ATA3271: An Armored, Next-Generation Off-The-Shelf, Allogeneic, Mesothelin-CAR T Cell Therapy for Solid Tumors

Abstract ID: 98

Jiangyue Liu, Xianhui Chen, Jason Karlen, Alfonso Brito, Tiffany Jehng, Philippe Foubert, Janani Krishnamurthy, Yannick Bulliard, Blake T. Aftab

Preclinical & Translational Sciences, Atara Biotherapeutics, Inc., Thousand Oaks, CA

ATARA BIO®

Background

Mesothelin (MSLN) is a glycosylphosphatidylinositol (GPI)-anchored membrane protein with high expression levels in an array of malignancies including mesothelioma, ovarian, non-small cell lung 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 preliminary investigator assessed efficacy results and acceptable safety profile [Adusumilli et al. AACR 2019] 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) demonstrating higher efficacy and persistence in animal models [Kiesgen et al. AACR 2020]. 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 with no evidence for T cell therapy-induced GvHD or 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 [Curran et al. TCT 2020]. Here we describe the first preclinical evaluation of ATA3271, a next-generation allogeneic CAR EBV T cell therapy targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

Generation of EBV T cells expressing MSLN-1XX CAR and PD1DNR

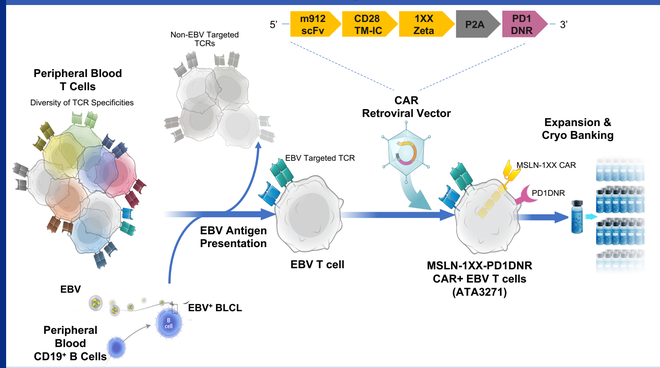

Figure 1 T and B cell fractions are separated from an unrelated donor via leukapheresis. The CD19+ fraction is transformed with EBV, generating an EBV+ lymphoblastoid cell line (BLCL). T cells are stimulated with BLCLs prior to retroviral introduction of mesothelin (MSLN)-targeted CAR with 1XX signaling domain and PD1DNR. The mesothelin scFv is derived from human anti-MSLN antibody m912. Continued expansion of MSLN-1XX-PD1DNR CAR+ EBV T cells (ATA3271) occurs with BLCL stimulation prior to harvest and cryopreservation for later use.

ATA3271 is transduced with high efficiency to express CAR and PD1DNR

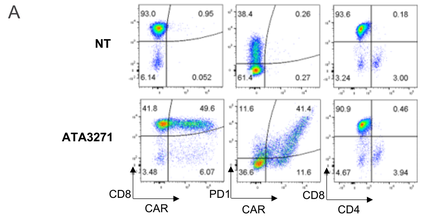

Figure 2 (A) Flow cytometric analysis after staining with CD4, CD8, CAR and PD1 demonstrates the successful transduction of EBV T cells with ATA3271 retroviral vector at high efficiency.

ATA3271 expresses MSLN-1XX CAR and PD1DNR and maintains viability post-transduction

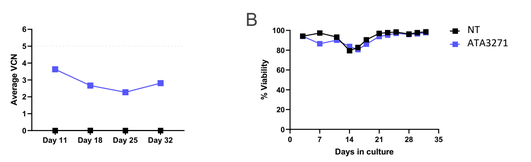

Figure 3 (A) Average level of MSLN-1XX-PD1DNR CAR vector copy number (VCN) and **(B)** viability of T cells during T cell production process demonstrates stable presence over time. Non-transduced T (NT) cells were used as control.

ATA3271 exhibits robust memory phenotype and shows high purity of T cells

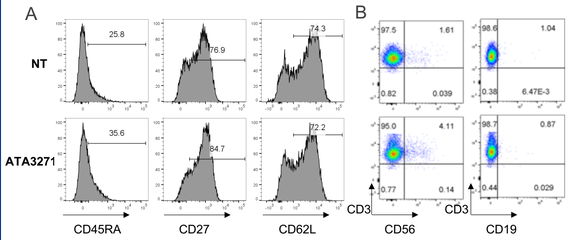

Figure 4 (A) Flow cytometric profiling of ATA3271 for CD45RA, CD27 and CD62L shows robust central memory and effector memory T cell phenotypes. **(B)** ATA3271 shows high purity of CD3+ T cells with negligible residual CD56+ NK cells and CD19+ B cells. NT cells were used as control.

ATA3271 demonstrates reduced alloreactivity towards HLA mismatched targets and potential for off-the-shelf use

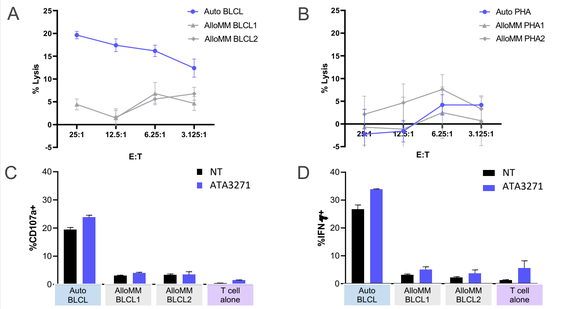

Figure 5 Alloreactivity was assessed *in vitro* by measuring the release of ^{51}Cr from labeled **(A)** EBV+ and MSLN- HLA-matched and mismatched BLCLs and **(B)** EBV- and MSLN- HLA-matched and mismatched PHA blasts after 4 hours in coculture with ATA3271. The expression of **(C)** stimulation marker CD107a and **(D)** cytokine IFN-γ in ATA3271 after 4 hours in coculture with BLCLs at the E:T ratio of 1:3 was analyzed by flow cytometry.

ATA3271 demonstrates MSLN-specific antitumor activity that is retained in the presence of high PD-L1 and repeat challenge

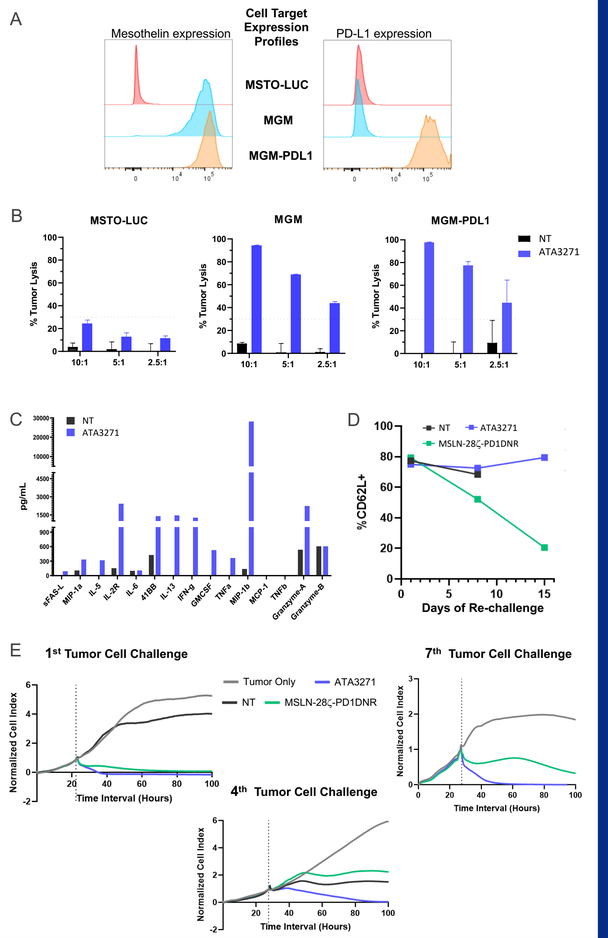

Figure 6 (A) Mesothelin and PD-L1 expression levels in target cell lines MSTO-LUC, MGM and MGM-PDL1. These three cell lines are derived from MSTO-211H human mesothelioma cell line. **(B)** Tumor cell lysis measured by luciferase-based cytotoxicity assay after 24 hours in coculture with target cells at the indicated Effector:Target (E:T) ratios and **(C)** cytokine secretion measured by Luminex assay after 24 hours in coculture with target cells at the E:T ratio of 10:1 demonstrate *in vitro* antitumor activity of ATA3271 that can be retained in the presence of high expression of PD-L1. In the serial killing study with T cells challenged by MGM-PDL1 cells every 2 or 3 days at the E:T ratio of 5:1, **(D)** CD62L+ memory phenotype and **(E)** tumor lysis capability of ATA3271 were well preserved during the 7 rounds of tumor cell challenges.

ATA3271 demonstrates potent *in vivo* antitumor activity, persistence and significant survival benefit without evidence of toxicity

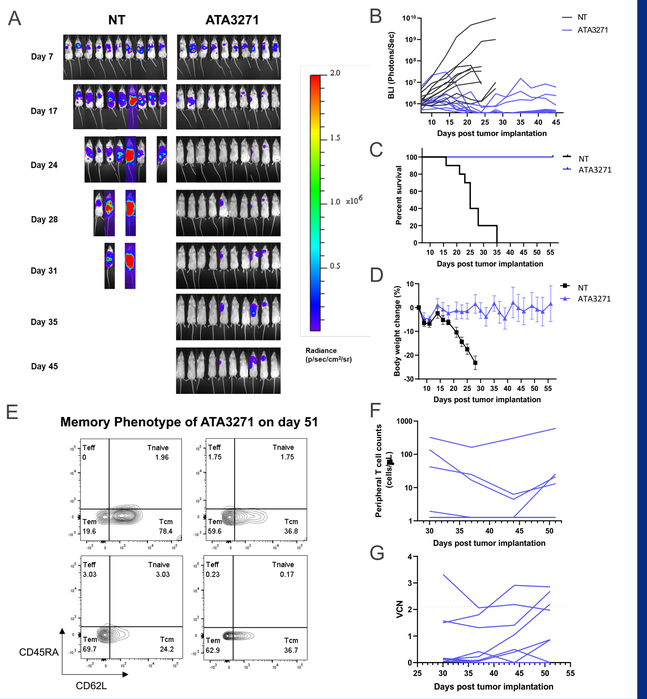

Figure 7 NSG mice implanted with $0.5x10^6$ MGM-PDL1 cells through intrapleural injection on day 0. Mice were randomized into NT group and ATA3271 group by BLI radiance on day 7 and treated with a single intrapleural injection of freshly thawed T cells. Mice were followed for another 48 days. **(A)** BLI radiance for each mouse was measured at various time points post tumor implantation. **(B)** BLI, **(C)** survival and **(D)** average body weight change of NT group and ATA3271 group were compared. **(E)** CD3+ CD62L+ CD45RA- central memory T cells were detected in the peripheral blood of 4 mice in ATA3271 group on day 51 (day 43 post treatment). **(F)** Human T cell count and **(G)** MSLN CAR vector copy number in the peripheral blood of ATA3271 group were analyzed at various time points by flow cytometry and qPCR, respectively.

Summary and Conclusions

- **ATA3271 is an armored, off-the-shelf allogeneic T cell therapy targeting MSLN via next generation CAR (1XX) and cell-intrinsic checkpoint resistance via PD1DNR built on our EBV T cell platform.**
- **With high efficiency, we engineered allogeneic EBV T cells to stably express MSLN CAR with 1XX signaling domain and PD1DNR (Fig 2, 3).**
- **ATA3271 demonstrates reduced alloreactivity against HLA mismatched targets *in vitro* (Fig 5).**
- **ATA3271 exhibits memory T phenotypes and demonstrates durable antigen-specific antitumor activity *in vitro* and *in vivo* (Fig 4, 6, 7).**
- **Overall, ATA3271 shows potent antitumor activity both *in vitro* and *in vivo*, with no evidence of allo-toxicity and represents a promising approach for the treatment of MSLN-positive cancers.**