construct further supports NK cell recruitment and may stimulate the recruitment of other immune cells. NK activation through the tri-specific engager is achieved through NKp46-mediated signaling. We are investigating the ability of the trifunctional engager to support and enhance NK cell-mediated cytotoxicity against GBM in vitro and in patient-derived GBM xenografts in vivo.

Results We hypothesize that taking advantage of our multifunctional engager, NK cells will exhibit, at once, superior persistence, infiltration and antitumor activity, simultaneously addressing three of the main limitations to the use of NK cells in immunotherapy of GBM and other solid tumors.

Conclusions N/A

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164 AGENT-797, A NOVEL ALLOGENIC AND 'OFF-THE SHELF' INKT CELL THERAPY PROMOTES EFFECTIVE TUMOR KILLING

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Background Harnessing both the innate and adaptive immune system could increase the efficiency of current cancer immunotherapies and promote durable anti-tumor immunity. Invariant natural killer T (iNKT) cells are innate-like lymphocytes that bridge innate and adaptive immune responses and promote anti-cancer immunity. iNKT cells are activated and respond rapidly via multiple signals such as recognition of lipid antigens through the invariant T cell receptor (TCR), pro-inflammatory cytokines or recognition of stress ligands. Here we describe, AgenT-797, a novel, allogeneic and 'off-the shelf' iNKT cell therapy, designed to promote effective anti-cancer immunity against a wide range of malignancies.

Methods iNKT cells isolated from healthy donors were expanded by stimulation of the invariant TCR with alpha-Galactosylceramide (αGalCer) and cytokines using the AgenTus manufacturing protocol. The phenotype and functional activity of the expanded unmodified iNKT cells, AgenT-797, were characterized by flow cytometry. The cytotoxic potential of AgenT-797 was assessed in tumor co-culture assays against CD1d-expressing cancer cell lines. To further direct anti-tumor responses, iNKT cells were engineered to express Chimeric Antigen Receptors (CARs), and the cytotoxic potential assessed against antigen-expressing cancer cells.

Results iNKT cells were rapidly expanded up to 2 × 1010 cells in 30 days, with over 99% purity. Expanded, unmodified iNKT cells, AgenT-797, were found to secrete both Th1 (IFNγ, TNFa, GM-CSF) and Th2 (IL4, IL13) type cytokines. After rapid expansion, AgenT-797, retained their inherent cytotoxic capacity against CD1d-expressing tumor cell lines. Further, killing of tumor target cells, in vitro, was mediated through their endogenous invariant TCR or engineered CAR receptor.

Conclusions AgenT-797 is an 'off-the-shelf' and allogenic cell therapy with effective cancer killing properties. Strategies to engineer iNKT cells using CAR technology further enhance the tumor killing potential of iNKT therapy.

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165 ACTIVATING ANTIGEN CARRIERS GENERATED WITH MICROFLUIDICS CELL SQUEEZING DRIVE EFFECTIVE ANTI-TUMOR RESPONSES

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Background Activation of T cell responses is essential for effective tumor clearance, however generating targeted, effective antigen presentation to stimulate T cell response remains challenging. We can harness the natural process of red blood cell (RBC) clearance from the body to activate the antigen-specific immune responses. Using the Cell Squeeze® microfluidics platform, we generate activating antigen carriers (AACs) from RBCs to drive antigen presentation and T cell activation in human and murine models.

Methods We loaded proteins or synthetic long peptide antigens together with adjuvants into murine or human RBCs with Cell Squeeze® (SQZ'ing) to generate AACs and investigated the effects of SQZ'ing on the RBC membrane. Following intravenous AAC injection into mice, we measured AAC clearance kinetics and characterized the site and cell type of AAC uptake. We investigated the regulation of activation markers on phagocytes that engulf AACs, clearance of endogenous RBCs in mice treated with AACs, and the effect of boosting with AACs on endogenous T cell responses. To determine the ability of AACs to control subcutaneously implanted tumors, we measured tumor growth rates in mice therapeutically treated and boosted with AACs. Finally, we observed in vitro uptake of human AACs loaded with adjuvant and resultant maturation of monocyte-derived dendritic cells (MODCs) to qualify adjuvant delivery. Peptide antigen delivery to human AACs was measured with flow cytometry and fluorescence microscopy.

Results We demonstrated that SQZ'ing effectively loads AACs without reducing CD47 expression. When administered into a mouse, AACs were cleared from circulation within one hour and were engulfed by professional phagocytes in both the spleen and liver. In vivo, AACs upregulated activation markers on macrophages and DCs, and administration of AACs does not affect clearance or half-life of endogenous RBCs. Therapeutic AAC administration to mice strongly impedes tumor growth and extends survival; the anti-tumor responses correlate with >10x increase in antigen-specific CD8+ tumor-infiltrating lymphocytes compared to untreated mice. Boosting enhances endogenous T cell responses and boosting at early time points in the tumor model enhances low dose vaccinations. In an in vitro human system, we demonstrated that human AACs can be loaded with peptide antigen and adjuvant such that upon engulfment, AACs stimulated MODC maturation.

Conclusions In summary, these results indicate that AACs loaded with antigen and adjuvant can effectively drive antigen presentation and prime a potent anti-tumor response in mice. These data support the further study of SQZ AACs as an immunotherapy for cancer treatment.

Ethics Approval All methods were performed in accordance with the relevant guidelines and regulations. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at SQZ Biotechnologies, using the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Office of Laboratory Animal Welfare. All activities were also