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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Durable Multiplex Epigenetic Editing for Generation of Allogeneic CAR T without Chromosomal Rearrangements

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CAR T therapies have provided significant breakthroughs for patients with CD19+ malignancies and multiple myeloma, but a major limitation is the need for individualized manufacturing of autologous T cells, increasing the cost and reducing the availability of these life-saving drugs. An allogeneic CAR T product, available off-the-shelf, could greatly reduce manufacturing burden and improve access to therapy. To date, allogeneic CAR T clinical trials have shown limited durable responses and have reduced only TCR expression to avoid a graft versus host response. Further editing to reduce MHC class I and II expression may produce a cell resistant to allogeneic CD4+ and CD8+ T cell rejection to potentially improve allogeneic CAR T persistence. Conventional genome editing technologies that rely on double or single stranded DNA breaks have the potential to introduce genomic changes including translocations, especially when multiple edits are introduced. Epigenetic editing, in contrast, enables durable modulation of gene expression without cutting or nicking the DNA sequence and is extremely well-suited for simultaneous targeting of multiple genes to enable generation of persistent allogeneic CAR T cells.

In this study, we evaluated the use of a dCas9-based epigenetic editor to silence TCR and MHC class I and II expression in primary T cells to produce allogeneic CAR T transduced for expression of CAR and HLA-E. Epigenetic editor mRNA and sgRNAs targeting the genes of interest were delivered via nucleofection to activated T cells. Edited T cells were then maintained in proliferative culture, with CD3/CD28 or PMA/ionomycin restimulation, and expression of the target genes was assessed by flow cytometry. Expression of TCR and MHC class I and II was durably suppressed in both single and multiple gene target silencing, demonstrating the robustness of epigenetic editing for ex vivo cell therapies. In addition, genomic integrity assessments demonstrated that epigenetic editing did not induce genomic changes as detected by either a quantitative sequencing-based assay or a fluorescence-based technique with single-cell resolution. In primary T cells, multiplex silencing did not cause genomic rearrangements, including translocations and truncations, above the level observed in controls, whereas Cas9 multiplex editing did.

Mixed lymphocyte reaction (MLR), specific killing, and degranulation assays were used to demonstrate allogeneic function of silencing edits and the overexpression of an NK shield in vitro. In these assays, epigenetically-edited cells performed similarly to control gene edited cells in which the same gene targets were knocked out. TCR-silenced T cells did not proliferate or produce IFN-γ in response to allogeneic PBMCs in an MLR assay, and MHC class I- and II-silenced T cells did not evoke a proliferative response or IFN-γ production from allogeneic CD4+ or CD8+ T cells. Furthermore, overexpression of the HLA-E NK shield protected HLA-E expressing cells relative to B2M KO cells in a specific killing assay and reduced the degranulation response of NKG2A+ NK cells without a corresponding increase in NKG2C+ NK cell degranulation observed with other single chain HLA-E constructs tested. Finally, multiplex epigenetically-edited allogeneic CAR T were produced by transduction for expression of an anti-BCMA CAR, followed by simultaneous editing to silence target genes. These cells displayed potent anti-tumor activity against the BCMA+ MM.1S cell line, comparable to activity of unedited CAR T cells.

These data support multiplex epigenetic editing to produce functional allogeneic CAR T cells that (1) do not exhibit a GVHD response; (2) resist CD8+ and CD4+ T cell alloresponses; and (3) resist the NK missing-self response normally consequent of reduced MHC class I expression without introduction of genotoxic events.

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