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

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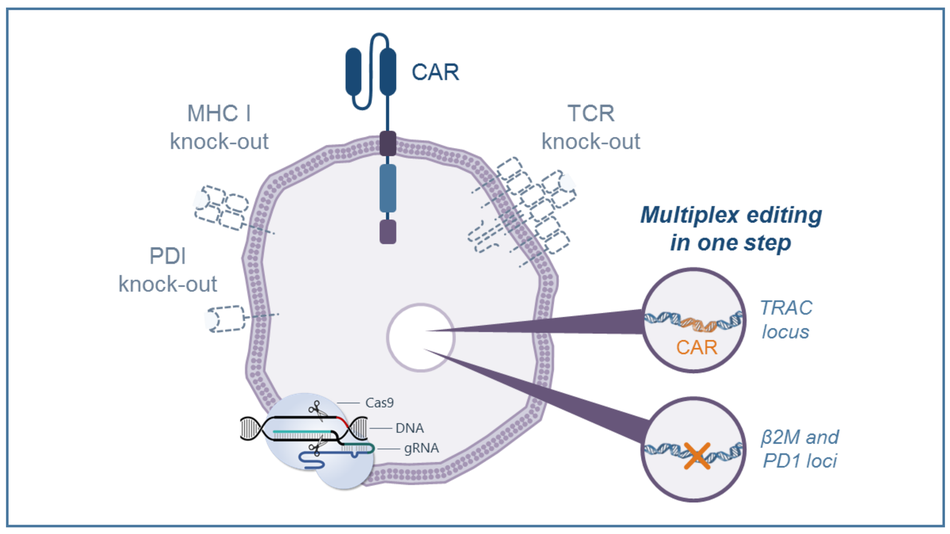
Abstract

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 remains (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 therapeutics 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

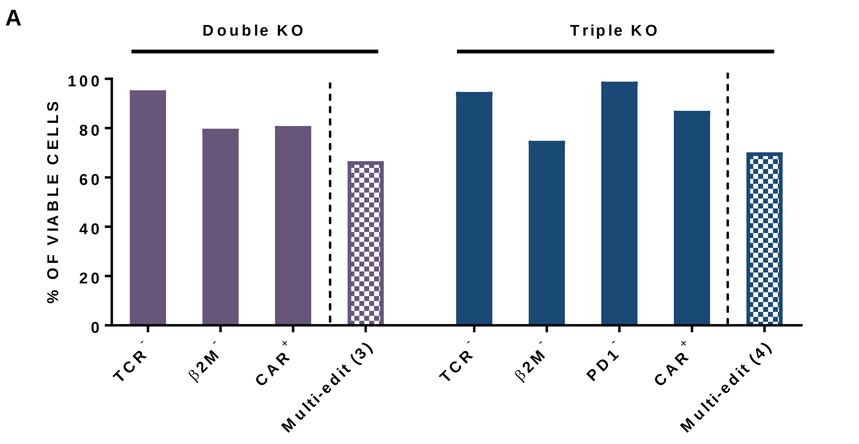


CRISPR/Cas9 genome editing of T cells from healthy donors is used to produce allogeneic CAR-T cells. To prevent GvHD, TCR expression is ablated by site-specific integration of an antigen-specific CAR construct into the *TRAC* locus by homology-directed repair after using CRISPR/Cas9 to introduce the double strand break. To enhance persistence of allogeneic cells, MHC I expression is eliminated by disrupting the *β2M* gene. In addition, an edit to knock-out PD1, as well as a fourth edit (“Edit4”), are made to enhance the anti-cancer properties of the multi-edited CAR-T cells.

Figure 2: CRISPR Therapeutics Allo CAR-T Pipeline

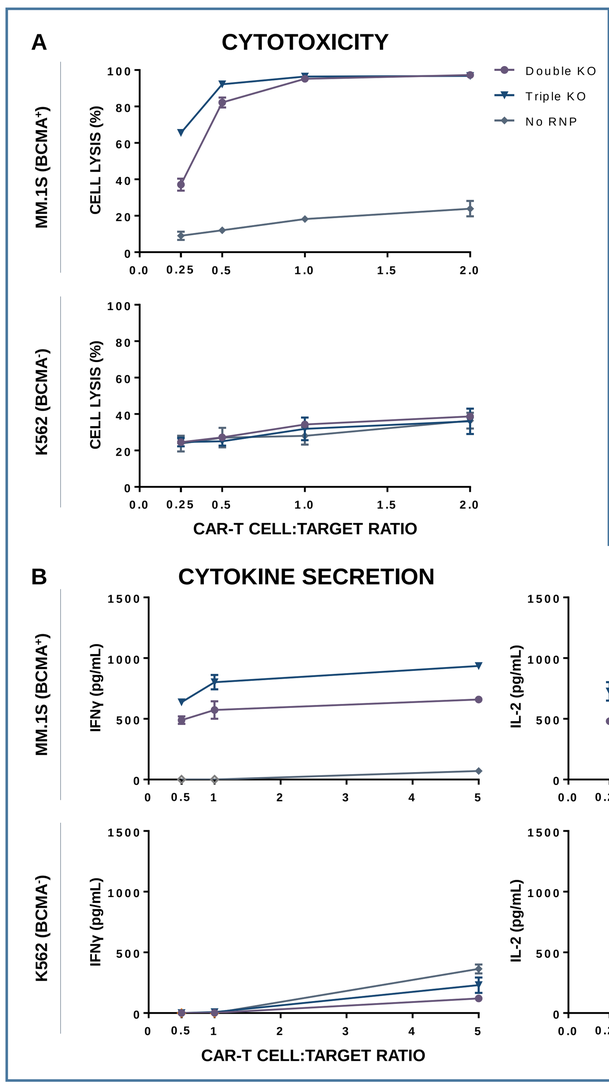
Program	Editing approach	Research	IND-enabling	Ph I/II	Partner
CTX101: Anti-CD19 allogeneic CAR-T	Disruption & Insertion			*IND filing Q4 2018*	Wholly-owned
CTX120: Anti-BCMA allogeneic CAR-T	Disruption & Insertion				Wholly-owned
CTX130: Anti-CD70 allogeneic CAR-T	Disruption & Insertion				Wholly-owned
Multiple solid tumor allogeneic CAR-T	Disruption & Insertion				Wholly-owned

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



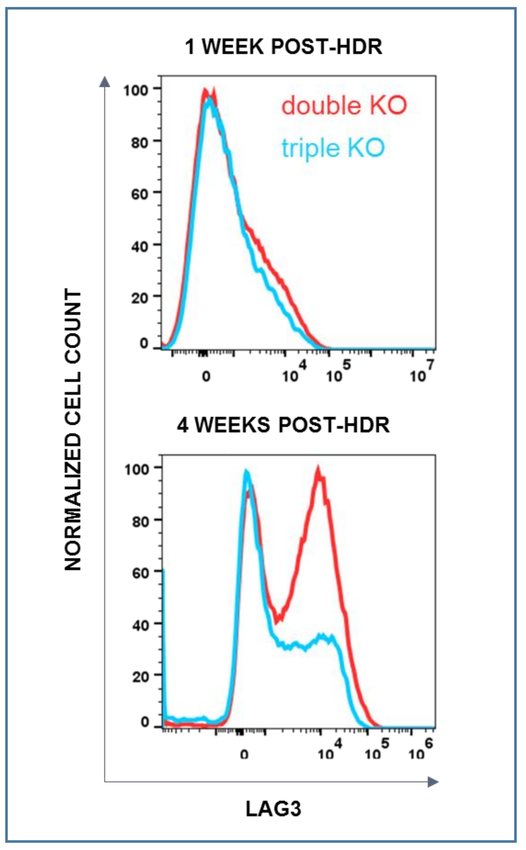
(**A**) Multi-editing results in decreased surface expression of TCR and MHC I, as well as high CAR expression. For both the double KO (TCR$^-$/β2M$^-$) and triple KO (TCR$^-$/β2M$^-$/PD1$^-$), more than 60% of T cells possess all 3 (TCR$^-$/β2M$^-$/CAR$^+$) or all 4 (TCR$^-$/β2M$^-$/PD1$^-$/CAR$^+$) desired modifications. (**B**) The CD4/CD8 ratio remains similar after multi-editing. (**C**) 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



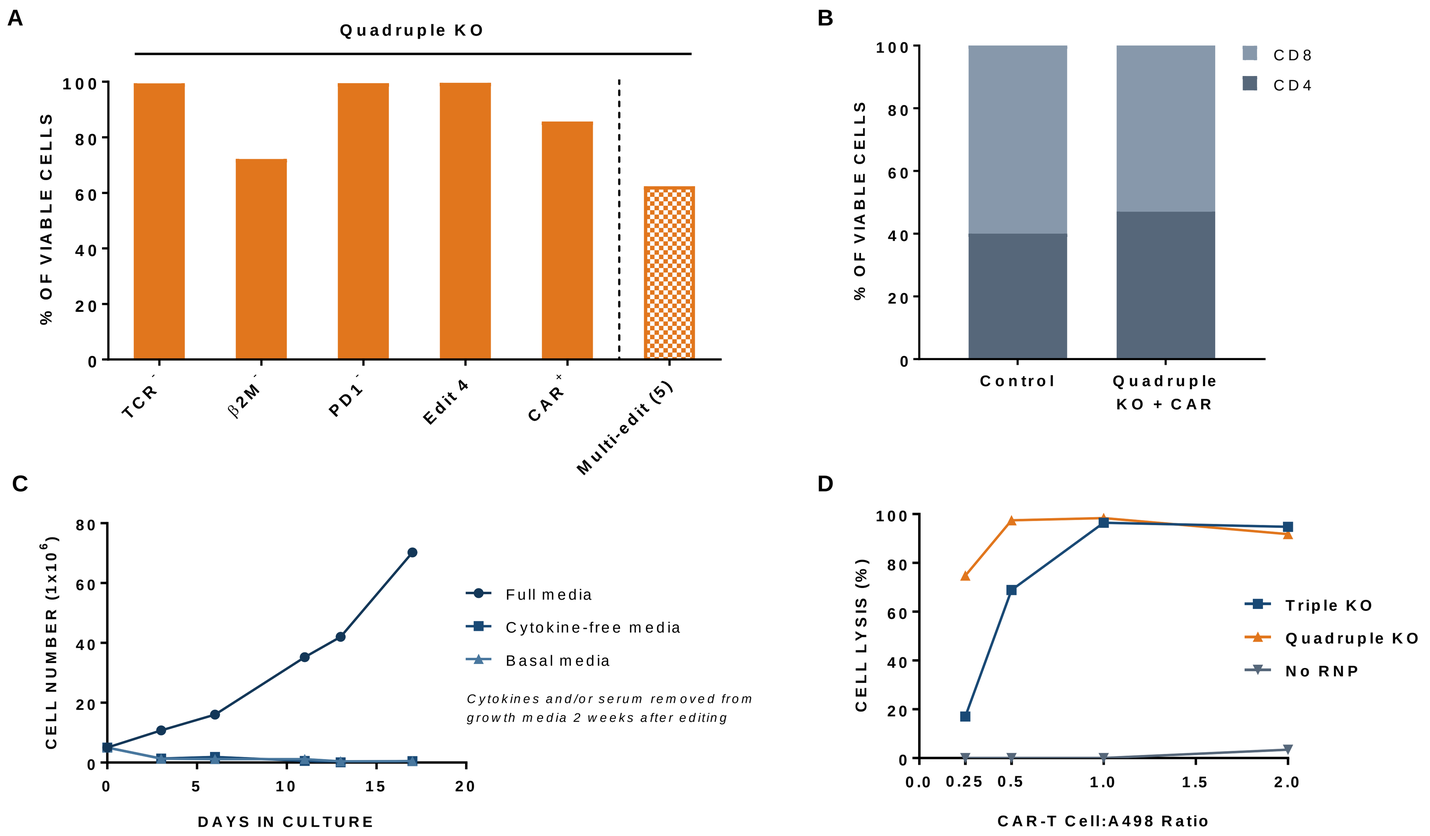
(**A**) Anti-BCMA CAR-T cells efficiently and selectively kill the BCMA-expressing multiple myeloma cell line MM.1S in a 4-hour cell kill assay, while sparing the BCMA$^-$ leukemic line K562. Differences in activity between double (TCR$^-$/β2M$^-$) and triple (TCR$^-$/β2M$^-$/PD1$^-$) KO CAR-T cells are notable at lower T cell concentrations. (**B**) The CAR-T cells also selectively secrete the T cell activation cytokines IFNγ 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 (“Edit4”) are achieved at high efficiency. More than 60% of T cells possess all 5 desired modifications (TCR$^-$/β2M$^-$/PD1$^-$/Edit4/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**