169 OFF-THE-SHELF, ENGINEERED IPSC-DERIVED NK CELLS MEDIATE POTENT CYTOTOXIC ACTIVITY AGAINST PRIMARY GLIOBLASTOMA CELLS AND PROMOTE DURABLE LONG-TERM SURVIVAL IN VIVO

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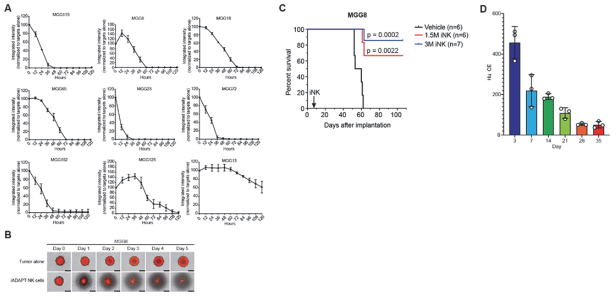
Background Glioblastoma multiforme (GBM) is a primary brain tumor with a high mortality rate and median survival of ~14 months. Although progress has been made in the development of available therapies, the treatment of GBM remains palliative.[1] Emerging results from preclinical studies support the concept that GBM cells may be highly susceptible to natural killer (NK) cell cytotoxicity.[2 3] However, sourcing donor-derived NK cells for adoptive cell therapy is limited by cell number and quality. To overcome these barriers, we developed a robust manufacturing system for the generation of high-quality off-the-shelf NK cells derived from induced pluripotent stem cells (iPSCs).[4]

Methods We generated triple gene-edited iPSCs designed for mass production of NK cells expressing a high affinity, non-cleavable version of the Fc receptor CD16a and a membrane-bound IL-15/IL-15R fusion protein along with knockout of the nicotinamide adenine dinucleotide (NAD+) hydrolase CD38. NK cells derived from these uniformly engineered iPSCs, termed iADAPT NK cells, displayed enhanced metabolic fitness, resistance to oxidative stress, broad natural cytotoxicity, and robust antibody-dependent cellular cytotoxicity (ADCC). To assess the cytotoxic capacity of iADAPT NK cells, we performed 3-dimensional (3-D) live imaging assays where iADAPT NK cell infiltration and cytotoxicity in response to 9 different primary, patient derived GBM spheroids was measured in real time over the course of 5 days. The in vivo persistence and antitumor function of iADAPT NK cells were also assessed using xenogeneic adoptive transfer models.

Results In 3-D live imaging assays, iADAPT NK cell efficiently infiltrated and eliminated patient-derived GBM spheroids (figure 1A, B). These in vitro results were recapitulated in vivo in xenogeneic experiments where human GBM cells were implanted intracranially into immunodeficient mice (n=19) followed by adoptive transfer of either 1.5x106 or 3x106 iADAPT NK cells. We show that adoptive transfer of iADAPT NK cells promoted survival in a dose-dependent manner (figure 1C). Importantly, we also found that iADAPT NK cells persisted at high levels in the brain for at least 21 days in the absence of exogenous cytokine support (figure 1D).



Abstract 169 Figure 1

Conclusions Triple gene-edited iPSCs can be used to robustly manufacture iADAPT NK cells. These off-the-shelf engineered NK cells exhibit potent cytotoxicity against primary, patient derived GBM cells. Work is in progress to further engineer iADAPT NK cells with chimeric antigen receptors incorporating defined targeting motifs to further enhance cytotoxicity against GBM cells. Our preclinical data provides proof-of-concept for a planned phase I clinical trial.

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Ethics Approval This project has been approved by the University of Minnesota IACUC. Approval ID: 1812-36595A

http://dx.doi.org/10.1136/jitc-2021-SITC2021.169