thorpean 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. Lete-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 measured using the NY-ESO-1 antibody single-chain antibody fragment and intracellular cytokine 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 lete-cel 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 anti-tumor effect in NSCLC.

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**REFERENCES**


At the core of our study, 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 ST8A, 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** GD3CAR construct generated included an anti-GD3 antibody single-chain antibody fragment and intracellular sequence of CD28 and CD3 zeta chain. 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 strength 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 attributed 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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Polyfunctional Activity of GD3CAR T-cells Against Tumors in Tuberous Sclerosis Complex

**Background** Gangliosides are glycosphingolipids that are involved in cellular functions, including signal transduction, cell proliferation, differentiation, adhesion, and angiogenesis. We
levels in an array of malignancies including mesothelioma, oварia, non-small cell lung cancer, and pancreatic cancers and is an attractive target antigen for immune-based therapies. Early clinical evaluation of autologous MSLN-targeted chimeric antigen receptor (CAR)-T cell therapies for malignant pleural mesothelioma has shown promising acceptable safety1 and have recently evolved with incorporation of next-generation CAR co-stimulatory domains and armoring with intrinsic checkpoint inhibition via expression of a PD-1 dominant negative receptor (PD1DNR).2 Despite the promise that MSLN CAR-T therapies hold, manufacturing and commercial challenges using an autologous approach may prove difficult for widespread application. EBV T cells represent a unique, non-gene edited approach toward an off-the-shelf, allogeneic T cell platform. EBV-specific T cells are currently being evaluated in phase 3 trials [NCT03934635] and, to-date, have demonstrated a favorable safety profile including limited risks for GvHD and cytokine release syndrome.3, 4 Clinical proof-of-principle studies for CAR transduced allogeneic EBV T cell therapies have also been associated with acceptable safety and durable response in association with CD19 targeting.5 Here we describe the first preclinical evaluation of ATA3271, a next-generation allogeneic CAR EBV T cell targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

Methods We generated allogeneic MSLN CAR+ EBV T cells (ATA3271) using retroviral transduction of EBV T cells. ATA3271 includes a novel 1XX CAR signaling domain, previously associated with improved signaling and decreased CAR-mediated exhaustion. It is also armored with PD1DNR to provide intrinsic checkpoint blockade and is designed to retain functional persistence.

Results In this study, we characterized ATA3271 both in vitro and in vivo. ATA3271 show stable and proportional CAR and PD1DNR expression. Functional studies show potent antitumor activity of ATA3271 against MSLN-expressing cell lines, including PD-L1-high expressors. In an orthotopic model of pleural mesothelioma, ATA3271 demonstrates potent antitumor activity and significant survival benefit (100% survival exceeding 50 days vs. 25 day median for control), without evident toxicities. ATA3271 maintains persistence and retains central memory phenotype in vivo through end-of-study. Additionally, ATA3271 retains endogenous EBV TCR function and reduced alloreactivity in the context of HLA mismatched targets.

Conclusions Overall, ATA3271 shows potent anti-tumor activity without evidence of alloreactivity, both in vitro and in vivo, suggesting that allogeneic MSLN-CAR-engineered EBV T cells are a promising approach for the treatment of MSLN-positive cancers and warrant further clinical investigation.

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