TAC receptors signal via the endogenous TCR, whereas CAR signaling is TCR-independent. Consistent with TCR-dependent signaling, ligation of TAC receptors resulted in the formation of conventional immunological synapses, whereas ligation of CARs produced unconventional synapses. Despite these functional differences, CARs and TAC receptors demonstrated a similar capacity to activate T cells against antigen-positive tumor cell targets. However, CAR-T cells displayed reactivity to antigen-negative cells, due to interaction with a cross-reactive antigen; TAC-T cells displayed no reactivity to antigen-negative cells.

**Conclusions** Tonic signaling in CAR-T cells reduces their activation threshold and increases their propensity to be activated by cross-reactive antigen. In contrast, TAC receptors do not deliver tonic signals, which increases the stringency of activation and reduces the likelihood of off-target responses. This feature of the TAC platform is advantageous to safeguard against the unexpected cross-reactivity that may occur when a new antigen-binding domain is deployed in vivo.

**Ethics Approval** Use of human materials was approved by the Hamilton Integrated Research Ethics Board (HiREB).

**REFERENCES**


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### INHIBITION OF AKT SIGNALING DURING EXPANSION OF TCR-ENGINEERED T-CELLS FROM PATIENT LEUKOCYTE MATERIAL GENERATES SPEAR T-CELLS WITH ENHANCED FUNCTIONAL POTENTIAL IN VITRO

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**Background** T-cells attributes for adoptive cell therapy of patients with advanced cancer can be optimized during ex vivo expansion culture. Autologous TCR-engineered T-cells targeting the MAGE-A4 antigen with Specific Peptide Enhanced Affinity Receptors (SPEAR T-cells) have shown promise in the clinic. The highly variable leukocyte material obtained from individual patients during apheresis can present a manufacturing challenge for autologous T-cell therapies. The degree of ex vivo expansion and the functional attributes of the expanded T-cell product impact therapeutic efficacy and can be suboptimal for some patient apheresis material. Both TCR and cytokine growth factor signals used for ex vivo T-cell expansion promote robust activation of AKT (Protein Kinase B) signaling, which drives T-cell activation, proliferation, and terminal differentiation. It is hypothesized that inhibition of AKT signaling during T-cell expansion may uncouple proliferation and terminal differentiation, leading to the generation of less differentiated T-cells that may have functional benefit in vivo. **Methods** We evaluated the use of an AKT inhibitor during SPEAR T-cell manufacturing using leukocytes from healthy donors and patients with advanced solid cancers.

**Results** AKT inhibition resulted in the generation of a more consistent expansion and phenotype of the final T-cell product. This was observed using two SPEAR T-cell constructs, ADP-A2M4 and ADP-A2M4CD8. Ex vivo SPEAR T-cell expansion in the presence of an AKT inhibitor generated CD8 + T-cells that maintained a less differentiated phenotype (based on CCR7+CD45RA+ and CD62L+ expression). AKT inhibition was associated with enhanced antigen-specific responses of SPEAR T-cells in vitro, including effector cytokine production, target-cell killing, ability to proliferate in response to prolonged antigen-stimulation and maintenance of cytotoxic activity following antigen re-stimulation.

**Conclusions** We plan to introduce AKT inhibition into the GMP manufacturing process, and evaluate the efficacy of the resulting products in ongoing clinical studies.

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**Ethics Approval** The experimental study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and was approved by local authorities. An independent ethics committee or institutional review board approved the clinical protocol at each participating center. All the patients provided written informed consent before study entry.

**REFERENCES**


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### DECITABINE GENE MODULATION SENSITIZES HUMAN NON–CELL-MEDiated LUNG CANCER (NSCLC) TO NY-ESO-1 TCR IMMUNOTHERAPY (LETETRESGENE AUTOLEUCEL; GSK3377794) IN VIVO

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**Background** NY-ESO-1–specific T cells (letetresgene autoleucel [lete-cell] GSK3377794) are autologous CD4+ and CD8+ T cells transduced to express a high-affinity T-cell receptor (TCR) capable of recognizing NY-ESO-1 and LAGE-1a antigens in complex with human leukocyte antigen (HLA)-A*02. NY-ESO-1 (CTAG1B) and LAGE-1a (CTAG2) are tumor-associated antigens (TAA) that share the SLLMWITQC peptide bound to human leukocyte antigen HLA-A*02 and are expressed in various cancers. Emerging evidence suggests that TCR-engineered T cells targeting NY-ESO-1 hold promise for patients with solid tumors. Approximately 75% of synovial sarcomas can over-express NY-ESO-1 vs 12% of NSCLC, however, NSCLC expression of NY-ESO-1/LAGE-1a may have
POLYFUNCTIONAL ACTIVITY OF GD3CAR T-CELLS AGAINST TUMORS IN TUBEROUS SCLEROSIS COMPLEX

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97 POLYFUNCTIONAL ACTIVITY OF GD3CAR T-CELLS AGAINST TUMORS IN TUBEROUS SCLEROSIS COMPLEX

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Background Gangliosides are glycosphingolipids that are involved in cellular functions, including signal transduction, cell proliferation, differentiation, adhesion, and angiogenesis.1 We confirmed marked overexpression of GD3 in tumors associated with Tuberous Sclerosis complex (TSC) and proposed to evaluate the use of T cells expressing a second-generation chimeric antigen receptor (GD3CAR T-cells) for patient treatment. To evaluate the potency of GD3CAR T-cells targeting solid tumor cells, we performed in vitro assays using Tsc2 knockout, GD3 overexpressing tumor cells isolated from mice heterozygous for Tsc2. HEK293 cells transfected or not with an expression plasmid encoding the enzyme SIAT8, responsible for converting GM3 to GD3, were used as controls. Cell were subjected to cytokotoxic assays using live cell imaging, and single cell cytokine secretonome analysis among CD4 or CD8 T-cell populations.

Methods GD3CAR construct generated includes an anti-GD3 antibody single-chain antibody fragment and intracellular sequence of CD28 and CD3 zeta chain.2 Mice T cells were transduced, and transduction was established by flow cytometry. GD3 expressing Tsc2/-/ tumor cells or HEK cells were co-cultured with untransduced or GD3CAR T-cells and cytokotoxicity was measured using the Incucyte S3 system. Cytokine secretion patterns of CD4 and CD8 subpopulations of CAR T-cells were measured after coculture in a single cell polyfunctional strength mouse Isocode Chip (IsoPlexis). Secretory profiles of single cells were analyzed by IsoSpeak Software. IFN gamma secretion was quantified by ELISA as a functional readout of T cell activity.

Results Transduction efficiencies observed were upward of 70% live GD3 CAR T-cells with 96% transduction efficiency of CD4 T cells and 90% of CD8 T cells. The cytokotoxic assay in the Incucyte live-cell imaging system indicated 4-fold increased apoptosis (p=0.038) when target cells were co-cultured with GD3CAR T-cells. Both CD8 and CD4 T cells were efficiently transduced to express the GD3CAR. In single-cell cytokine analysis, both T cell subsets showed enhanced polyfunctionality with increased polyfunctional strength index (PSI) by 9 and 10-fold in the GD3CAR T-cells in the CD4 and CD8 populations, respectively. This was mainly attributed to effector, chemo-attractive and stimulatory cytokines IFN gamma production was increased significantly in response to target cells expressing GD3.

Conclusions Both CD4 and CD8 GD3CAR T-cells express polyfunctional cytokine profiles in response to GD3 expressing tumor cells, and CAR T-cells were selectively cytotoxic to relevant tumor cells. The data suggests that GD3CAR T-cells may reduce tumor growth observed in patients with TSC.

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98 ATA3271: AN ARMORED, NEXT-GENERATION OFF-THE-SHELF, ALLOGENEIC, MESOTHELIN-CAR T CELL THERAPY FOR SOLID TUMORS


Background Mesothelin (MSLN) is a glycosylphosphatidylinositol (GPI)-anchored membrane protein with high expression...