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-cel therapy. The aim of this study was to assess enhancement of combination therapy with lete-cel 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-cel 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. Lete-cel in combination with DAC significantly enhanced antitumor efficacy in vivo compared with lete-cel 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/Ia, 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-cel to improve adoptive T-cell therapy by increasing levels of target antigens and antitumor effect in NSCLC.

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Abstracts

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

1Anny Thomas, 2Emilia Dellacecca, 3Rohan Shivde, 4Nicola Lanki, 5Levi Barse, 1Anny Thomas, 1Agnes Lo, 1Denise M Scholtens, 2Daniel F Dilling, 1Richard Junghans, 2Angelo SP, Melchiori L, Merchant MS, et al. Cancer Discov. 2018;8:944–957.

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 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 cultured with or without DAC to determine if DAC treatment resulted in induction of NY-ESO-1 and LAGE-1a. 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 are selectively cytotoxic to relevant tumor cell lines. The data suggests that GD3CAR T-cells may reduce tumor growth observed in patients with TSC.

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Abstracts

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
levels in an array of malignancies including mesothelioma, ovari,a non-small cell lung cancer, and pancreatic cancers and is an attractive target antigen for immune-based therapies. Early clinical evaluation of allogeneic MSLN-targeted chimeric antigen receptor (CAR)-T cell therapies for malignant pleural mesothelioma has shown promising acceptable safety and have recently evolved with incorporation of next-generation CAR co-stimulatory domains and arming with intrinsic checkpoint inhibition via expression of a PD-1 dominant negative receptor (PD1DN R).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 [NCT03394365] and, to-date, have demonstrated a favorable safety profile including limited risks for GvHD and cytokine release syndrome. 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 therapy targeting MSLN and incorporating PD1DN R, 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 PD1DN R 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 PD1DN R expression. Functional studies show potent antitumor activity of ATA3271 against MSLN-expressing cell lines, including PD-L1-high expressors. In an orthotopic mouse 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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99 STRUCTURAL OPTIMIZATION OF ANTI-CEA-GITR-CAR TO REDUCE TONIC SIGNALING AND IMPROVE ANTIGEN-SPECIFIC REACTIVITY

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Background Adoptive immunotherapy using chimeric antigen receptor (CAR) is recently reported as one of the effective cancer therapy. Especially CAR-T cell therapy targeting CD19 antigen in B-cell tumors have shown impressive clinical results and CAR-T cell products targeting CD19 have already approved. However as the high relapse rate is still the problem and the clinical efficacy of CAR-T cell therapy for solid tumors is currently inadequate, further improvement of CAR design is required. It is known that the design of CAR construct affects the function of CAR-T cells. For example co-stimulatory domain such as CD28 and 4-1BB is used in the second generation CARs, CD28z-CAR-T cells show higher anti-tumor activity, whereas 4-1Bbz-CAR-T cells demonstrate superior in vivo persistence. To enhance survival of T cells, several attempts had been made to optimize the signaling domains. Recently, we have developed the novel CARs incorporated GITR (glucocorticoid-induced tumor necrosis factor receptor family-related protein) intracellular domain for T cell survival prolongation and inhibition of regulatory T cells’ suppressive activity. It is also reported that the antigen-nonspecific activation of CAR-T cells (tonic signaling) is influenced by the CAR design, and excessive T cell activation leads exhaustion of CAR-T cells. Previously, we have found that the design of CAR, not only single chain variable fragments (scFvs), affect the strength of tonic signaling. Thus, the optimization of CAR construct is essential to induce antigen-specific response with minimal non-specific activation, which results in maximal efficacy.

Methods We have optimized the structure of anti-CEA-GITR-CAR targeting CEA antigen expressing solid tumor such as gastric and pancreatic cancer. We have constructed several CARs with the different composition such as hinge region, transmembrane domain, and the order of VL/VH in scFv region, and compared the tonic signaling and antigen-specific activity in CAR-T cells.

Results The property of CAR-T cells was largely affected by the CAR construct, especially the hinge region. The CAR-T cells with CD80 hinge showed strong tonic signaling, the CAR-T cells with short hinge-CAR lost antigen specificity, and elimination of hinge region lowered the CAR expression level and antigen reactivity. Furthermore, GITR-CAR-T cells showed higher proportion of CCR7+/CD45RA+ cells and lower expression of exhaustion markers (PD1, Tim3, and LAG3) compared to CD28z-CAR-T cells.

Conclusions Our CEA-GITR-CAR with the optimized scFv design and CD28-hinge demonstrated improved antigen-