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.1 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 factors used for ex vivo T-cell expansion promote robust 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.2 3

Methods We evaluated 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.

Acknowledgements We are extremely grateful to the patients, who were previously enrolled in our clinical trials, and healthy donors for their consent for R&D studies. This was a collaborative cross-functional project, and we are grateful for the contributions of the following Scientists: Garth Hamilton, Adel Toth, Abigail Kay, Sophie Badie, Josh Griffiths, Kaushik Sarkar, Anoon Chandran.

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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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NOC

DEGCTABLE GENE MODULATION SENSITIZES HUMAN NON–SMALL CELL LUNG CANCER (NSCLC) TO NY-ESO-1 TCR IMMUNOTHERAPY (LETETREGENE AUTOLEUCE; GSK3377794) IN VIVO

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Background NY-ESO-1–specific T cells (letetregene 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.1 Approximately 75% of synovial sarcomas can over-express NY-ESO-1 vs 12% of NSCLC,2 however, NSCLC expression of NY-ESO-1/LAGE-1a may have
therapeutic potential. A separate study using engineered T cells targeting NY-ESO-1 has shown a partial response in a patient with advanced lung adenocarcinoma. Decitabine (DAC) is a hypomethylating agent and potent inducer of TAA, including NY-ESO-1. We have reported in vitro use of DAC to selectively modulate TAA expression in TAA low-expressing tumor cell lines in order to enhance lete-cell therapy. The aim of this study was to assess enhancement of combination therapy with lete-cell and DAC in an in vivo NSCLC model.

**Methods** NOD scid gamma (NSG) mice were injected subcutaneously with the human NSCLC tumor cell line NCI-H1703. Upon engraftment, tumor-bearing mice were treated with a 5-day course of DAC or vehicle control followed by 2 days of rest. Lele-cell was infused on Day 8. RNA was isolated from tumor formalin-fixed paraffin-embedded blocks, and levels of NY-ESO-1 and LAGE-1a transcript were measured by RT-qPCR. Expression pattern of the NY-ESO-1 protein was assessed via immunohistochemistry. Efficacy was defined by changes in tumor volume and systemic IFN-γ secretion.

**Results** Consistent with our previous in vitro studies, DAC treatment in vivo resulted in induction of NY-ESO-1 and LAGE-1a in NSCLC tumors. Lele-cell in combination with DAC significantly enhanced antitumor efficacy in vivo compared with lete-cell alone. This was associated with increased interferon-γ secretion. Mice that received DAC treatment only did not show statistically significant tumor reduction compared with untreated mice.

**Ethics Approval** All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals. Human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an Institutional Review Board/ Ethics Committee approved protocol.

**Conclusions** GSK is currently enrolling a Phase Ib/IIa, multi-arm, open-label pilot study (NCT03709706) of lele-cell as a monotherapy or in combination with pembrolizumab in HLA-A*02-positive patients with NSCLC whose tumors express NY-ESO-1/LAGE-1a. This work may support rationale for the use of DAC in combination with lete-cell to improve adoptive T-cell therapy by increasing levels of target antigens and antitumor effect in NSCLC.

**Acknowledgements** Funding: GSK

**REFERENCES**


**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 cytotoxicity assays using live cell imaging, and single cell cytokine secretome analysis among CD4 or CD8 CAR T-cells.

**Methods**

1. **GD3CAR** construct generated includes an anti-GD3 antibody single-chain antigen fragment and intracellular sequence of CD28 and CD3 zeta chain. 2 Mouse 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 cytotoxicity 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 assay using mouse Isocode Chip (IsoPlexis). Secretory profiles of single cells were analyzed by IsoSpeak Software. IFNγ 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 cytotoxicity 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 attributable to effector, chemo-attractive and stimulatory cytokines IFNγ 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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**REFERENCES**


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

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