Clinical-Scale Production and Characterization of NTLA-5001 – a Novel Approach to Manufacturing CRISPR/Cas9 Engineered T Cell Therapies

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ABSTRACT

Adoptive T cell therapy has shown exciting efficacy in the treatment of certain hematological malignancies, particularly B cell tumors. However, with other cancers there has been limited success to date, and there remain significant challenges to develop safe and effective advanced cell therapies. Therefore, Intellia Therapeutics is leveraging its proprietary genome editing and cell engineering capabilities to develop a next-generation T cell therapy for the treatment of acute myeloid leukemia (AML). NTLA-5001 is an autologous T cell drug product genetically modified using CRISPR/Cas9 to eliminate endogenous T cell receptor (TCR) expression and transduced with AAV to site-specifically integrate a transgene encoding a Wilms' Tumor 1 (WT1)-targeting TCR into the *TRAC* locus. The TCR recognizes an HLA-A*02:01 restricted epitope of WT1.

To overcome manufacturing difficulties often seen in autologous cell therapies, we developed a robust, electroporation-free, functionally closed-system manufacturing process capable of producing large numbers of minimally differentiated T cells with high editing rates, robust transduction efficiency, low translocation rates, and high viability. The manufacturing process begins with the enrichment of CD8+ and CD4+ T cells from patient apheresis to facilitate an optimum CD8:CD4 ratio at culture initiation. After incubation, T cells are stimulated using an αCD3 αCD28 activation reagent followed by disruption of the TCRβ chain by CRISPR/Cas9 via a lipid nanoparticle (LNP) containing mRNA encoding SpCas9 and sgRNA targeting *TRBC*. TCRα is subsequently knocked out in the same manner using an LNP containing SpCas9 mRNA and a sgRNA targeting the *TRAC* locus followed by delivery of the WT1-TCR transgene using AAV-6 for site-specific integration of the WT1-TCR template into the *TRAC* locus via homology directed repair. T cells are then expanded for several days under constant perfusion using a chemically defined expansion media in a rocking bioreactor until harvest, formulation, and cryopreservation.

To date, clinical-scale production of NTLA-5001 at Intellia using healthy donors (n = 6) averaged 9.2 days in length. In that time, the process produced an average of 24.3 x 10^9 total T cells with an average viability of 93%. Although T cells underwent rapid expansion, they retained a minimally differentiated phenotype, with >90% of T cells at harvest expressing CD62L. Using our novel LNP-mediated cell engineering approach, we were able to achieve an average of 98.0% knockout of the endogenous TCR while simultaneously expressing the WT1-TCR in an average of >50% of T cells. This sequential editing approach reduced TRBC/TRAC translocation rates to near background levels. In addition, NTLA-5001 drug product displayed cytotoxic functionality when exposed to cell lines presenting the target WT1 peptide.

The NTLA-5001 clinical-scale manufacturing process is a controlled and robust platform for the generation of minimally differentiated T cells with high rates of editing and transgene expression.

NTLA-5001 – An Engineered T Cell Therapy for the Treatment of Acute Myeloid Leukemia (AML)

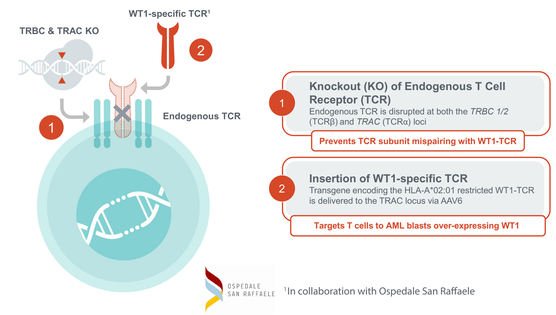

Figure 1. NTLA-5001 is an autologous T cell drug product genetically modified using CRISPR/Cas9 for the treatment of AML.

Schematic of the NTLA-5001 Manufacturing Process

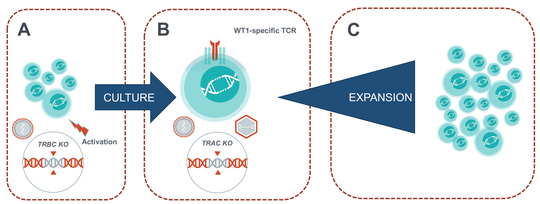

Figure 2. A. T cells are stimulated using an αCD3 αCD28 activation reagent followed by disruption of the TCRβ chain by CRISPR/Cas9 via a lipid nanoparticle (LNP) containing mRNA encoding SpCas9 and sgRNA targeting TRBC. **B.** TCRα is knocked out using an LNP containing SpCas9 mRNA and a sgRNA targeting the TRAC locus followed by delivery of the WT1-TCR transgene using AAV6 **C.** T cells are expanded for several days under constant perfusion using a chemically defined expansion media in a rocking bioreactor until harvest

In-process Cell Counts Demonstrate a Robust Manufacturing Process

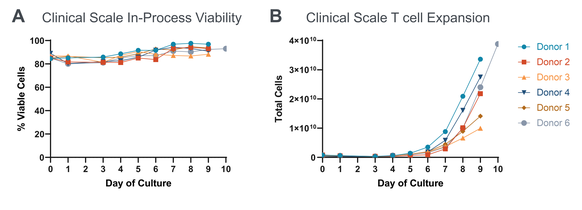

Figure 3. A. CRISPR/Cas9 editing via LNP prevents electroporation-derived cell death, resulting in consistently high in-process viability. **B.** Optimized chemically-defined expansion media and bioreactor parameters drives rapid T cell expansion, reaching at least 10 x 10^9 total T cells by Day 9, more if expanded an additional 1-2 days.

CRISPR/cas9 Editing via LNP Enables High Editing Rates While Maintaining Low Translocation Events

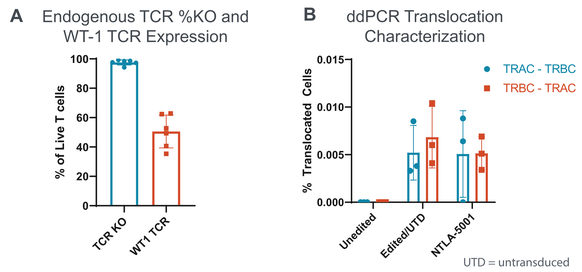

Figure 4. A. An average of 98% endogenous TCR KO and 50% WT1-TCR transgene insertion is achieved under functionally closed system conditions, as determined by flow cytometry. **B.** Quantitation of translocations (n=3) via ddPCR demonstrates minimal translocations as a result of sequential TRBC and TRAC LNP editing

NTLA-5001 Process is Optimized to Expand Large Populations of Memory T Cells

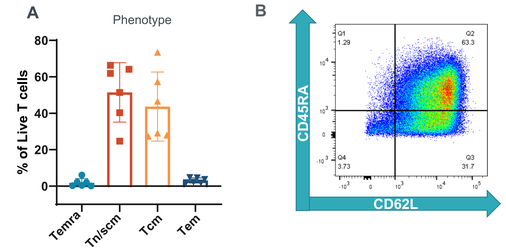

Figure 5. A. Despite rapid expansion T cells maintain a favorable, minimally differentiated phenotype at harvest, with most cells expressing CD62L and large populations of cells with a more naïve or stem cell memory-like expression of CD62L+CD45RA+.
B. Example of CD62LxCD45RA gating strategy.

NTLA-5001 Demonstrates Robust and Reproducible Cytotoxic Functionality Towards Target Cells

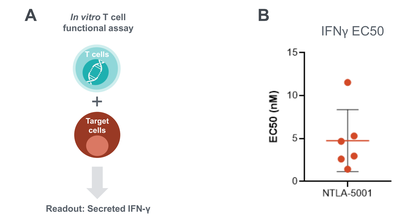

Figure 6. A. Schematic of *in vitro* functional assay.
B. Relative potency toward target cell lines pulsed with varying concentrations of WT1 peptide.

KEY TAKEAWAYS

- Developed a robust, functionally closed-system manufacturing process for clinical production of NTLA-5001
- The process utilizes Intellia's proprietary LNP-based cell engineering technology to sequentially edit T cells at-scale, resulting in high editing rates with minimal translocations
- The NTLA-5001 process produces large numbers of potent, minimally differentiated WT1-TCR T cells in approximately 9-10 days
- An IND for a first-in-human trial of NTLA-5001 has been approved.