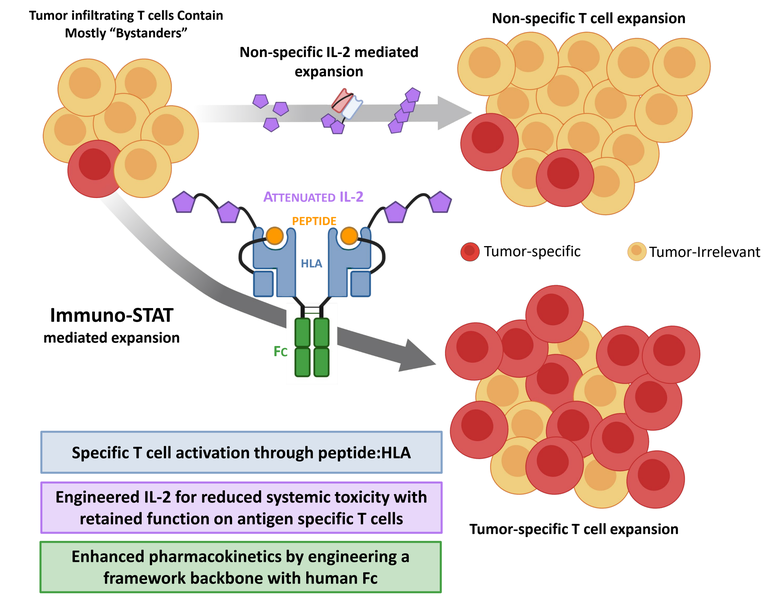
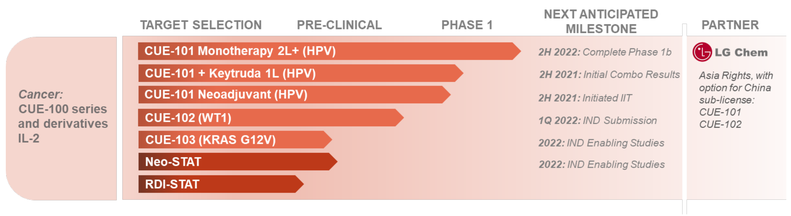


Figure 1. Immuno-STATs selectively expand antigen specific (AgS) T cells. Moieties that systemically or locally deliver IL-2 expand T cells in a non-AgS manner. Alternatively, Immuno-STATs target AgS cells through coincident delivery of rationally attenuated IL-2[1] and cognate TCR:HLA signaling that is pharmacologically enhanced by fusion to a human Fc fragment[2].

CUE-100 Series in Oncology



CUE-101: Human papilloma virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC)
CUE-102: Wilms' tumor 1 (WT1) positive cancers (e.g., leukemia and multiple solid cancers)
CUE-103: KRAS G12V is a KRAS mutation associated with many cancer types
IIT: Investigator-initiated trial

Figure 2. Oncology pipeline of CUE-100 series Immuno-STATs. CUE-101 is a novel fusion protein that incorporates an HLA-A*0201 allele bound to an epitope from the HPV16 E7 protein ($E7_{11-20}$) and is designed to activate and expand tumor-specific T cells that target HPV16-driven malignancies. In September 2019 we commenced our first-in-human, dose-escalation and expansion Phase 1 clinical trial of CUE-101 in patients with HPV-positive recurrent or metastatic head and neck squamous cell carcinoma (NCT03978689). In February 2021 we commenced a dose-escalation and expansion Phase 1 clinical trial of CUE-101 in combination with KEYTRUDA® (pembrolizumab) in newly diagnosed patients with HPV-positive recurrent or metastatic head and neck squamous cell carcinoma (NCT03978689). CUE-102 is the second clinical candidate from the CUE-100 series and incorporates a peptide:HLA derived from the Wilms' Tumor protein (WT1) and will be submitted for IND in 1Q 2022. CUE-103 will leverage the CUE-100 framework to elicit T cells specific for mutant KRAS G12V against numerous cancer types. See **poster #438** for more information on the clinical development of CUE-101 and **poster #720** for progress on the preclinical development of CUE-102.

Neo-STAT Design and Rationale

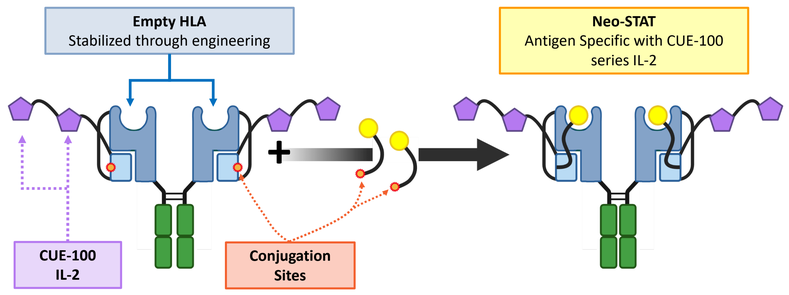


Figure 3. Neo-STAT (NSTs) is a rationally engineered 100-series "empty" Immuno-STAT. NSTs were designed to allow the chemical conjugation of a peptide into an "empty" HLA molecule. The HLA component of NSTs are modified to be stable in the absence of peptide but remain amenable to downstream incorporation of any peptide. This characteristic affords the ability to mass-produce NSTs from a single master cell bank, enabling the robust and cost-effective production of 100-series based Immuno-STATs bearing the clinically de-risked Cue engineered IL-2. NSTs therefore enable a therapeutic path for selective activation of T cells of any antigen specificity.

Neo-STATs and Immuno-STATs exhibit comparable activity

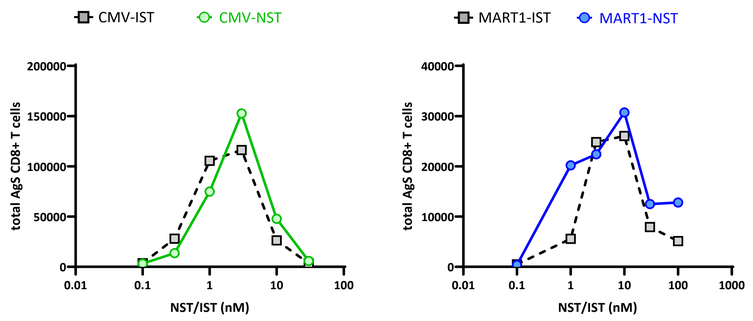


Figure 4. Neo-STATs (NSTs) exhibit comparable activity to CUE-100 series Immuno-STATs (ISTs). Primary human PBMC were isolated from leukopaks derived from healthy HLA-A02+ donors and then exposed to a dose-response of either the respective IST or NST for 10 days. The number of antigen specific CD8+ T cells was assessed by tetramer staining for the indicated antigen by flow cytometry. Data are representative of multiple donors across two independent experiments. Antigen peptides used in either IST or NST constructs were human cytomegalovirus (CMV) peptide NLVPMVATV (left) or melanoma associated antigen recognized by T cells (MART1) peptide ELAGIGILTV (right).

Neo-STAT Manufacturability of Multiple HLA Alleles

		HLA Allele		
		A-24	A-11	A-02
Engineering	Peptide	Peptide-less		
	IL-2	Attenuated		
Production	Transient expression titer (mg/L)	≥150	≥150	≥150
	Concentration (mg/mL)	≥10	≥10	≥10
	Endotoxin (eu/mg)	< 0.07	< 0.07	< 0.07
Biophysical	Final SEC (% monomer)	≥95	≥95	≥95
	Freeze-thaw (3x)	Stable, No Change	Stable, No Change	Stable, No Change
	Intact mass (LC-MS)	Confirmed	Confirmed	Confirmed
Biological Activity	CD8+ T cell Expansion	Pending	Confirmed	Confirmed
	Antigens Conjugated	SCV2, WT1	mKRAS	SCV2, CMV, MART1

Table 1. Neo-STATs (NSTs) can be manufactured to contain multiple different HLA alleles. Data addressing the manufacturability of NSTs with the indicated HLA allele.

NSTs provide a means to rapidly generate therapeutics

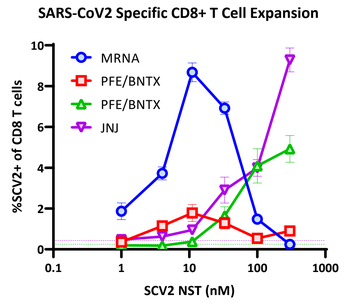


Figure 5. Neo-STATs can be implemented rapidly to expand SARS-CoV2 (SCV2) specific T cells. Primary human PBMC were isolated from leukopaks derived from healthy HLA-A02+ donors collected after March 20th, 2021. Donors reported having at least one dose of a SCV2 vaccine developed by the indicated company ticker label. PBMC were exposed to NST loaded with the immunodominant[3] HLA-A02 peptide YLQPRTFLL for 10 days. Antigen specific CD8+ T cells were assessed by tetramer staining for the indicated antigen by flow cytometry. Data are from a single experiment, with error bars representing the mean percentage of SCV2 reactive CD8+ T cells from triplicate wells.

Re-Directed Immuno-STATs (RDI-STATs) Rationale

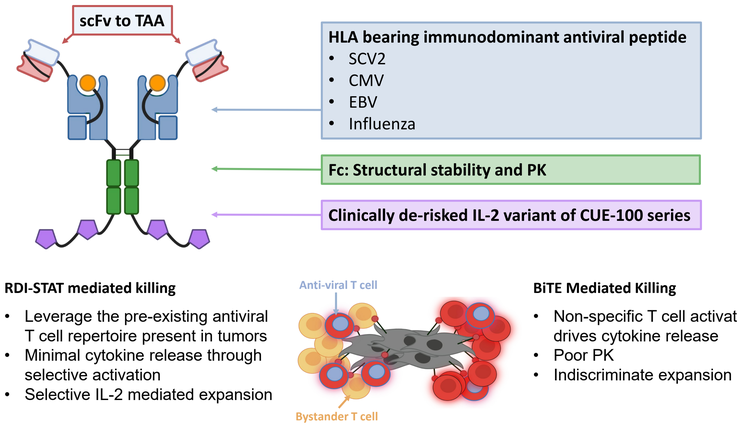


Figure 6. RDI-STATs enable tumor killing through pre-existing antiviral T cells in tumors. Tumors are populated with bystander T cells that are not specific to the tumor but instead are memory cells specific for viruses[4,5]. RDI-STATs are engineered to specifically activate antiviral T cells against tumors by leveraging the CUE-100 series Immmuno-STAT ability to activate antigen specific T cells through peptide:HLA, and attenuated IL-2. RDI-STATs offer an approach distinct from other T cell engagers that are hampered by the liabilities of non-specific activation, cytokine release syndrome, and poor PK.

Rational Engineering of RDI-STATs

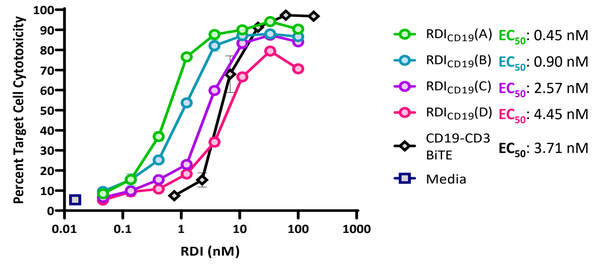


Figure 7. RDI-STAT molecular framework optimization increases killing potency. RDI-STATs were engineered for optimal killing by varying components of the RDI-STAT framework while retaining the CUE-100 series derived IL-2. Primary human PBMC were isolated from leukopaks derived from healthy HLA-A02+ donors and CD8+ T cells expanded using antiviral peptide and native IL-2 for 10 days. CD8+ T cells were isolated by magnetic beads and cultured with RAMOS cells at a ratio of 20:1 CD8 T cells:RAMOS cell. Mixtures were incubated with a dose-response of RDI-STAT framework variants (denoted as A-D) specific to an immunodominant viral epitope compromising an anti-CD19 scFv fragment, or a bi-specific scFv against CD3:CD19 Bi-Specific T cell Engager (CD3:CD19 BiTE, Invivogen). Cells were cultured for 24 hours and killing determined by the percentage of live CD20+ (RAMOS) cells remaining in culture. Data represent two independent experiments with the mean values +/- SEM of duplicate conditions plotted.

RDI-STATs Leverage Anti-Viral Responses Against Cancer

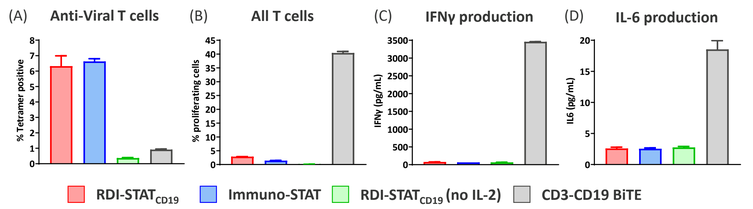


Figure 8. RDI-STATs specifically expand anti-viral T cells and minimize cytokine release. PBMC from a donor with a basal (1% of circulating CD8+ T cells reactive to immunodominant epitope) antiviral T cell response were exposed to viral specific RDI-STAT with scFv specific to CD19 (RDI-STAT$_{CD19}$), virus specific Immuno-STAT, RDI-STAT$_{CD19}$ without IL-2, or a CD3:CD19 BiTE (Invivogen). Cultures were incubated for 5 days, a portion of supernatants removed, and continued in culture for an additional 5 days. Percentage of antiviral CD8+ T cells or non-specific CD8+ T cells were determined by tetramer staining with flow cytometry (A and B, respectively). At the dosage of maximal killing of endogenous B cells in PBMC at day 10, supernatants from day 5 were analyzed for IFNγ (C) and IL-6 levels by MSD analysis. Data are representative of two experiments, with the mean of duplicate measurements +/- SEM depicted.

Neo-STAT and RDI-STAT: Evolution of the CUE-100 Series

- The CUE-100 series leverages Immuno-STATs, fusion proteins designed to expand and activate disease relevant T cells through coincident delivery of HLA-presented antigen and IL-2.
- The attenuated IL-2 on the CUE-100 series has been de-risked through clinical studies with CUE-101.
- Neo-STATs represent a rational evolution of the CUE-100 series, using the same IL-2 but an empty HLA adptable to multiple alleles and peptides.
- Neo-STATs afford the ability to address multiple antigen specificities across many alleles, vastly expanding the reach of the CUE-100 series across many indications.
- RDI-STATs leverage the potency and ubiquity of the antiviral T cell repertoire to re-direct T cell responses against cancer.
- Using the CUE-100 series IL-2, RDI-STATs specifically activate and expand only antiviral T cells and thus circumvent the current liabilities of indiscriminate T cell redirection.

References

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Presented November 12th, 2021 at the 36th Annual Meeting of Society for Immunotherapy of Cancer (SITC)