A Novel Strategy for Off-The-Shelf T Cell Therapy which Evades Host T Cell and NK Cell Rejection

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ABSTRACT

Introduction

Despite the success of autologous chimeric antigen receptor (CAR)-T cells, barriers to a more widespread use of this engineered cell therapy include manufacturing failures and the high cost of individualized production. There is a strong desire for an immediately available cell therapy option; however, development of "off-the-shelf" T cells is challenging. Alloreactive T cells from unrelated donors can cause graft versus host disease (GvHD) for which researchers have successfully used nucleases to reduce expression of the endogenous T cell receptor (TCR) in the allogeneic product. The recognition of allogeneic cells by the host is a complex issue that has not been fully solved to date. Some approaches utilize prolonged immune suppression to avoid immune rejection and increase persistence. However showing responses in the clinic, this approach carries the risk of infections and the durability of the adoptive T cells is uncertain. Other strategies include deletion of the *B2M* gene to remove HLA class I molecules and avoid recognition by host CD8 T cells. However, loss of HLA class I sends a "missing-self" signal to natural killer (NK) cells, which readily eliminate *B2M*^{null} T cells. To overcome this, researchers are exploring insertion of the non-polymorphic *HLA-E* gene, although this provides partial but not full protection from NK cell-mediated lysis. Because activated T cells upregulate HLA class II, rejection by alloreactive CD4 T cells should also be addressed.

Methods

Here, we developed an immunologically stealth "off-the-shelf" T cell strategy by leveraging our CRISPR/Cas9 platform and proprietary sequential editing process. To address the issue of rejection by alloreactive CD4 and CD8 T cells, we knocked out (KO) receptor X and class II expression with a sequential editing process. Additionally, we utilize potent TCR- a and -β constant chain (*TRAC, TRBC*) gRNAs that achieve >99% KO of the endogenous TCR, addressing the risk of GvHD. An AAV-mediated insertion of a CAR or TCR into the *TRAC* locus is used in parallel with the *TRAC* KO step to redirect the T cells to tumor targets of interest. Alloreactivity by CD4 and CD8 T cells, NK killing, GvHD induction and T cell function was assessed *in vitro* and/or *in vivo*.

Results

By reducing the expression of two proteins, we were able to avoid host CD4- and CD8-T cell-mediated recognition. Edited T cells were protected from host NK cells, both *in vitro* and in an *in vivo* model engrafted with functional human NK cells. *TRAC* edited donor T cells did not induce GvHD in an immune compromised mouse model over the 90-day evaluation period. Using our proprietary T cell engineering process, we successfully generated allogeneic T cells with sequential KOs and insertion of a tumor-specific TCR or CAR with high yield. Importantly, these allogeneic T cells had comparable functional activity to their autologous T cell counterparts in *in vitro* assays (tumor cell killing and cytokine release) as well as *in vivo* tumor models. With a relatively small number of donors, we can provide an "off-the-shelf" CAR or TCR-T cell solution for a large proportion of the population.

Conclusions

We have successfully developed a differentiated "off-the-shelf" approach, which is expected to be safe and cost-effective. It is designed to provide long-term persistence without the need for an immune suppressive regimen. This promising allogeneic strategy is being applied to our T cell immuno-oncology and autoimmune research candidates.

BACKGROUND

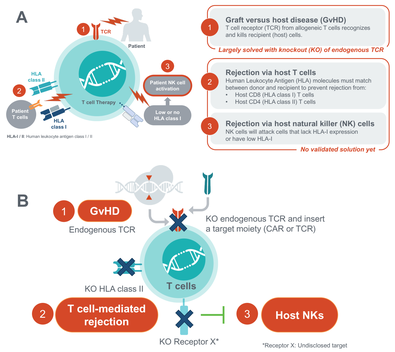

Figure 1. Three immune concerns are to be addressed by allogeneic cell therapies and Intellia's differentiated allogeneic approach

A. The first hurdle is GvHD which results from the presence of the TCR on the allogeneic T cell. Besides the patient's CD8 and CD4 T cells mediated rejection, the patient's NKs recognize the allogeneic T cells with low or no HLA class I expression as a "non-self" threat and reject them.

B. Using our CRISPR technology, a targeting moiety is specifically inserted into the TRAC locus by AAVs, minimizing random insertions and potential mutagenicity concerns.

By knocking out a transcription factor in the HLA class II pathway and Receptor X, both CD4 and CD8-mediated allo-reactivity can be addressed. Most importantly, in contrast to B2M KO T cells, our allogeneic T cells are well-protected from host NK cells.

With these edits, we believe our Allo approach has several key advantages. First, it can be applied to engineer both CAR-T and TCR-T cells, and potentially other cell types beyond T cells. Second, these Allo T cells will not trigger activation of host T cell or NK cell response. Finally, our approach avoids long-term immune suppression by not requiring enhanced or prolonged lymphodepletion, thereby preventing potential risk of severe infection.

DATA

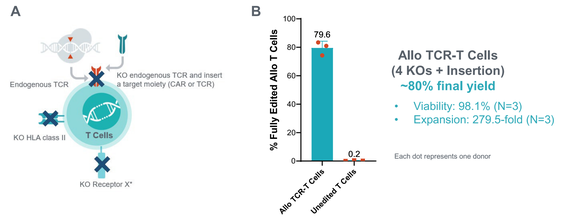

Figure 2. High editing efficiency in Allo TCR-T cells and Allo CAR-T cells by leveraging Intellia's proprietary LNP-based multiplex editing platform

A. Schematic of engineered Allo TCR-T cells

B. By utilizing our proprietary cell engineering process, we can achieve more than 95% editing efficiency for each step, which enables us to reach close to 80% for combined editing efficiency in Allo TCR T cells and 86% in Allo CAR T cell (data not shown). Our sequential editing process also minimizes translocations between on-target loci (data not shown).

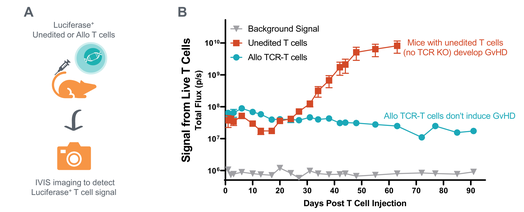

Figure 3. Mice engrafted with Allo TCR-T cells did not develop GvHD

A. Schematic of *in vivo* GvHD study workflow

B. After generating luciferase-expressing wild-type T cells or engineered Allo TCR-T cells, the cells were injected into NOG-IL15 immunodeficient mice. IVIS imaging was used to monitor T cell activation and proliferation *in vivo*. After exposure to host mouse antigens, Allo TCR-T cells did not show uncontrolled proliferation, indicating protection from GvHD, in contrast to unedited T cells.

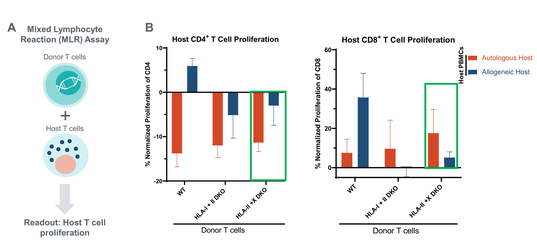

Figure 4. Allo T Cells do not elicit host CD4 or CD8 T cell responses

A. Schematic of *in vitro* MLR assay

B. In addition to T cells lacking Receptor X and HLA class II, WT T cells were prepared as positive control; B2M and HLA class II double KO T cells were prepared as negative control of stimulator cells. For responder cells, we included host PBMCs from allogeneic and autologous donors in the assay. After we co-cultured donor T cells with host PBMCs for 8 days, we measured host CD4 T cell proliferation and host CD8 T cell proliferation by examining CellTrace Violet dilution in host T cells.

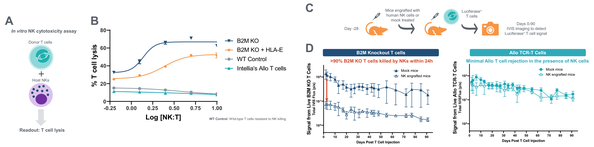

Figure 5. Intellia's Allo T cells are protected from NK cell killing

A. Schematic of *in vitro* NK cytotoxicity assay

B. After incubating allogeneic T cells or wild-type T cells with host NK cells, T cell lysis was measured. T cells lacking HLA class I (B2M KO) are killed by NK cells, and only partially rescued by insertion of HLA-E. In sharp contrast, Intellia's Allo T cells are well-protected from NK cell elimination.

C. Schematic of *in vivo* NK cytotoxicity assay

D. After injecting human primary NK cells into immunodeficient NOG-IL15 mice, we injected B2M KO T cells or Allo TCR-T cells expressing luciferase into NK engrafted or mock mice. T cell survival was measured using IVIS imaging.

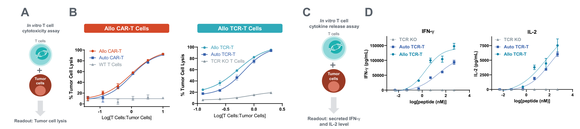

Figure 6. Allo T cells and autologous T cells show comparable *in vitro* tumor cell killing activity and cytokine secretion

A. Schematic of *in vitro* T cell cytotoxicity assay

B. After we generated different T cells (CAR T cells or TCR T cells), we co-cultured T cells with target tumor cells for 18 hours, and performed *in vitro* T cell cytotoxicity assay.

C. Schematic of *in vitro* cytokine release assay

D. After we generated different T cells (CAR T cells or TCR T cells), we co-cultured T cells with target tumor cells for 18 hours, and measured IFN-γ and IL-2 production.

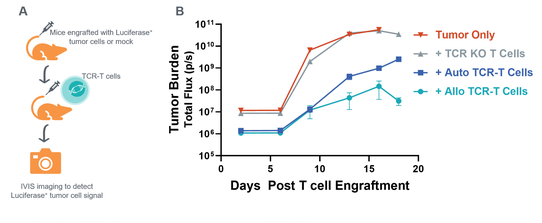

Figure 7. Allo TCR-T cells and autologous TCR-T cells have comparable anti-tumor efficacy *in vivo*

A. Schematic of *in vivo* tumor control model

B. Autologous and allogeneic version of engineered TCR T cells were generated and injected into tumor-bearing NOG mice along with a negative control T cells. Tumor burden was monitored by IVIS imaging.

CONCLUSIONS

Immune Concerns Unaddressed by Current Allogeneic Solutions

Approach	Employ intense lymphodepletion regimen	Knockout (KO) HLA-I (B2M)	KO HLA-I & express NK inhibitor (HLA-E)	Intellia's Approach: KO HLA-II & Receptor X*
Avoid rejection of cell therapy by host CD8 T cells	✓	✓	✓	✓
Avoid rejection of cell therapy by host CD4 T cells	✓	✗	✗	✓
Avoid rejection of cell therapy by host NK cells	✓	✗	✗	✓
Avoid profound immunosuppression	✗	✓	✓	✓

*Receptor X: Undisclosed target
B2M: Beta-2-microglobulin
HLA-E: Human leukocyte antigen class E

Intellia has developed a differentiated allogeneic platform

- Highly efficient TCR knockout eliminates GvHD concerns
- Allogeneic T cells avoid recognition by host CD4 and CD8 T cells
- Allogeneic T cells are protected from NK cell killing *in vitro* and *in vivo*
- Approach does not depend on immune suppression of the host

Allogeneic platform is readily deployable for TCR-T and CAR-T cell therapy

- Allo TCR-T and CAR-T cells have comparable anti-tumor activity to autologous counterparts *in vitro*
- Both Allo TCR-T cells and autologous TCR-T cells suppress tumor growth *in vivo*

Expect to nominate Intellia's first allogeneic development candidate by 1H 2022