T CELLS ENGINEERED WITH CHIMERIC ANTIGEN RECEPTORS TARGETING NKG2D LIGANDS DISPLAY LETHAL TOXICITY IN MICE

Heather VanSeggelen[1*], Joanne A. Hammill[1*], Daniela G.M.Tantalo[1], Anna Dvorkin-Gheva[2], Jacek M. Kwiecien[3], Galina F. Denisova[1], Brian Rabinovich[4], Yonghong Wan[1], Jonathan L. Bramson[1]

[1]Dept. of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON

[2]Dept. of Biochemistry and Biomedical Sciences, Centre for Functional Genomics, McMaster University, Hamilton, ON

[3]Department of Pathology and Molecular Medicine, Central Animal Facility, McMaster University, Hamilton, ON

[4]Division of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX

[*]*These authors contributed equally to this work*

T cells engineered to express a chimeric antigen receptor (CAR), called CAR-T cells, have shown significant promise as an adoptive transfer therapeutic for cancer. We have explored the use of CARs which target tumors via the NKG2D receptor; given that NKG2D ligands are overexpressed on stressed cells, such as tumor cells, they are an attractive therapeutic target. We evaluated the use of two unique NKG2D-based CAR constructs: i) a fusion of the full length NKG2D receptor and cytoplasmic CD3ζ, and ii) the extracellular domain of NKG2D on the scaffold of a conventional second-generation CAR (intracellular CD28 and CD3ζ domains). In addition, we combined the NKG2D-CD3ζ fusion with co-expression of the adaptor protein DAP10 in order to enhance CAR surface expression. All three of these CAR constructs were expressed on murine T cells from BALB/c and C57BL/6 hosts and their functionality compared. *In vitro*, BALB/c derived CAR-T cells showed increased functionality and surface expression of the CARs, suggesting strain-specific differences exist when using NKG2D-based CARs. Upon adoptive transfer of the NKG2D-CAR-T cells into their respective syngeneic hosts, we observed evidence of rapid, severe toxicity resulting in morbidity and mortality. The severity of this toxicity paralleled *in vitro* CAR expression levels; NKG2D-based CARs with higher levels of cell surface expression showed exacerbated toxicity as did those in BALB/c hosts, supporting CAR configuration and strain specific differences. Pre-treatment of mice with a chemotherapeutic agent, cyclophosphamide, prior to adoptive transfer of NKG2D-CAR-T cells exacerbated toxicity, particularly in BALB/c mice. These data demonstrate that enhancing the surface expression of CARs may be hazardous when targeting ligands which are not tumor specific. In addition, these data demonstrate that NKG2D-based CARs have the potential to drive serious off-tumor toxicities and urge extreme caution be used in their continued development.