Development of a CD8 Co-receptor Independent T Cell Receptor Specific for Tumor-Associated Antigen MAGE-A4 for Next Generation T Cell-Based Immunotherapy

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Background
The cancer-testis antigen MAGE-A4 is an attractive target for T cell-based immunotherapy, especially for indications with unmet clinical need like non-small-cell lung carcinoma or triple-negative breast cancer. Overcoming high tumor burden using adoptive transfer of T cells modified to express a transgenic T cell receptor (TCR) demands optimal recognition of the corresponding target on tumor cells by the TCR-modified T cells (TCR-Ts). Here we describe the isolation and pre-clinical characterization of high avidity TCR-Ts expressing a human leukocyte antigen (HLA)-A*02:01-restricted MAGE-A4-specific TCR that is fully functional in T cells irrespective of CD4 or CD8 co-receptor expression.

Methods
An unbiased CD137-based sorting approach was first used to identify an immunogenic MAGE-A4-derived candidate epitope that was properly processed and presented on HLA-A2 molecules encoded by the HLA-A*02:01 allele. To isolate high avidity T cells via subsequent multimer sorting, an in vitro priming approach using HLA-A2-negative donors (allo-geic-HLA-restricted priming approach) was conducted to bypass central tolerance to this self-antigen. Pre-clinical parameters of safety and activity were assessed in a comprehensive set of in vitro and in vivo studies of the lead TCR candidate derived from a selected T cell clone.

Results
A TCR recognizing the MAGE-A4-derived decapeptide GVYDGRHTV was isolated from primed T cells of a non-tolerant HLA-A2-negative donor. The respective TCR-T cell product bbT485, expressing the lead TCR in T cells from healthy donors, was demonstrated pre-clinically to have a favorable safety profile and superior in vivo potency compared to TCR-Ts made using a TCR derived from an HLA-A2-positive donor bearing a tolerized T cell repertoire to self-antigens. The natural high avidity allogeic (allo)-derived TCR was found to be CD8 co-receptor-independent, allowing effector functions to be elicited in transgenic CD4+ T helper cells. These CD4+ TCR-T cells not only supported an anti-tumor response by direct killing of MAGE-A4-positive tumor cells, but also upregulated hallmarks associated with helper function, such as CD154 expression and release of key cytokines upon tumor-specific stimulation.

Conclusions
The extensive pre-clinical assessment of safety and in vivo potency of this non-mutated high avidity, CD8 co-receptor-independent, MAGE-A4-specific HLA-A2 restricted TCR provide the basis for its use in clinical TCR-T immunotherapy studies. The ability of this co-receptor-independent TCR to activate all transduced T cells (irrespective of CD4 or CD8 expression) could potentially provide enhanced cellular responses in the clinical setting through the induction of functionally diverse T cell subsets that goes beyond what is currently tested in the clinic.

Reference
1. NA

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NICE: NEOANTIGEN-CYTOKINE-CHEMOKINE MULTIFUNCTIONAL ENGAGER FOR NK CELL IMMUNOTHERAPY OF SOLID TUMORS

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Background
The effectiveness of natural killer cell-based immunotherapy against solid tumors is limited by the lack of specific antigens and the immunosuppressive tumor microenvironment. To improve the clinical efficacy and specificity of NK cell therapy, we are designing, developing, and characterizing a new generation of multi-specific killer engagers, which consists of a neoantigen-targeting moiety, together with cytokine and chemokine-producing domains.

Methods
Targeting a neoantigen-an antigen formed specifically in response to tumor genome mutations-enables substantially enhanced tumor specificity to be achieved. We evaluated the responsiveness of NK cells to Wilms Tumor 1 (WT1) antigen in GBM by synthesizing an antibody that is able to recognize the WT1/HLA complex. Incorporation of cytokine (namely IL-2, IL-15, and IL-21)-essential for the maturation, persistence, and expansion of NK cells in vivo-favors the proliferation and survival of NK cells in the tumor microenvironment, thereby leading to more sustained anti-tumor responses. Additionally, our data have indicated that the chemokine CXCL10 plays an important role in the infiltration of immune cells into GBM, yet the chemokine itself is expressed at low levels in GBM. Incorporation of a CXCL10-producing element into our
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-Galactosyleramidase (αGalCer) and cytokines using the AgenTis 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 cell lines.

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, TNFα, 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 allogeneic 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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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 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 impeded 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