therapeutic potential.³ A separate study using engineered T cells targeting NY-ESO-1 has shown a partial response in a patient with advanced lung adenocarcinoma.⁴ Decitabine (DAC) is a hypomethylating agent and potent inducer of TAA, including NY-ESO-1.⁵ We have reported in vitro use of DAC to selectively modulate TAA expression in TAA low-expressing tumor cell lines in order to enhance let-7 therapy.³ The aim of this study was to assess enhancement of combination therapy with let-7 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. Let-7 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. Let-7 in combination with DAC significantly enhanced antitumor efficacy in vivo compared with let-7 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 let-7 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 let-7 to improve adoptive T-cell therapy by increasing levels of target antigens and anti-tumor effect in NSCLC.

Acknowledgements Funding: GSK

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<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0096>

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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. Cells 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 includes an anti-GD3 antibody single-chain antibody 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 strength mouse Isocode Chip (IsoPlexis). Secretory profiles of single cells were analyzed by IsoSpeak Software. IFN γ 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 IFN γ 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.

Acknowledgements This study is supported by a Department of Defense grant W81XWH-17-TSCR-CTRA-TS170066 to CLP.

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<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0097>

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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 glycosylphosphatidylinositol (GPI)-anchored membrane protein with high expression