Allogeneic CAR-T Cells with Multiple Therapeutically Favorable Edits Can Be Created Efficiently Using CRISPR/Cas9

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Abstract

Remarkable therapeutic benefit of CAR-T cells has been observed for hematologic tumors across multiple indications and with different antigen targets. The most advanced systems are lentivirus-derived autologous CAR-Ts as seen with the approval of Kymriah and Yescarta and the reported clinical trial data from CAR-T cells targeting BCMA. Despite these significant advancements, there are (as ever in oncology) scope for improvement; in this case around supply and consistent product as well as the usual efficacy and safety profiles. Allogeneic (off-the-shelf) CAR-T cells created using gene editing techniques offer the opportunity to improve all of those aspects. Indeed TALEN based gene editing has been used to generate "off-the-shelf" CAR-T cell therapeutic targeting CD19. However, the CRISPR/Cas9 system provides an unprecedented opportunity to rapidly improve the properties of CAR-T cell therapeutics to treat solid tumors. Using CRISPR/Cas9 gene editing, homology-based guide RNAs can be assayed for functionality within weeks so that the most relevant targets can be validated. Furthermore, T cells are very tolerant of multiplex CRISPR based editing, including knock-out and knock-in editing events. Here we show selection of multiple candidate T cell edits that improve T cell function without damaging T cell properties.

Figure 1: Allogeneic CAR-T Cells Produced with CRISPR/Cas9

CRISPR/Cas9 genome editing of T cells from healthy donors is used to produce allogeneic CAR-T cells. To prevent off-target expression is ablated by site-specific integration of an antigen-specific CAR construct into the TRAC locus by homology-directed repair after using CRISPR/Cas9 to introduce the double strand break. To enhance persistence of allogeneic cells, MHC I expression is achieved by disrupting the I2M gene. In addition, an edit to knock-out PD1, as well as a fourth edit (ESM1), are made to enhance the anti-cancer properties of the multi-edited CAR-T cells.

Figure 2: CRISPR Therapeutics Allo CAR-T Pipeline

Program: CRISPR Therapeutics Allo CAR-T Pipeline

Table: CRISPR Therapeutics Allo CAR-T Pipeline

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Figure 3: High Efficiency Multi-Editing by CRISPR/Cas9 to Produce Anti-BCMA CAR-T Cells

Multi-editing results in decreased expression of TRAC and MHC I as well as high CAR expression. For both the double KO (TCR β2M and triple KO (TCR β2M/PD1), more than 80% of T cells possess all 3 (TCR β2M/PD1) or all 4 (TCR β2M/PD1/CD70) desired modifications. The CD4/CD8 ratio remains similar after multi-editing. Anti-BCMA CAR-T cells remain dependent on cytokines for growth after CRISPR/Cas9 multi-editing.

Figure 4: Multi-Edited Anti-BCMA CAR-T Cells Show Improved Anti-Cancer Properties

(A) Anti-BCMA CAR-T cells efficiently and selectively kill the BCMA-expressing multiple myeloma cell line MM.1S in a 4-hour cell kill assay, while sparing the BCMA isogenic line K462. Differences in between double (TCR β2M) and triple (TCR β2M/PD1) KO CAR-T cells are notable at lower T cell concentrations. (B) The CAR-T cells also specifically secrete the T cell activation cytokine IFN-γ and IL-2, which are upregulated in response to induction only by BCMA+. Cells, again, the triple KO outperforms the double.

Figure 5: PD1 KO Reduces LAG3 Exhaustion Marker Expression in Long-Term Cultured CAR-T Cells

Following 4 weeks of in vitro culture, triple KO (TCR β2M/PD1) anti-BCMA CAR-T cells show low expression of the exhaustion marker LAG3 relative to double KO (TCR β2M) anti-BCMA CAR-T cells, which lack the edit to eliminate PD1.

Figure 6: High Efficiency Quadruple Knock-Out Plus CAR Insertion by CRISPR/Cas9 to Produce Anti-CD70 CAR-T Cells with Enhanced Cytotoxicity

(A) Quadruple multi-editing results in decreased surface expression of TCR and MHC I, as well as high CAR expression. In addition, an edit to eliminate expression of PD1 and a fourth edit (ESM1) are achieved at high efficiency. More than 60% of T cells possess all 5 desired modifications (TCR β2M/PD1/CD70/CD84/CAR). (B) The CD4/CD8 ratio remains similar after multi-editing. (C) Triple KO (TCR β2M/PD1) anti-CD70 CAR-T cells remain dependent on cytokines for growth following CRISPR/Cas9 multi-editing. (D) Anti-CD70 CAR-T cells show potent killing activity against the CD70+ A498 renal cell carcinoma line. Quadruple KO CAR-T cells show higher potency than those with the triple KO at the lower effector-target ratios.

Summary and Conclusion

- Multi-edited antigen-specific CAR-T cells can be generated using CRISPR/Cas9 genome editing
- More than 60% of T cells possess all desired modifications, whether performing double, triple, or quadruple KO, plus CAR insertion.
- PD1 knock-out reduces expression of the exhaustion marker LAG3 in long-term in vitro culture of multi-edited anti-BCMA CAR-T cells.
- Both anti-BCMA and anti-CD70 multi-edited CAR-T cells:
  - Display antigen-specific effector functions
  - Have a similar CD4/CD8 ratio as controls
  - Maintain characteristic dependence on cytokines for growth, suggesting that no transformation has occurred as a result of the editing process.