250 MANIPULATING T CELL EPIGENETIC PROGRAMS TO IMPROVE PERSISTENCE AGAINST GROUP 3 PEDIATRIC MEDULLOBLASTOMA

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Background Safe, effective therapies are desperately needed to improve survival and long-term quality of life for patients with medulloblastoma (MB), a malignant pediatric brain cancer. Chimeric antigen receptor (CAR) T cells are a promising therapeutic option as they specifically target T-cell cytotoxic potential to surface antigens only presented by tumor cells. All subgroups of MB highly express the surface protein B7 homolog 3 (B7-H3) whereas healthy brain tissue does not, making it an ideal target for CAR T cell therapy. However, a major roadblock to long-term tumor control is the inability of CAR T cells to persist, suggesting that genetic modifications to improve persistence will benefit overall therapeutic efficacy. The goal of this study was to compare head-to-head the impact of knocking out DNMT3A and TET2 negative regulators of CAR T cell proliferation and persistence against group 3 MB (G3MB), the most malignant subtype.

Methods We generated second generation B7-H3 CAR T cells with CD28ζ signaling domain and performed CRISPR/Cas9 knockout (KO) of DNMT3A, TET2, and AAVS1 as a control. We compared resulting CAR T cell persistence via repeat stimulation assays and tumor killing capacity via MTS cytotoxicity assays *in vitro*. We further compared genetically modified CAR T cells in NSG mice *in vivo* against the HDMB03 G3MB cell line.

Results Our data shows that KO of DNMT3A in B7-H3 CAR T cells consistently improves persistence against G3MB cell lines *in vitro*. KO of TET2, however, shows inconsistent improvement of persistence that is dependent on the healthy donor used to generate the CAR T cells. In 2 out of 4 donors tested, TET2 KO CAR T cells persisted longer than AAVS KO control CAR T cells and DNMT3A KO CAR T cells. In the other 2 donors, TET2 KO did not improve CAR T cell persistence. In donors where TET2 did produce a persistence benefit, TET2 KO CAR T cells failed to elicit strong tumor killing after multiple repeat stimulations compared to DNMT3A KO.

Conclusions Our study demonstrates that genetic KO of negative regulators of CAR T cell persistence can improve their anti-tumor function against G3MB. We further conclude that for the CD28ζ B7-H3 CAR, DNMT3A KO is superior for achieving improved persistence over TET2 KO. We are currently validating this result in other G3MB cell lines with differential B7-H3 antigen density to determine how antigen quantity impacts the performance of DNMT3A and TET2 KO CAR T cells.

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