MAYO CLINIC

Saad S. Kenderian
Assistant Professor of Medicine, Oncology and Immunology
Senior Associate Consultant, Hematology
Mayo Clinic College of Medicine
Phone: 507-284-2865
Kenderian.saad@mayo.edu

Editors of *JoVE*

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Re: Using CRISPR/Cas9 to edit CAR-T cells

Dear Editors,

Here we re-submit editor revisions for consideration of a methods paper at your invitation (JoVE59629R1) entitled “Using CRISPR/Cas9 to edit CAR-T cells” by Rosalie M. Sterner, Michelle J. Cox, Reona Sakemura, and Saad S. Kenderian.

CAR-T cell therapy is a new potent modality in the treatment of cancer that was FDA approved in 2017 for B cell malignancies. However, its wider application is limited by the development of toxicities and need for enhanced efficacy in certain situations.

Here we describe one strategy to enhance CAR-T efficacy and/or control toxicities of CAR-T cells by editing the genome of the CAR-T cells themselves during CAR-T cell manufacturing. We detail the use of CRISPR/Cas9 gene editing in CAR-T cells via transduction with a lentiviral construct containing a guide RNA to granulocyte macrophage colony-stimulating factor (GM-CSF) and Cas9. As an example, we describe CRISPR/Cas9 mediated knockout of GM-CSF. We have shown that these GM-CSF$^{k/o}$ CAR-T cells effectively produce less GM-CSF while not inhibiting other critical T cell cytokines, and *in vivo* enhance anti-tumor activity and survival compared to wildtype CAR-T cells.

Here we describe a methodology to edit CAR-T cells with CRISPR/Cas9 technology. We believe this methods paper would address an unmet need and is quite timely as CAR-T cell therapy access and care is a major issue. This work is well suited for the widespread viewership of *JoVE.* This is an original work that has not been published or submitted in the past. This work was supported through grants from K12CA090628 (SSK), the National Comprehensive Cancer Network (SSK), the Mayo Clinic Center for Individualized Medicine (SSK), the Predolin Foundation (SSK), the Mayo Clinic Office of Translation to Practice (SSK), and the Mayo Clinic Medical Scientist Training Program Robert L. Howell Physician-Scientist Scholarship (RMS). SSK is an inventor on patents in the field of CAR T-cell therapy that are licensed to Novartis (under an agreement

between Mayo Clinic, University of Pennsylvania, and Novartis). These studies were funded in part by a grant from Humanigen (SSK). RMS, MJC, RS, and SSK are inventors on patents related to this work. The laboratory (SSK) receives funding from Tolero, Humanigen, Kite, Lentigen, Morphosys, and Actinium.

We would like to thank the editor for their timely, thoughtful, and insightful comments. Below we have addressed the editor's comments.

Thank you for your consideration,

Saad S. Kenderian, M.B., Ch.B.

Editorial comments:

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.
The formatting has been retained as requested.

2. Please address specific comments marked in the manuscript.
The comments have been addressed and explained in the manuscript as requested.

3. For the protocol section, please remove the redundancy and write exactly how the individual action was performed. Please write the steps in order of it being performed. Please make the steps crisp.
The steps have been edited as requested.

4. Please proofread the manuscript once done.
The manuscript has been proofed as requested.

5. Once done please highlight 2.75 pages of the protocol including headings and spacings for filming purpose.
The manuscript has been highlighted as requested.

6. Please expand the result section.
The results section has been expanded as requested.

7. Please alphabetically sort the materials table.
The materials table has been sorted as requested.