CRISPR/Cas9 Enables the Efficient Production of Allogeneic CAR-T Cells Engineered to Contain Multiple Genome Edits to Enhance Therapeutic T Cell Function

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Abstract

The CRISPR/Cas9 system allows for rapid assessment of the consequences of perturbing many genes while at the same time deriving potentially lead molecules for cell and gene therapies. We have applied this technology to discover the following in the allogeneic CAR-T cell setting:

1. multiple edits (>5) can be applied efficiently to produce stable non-transformed CAR-T cells;
2. the effects of single and multiple edits on CAR-T function can be examined efficiently to determine gene edits that improve CAR-T cell function;
3. the consequence of these effects for on-target and off-target activity can be used to rapidly generate lead gRNAs; and
4. next-generation cell therapies can be defined towards targeting solid tumors with allogeneic CAR-T cells.

Here we show the effects in vitro and in vivo of knocking-out multiple genes singly and in combination, including the response to multiple antigen challenges and the ability to overcome PD-L1 induced resistance. Producing CAR-T cells with multiple edits could be an important step towards enhancing the ability of this therapeutic class to tackle solid tumors with improved efficacy over the current therapeutics.

Figure 1: Anti-CD70 CAR-T Cells with Multiple KOs Show High Cytotoxic Activity against an RCC Tumor Cell Line

Figure 2: Additional KOs Confer Superior Cytotoxic Activity Against an RCC Tumor Cell Line Overexpressing PD-L1

Figure 3: Additional KOs Confer Superior Cytotoxic Activity During In Vitro Re-Challenge

Figure 4: 2X KO and 4X KO Anti-CD70 CAR-T Cells Both Eliminate an RCC Tumor Model In Vivo, but 4X KO CAR-T Cells Exhibit Superior Efficacy Following In Vivo Re-Challenge

Figure 5: High Efficiency Sixtule KO Plus CAR Insertion by CRISPR/Cas9 to Produce CAR-T Cells that Exhibit Good Health and Target-Specific Cytotoxicity

Conclusions

- The efficiency of the CRISPR/Cas9 system enables rapid screening of a number of different target genes to identify those that improve CAR-T function in a model solid tumor immunosuppressive environment.
- Multi-edited CAR-T cells containing these edits show impressive efficacy in both in vitro and in vivo re-challenge models.
- 7 edits (6 knock-outs and 1 knock-in) can be performed in a single experiment, generating functional and non-transformed CAR-T cells.
- These septuple-edited CAR-T cells show high efficiency and specificity cytotoxicity and cytokine response.
- The CRISPR/Cas9 system enables the selection and incorporation of edits that can help overcome the immunosuppressive environment of solid tumors.
- The viability and CD4/CD8 subset ratios (assessed 1 week after HDR) of sixtule KO CAR-T cells remain similar to unedited controls. Sixtule KO CAR-T cells still remain dependent on cytokines for growth and survival following multi-editing, suggesting no oncogenic transformation has occurred.

(2X KO anti-CD70 CAR-T cells retain cytotoxicity, while 2X KO anti-CD70 CAR-T cells lose their ability to mount a cytotoxic response and eventually stop proliferating. 2:1 T cell:A498 ratio.)