Allogeneic CAR-T cell products containing 10 gene edits using CRISPR/Cas9 can retain full functionality in vivo and in vitro

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Abstract

Hematologic malignancies have proven most responsive to CAR-T therapy. However, there is significant variability in the depth and duration of response to autologous CAR-Ts. Further, CAR-T responses in solid malignancies have been minimal. T cells from cancer patients can be compromised by the disease and prior treatments, and the use of allogeneic CAR-T products could potentially provide a more robust therapy. However, even CAR-T cells from healthy donors may benefit from additional gene edits that could enhance effector function, increase durability, evade immune mechanisms and/or combat the tumor microenvironment.

These types of additional CAR-T features are readily enabled by CRISPR/Cas9 technology. While recent clinical data has demonstrated the persistence of therapeutic T cells that have been genetically engineered via CRISPR/Cas9 in patients with lymphoma, the extent of gene editing in a cell product can vary from patient to patient. Here, we have generated CAR-T cells with 10 gene edits, loss of function alleles produced in 9 genes and a Cancer associated gene was knocked into the TRAC locus via homology-directed repair. CD19-directed CAR-T cells with these 10 gene edits were fully functional in vitro, as shown by their ability to expand and kill target cells and secrete cytokines while remaining non-transformed. These cells were also highly efficacious in an in vivo model of CD19+ malignancy. Importantly, these edits, which produced insertions and deletions in target genes, could be detected in the blood of mice several months post infusion. These data demonstrate that CRISPR/Cas9 can be used to create highly edited allogeneic CAR-Ts, unlocking the potential to enhance functions relevant to CAR-T health and anti-tumor activity.

Figure 1: Performing up to 10 gene edits using CRISPR/Cas9 can improve allogeneic CAR-T function

Collect blood and isolate DNA for analysis of gene editing

Inject mice tumor models with multi-edited CAR-T cells

In vitro assays

Multi-edited CAR-T cells showed enhanced expansion in mice

Mouse treated with multi-edited CAR-T cells survived longer

Figure 2: 10-edit allogeneic CAR-T cells show polyclonal expansion in vivo

A) Lines representing persistence of multi-edited CAR-T cell variants across time in mice

B) KO2 indel frequency over time

C) KO6 indel frequency over time

D) Example plot for tracking indel patterns over time in vivo. Examples of polyclonal and monoclonal expansion are shown including early and lateclone expansion overlapping the CAR-T compartment. (B) & (C) indel patterns were assessed from two genes in rapid 10-edit CAR-T cells and in mice in the same system. T cells bearing indel frequencies (as described in figure 1) in indel patterns fluctuated over the length of the study with specific patterns observed in (B) & (C) T cells. This model of targeted multi-clonistearry CAR-T function in vivo and dynamic fluctuations in these clones. The lack of monoclonal expansion suggests that the process of producing 10-edit CAR-T cells with CRISPR/Cas9 does not produce transformed cells in vivo over this observation period.

Figure 3: 10-edit allogeneic CAR-T cells do not grow in the absence of cytokines

Allogeneic CAR-T cells were produced and implanted in media that contained serum and cytokines or serum alone (no cytokines). The total number of cells was assessed over the indicated days in culture. Cells that required up to 10 edits remained cytokine dependent, regardless of the absence of serum, consistent with earlier reports.

Figure 4: Specific edits identified can enhance the potency and persistence of CAR-T cells in vivo

A) Leukemia and lymphoma: Nalm6 primary tumor and Raji re-challenge

B) MM: RPMI-8226 primary tumor and re-challenge

C) RCC: A498 primary tumor and Caki-1 re-challenge

Conclusions from preclinical studies

• Allogeneic CAR-T cells bearing additional, potency-enhancing edits identified in the experiment described in Figure 2. These cells show increased aggressive cancer re-challenges in liquid and solid tumor xenograft models in a preclinical setting.

• The addition of multi-edited allogeneic CAR-T cells results in T-cell expansion in vivo, providing total body tumoricidal. 30 days after CAR-T cell expansion was re-challenged with CD19+ leukemic cells bearing Nalm6 (luciferase expressing) lymphoma cells delivered intraperitoneally. This model that produced effective CAR-T cells with the additional scalability control of lymphoma growth, while also maintaining the ability to develop lethal lymphoma. Similar clearance of ovarian tumors on re-challenge was observed in additional subcutaneous xenograft models (B) multiple myeloma (MM) and (C) renal cell carcinoma (RCC) using CAR-T cells targeting MAGE-A3 and CD70, respectively, and engineered with an additional edit.

• 10-edit allogeneic CAR-T cells do not show signs of oncolytic transformation in vitro or in vivo

• Multiple edited CAR-T cells can be engineered for enhanced potency and persistence in vivo