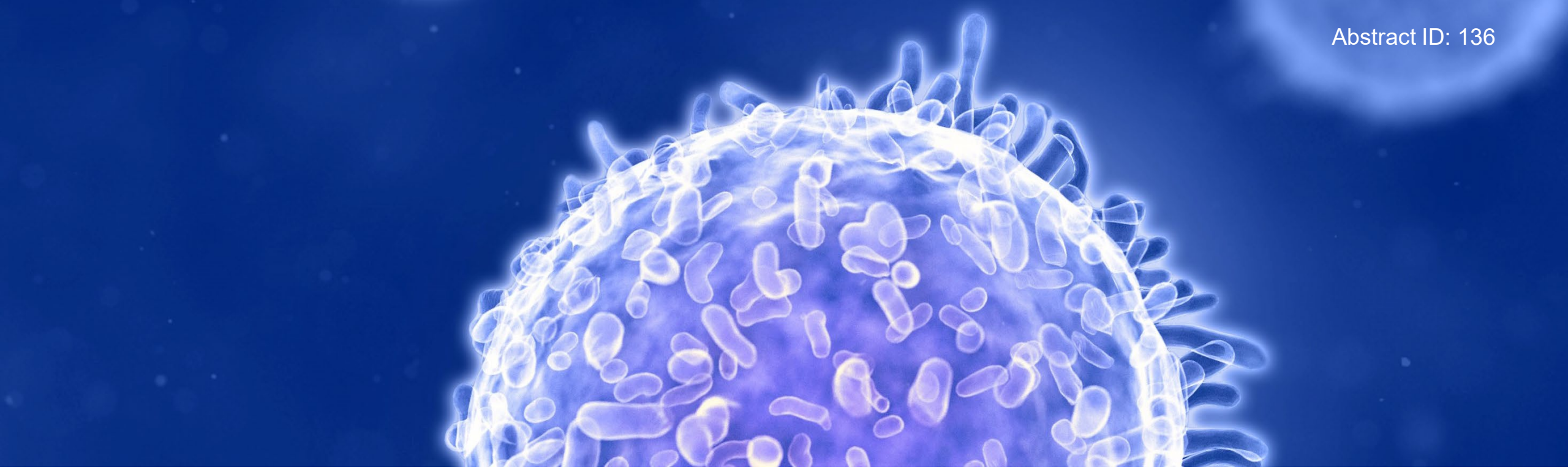

Abstract ID: 136

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 GPI-anchored membrane protein with high expression levels in an array of malignancies including mesothelioma and is an attractive target antigen for tumor surface antigen-targeting therapies. Regional administration of autologous, 2nd-generation MSLN-targeted CAR-T cells for malignant pleural mesothelioma has shown promise in early clinical evaluation [1,2]. More recently, a next-generation MSLN-targeted, autologous CAR-T therapy leveraging 1XX CAR signaling and PD1DNR is currently under investigation for advanced mesothelioma [NCT04577326]. Although autologous MSLN CAR-T holds promise, an allogeneic approach may have more widespread application.
- EBV T cells represent a unique, non-gene edited approach for allogeneic T cell therapy. EBV-specific T cells are currently in a phase 3 trial for EBV-positive post-transplant lymphoproliferative disease [NCT03394365] and, to-date, have demonstrated a favorable safety profile with no evidence for GvHD and cytokine release syndrome attributable to EBV T cells. Clinical proof-of-principle studies for CAR transduced CD19-targeted allogeneic EBV T cell therapies have shown acceptable safety and durable response [3]. The first preclinical evaluation of ATA3271 was reported last year [4]. Here, we describe updated preclinical data for this potential off-the-shelf, allogeneic cell therapy.

Figure 1. Generation of EBV T Cells Expressing MSLN-1XX CAR and PD1DNR

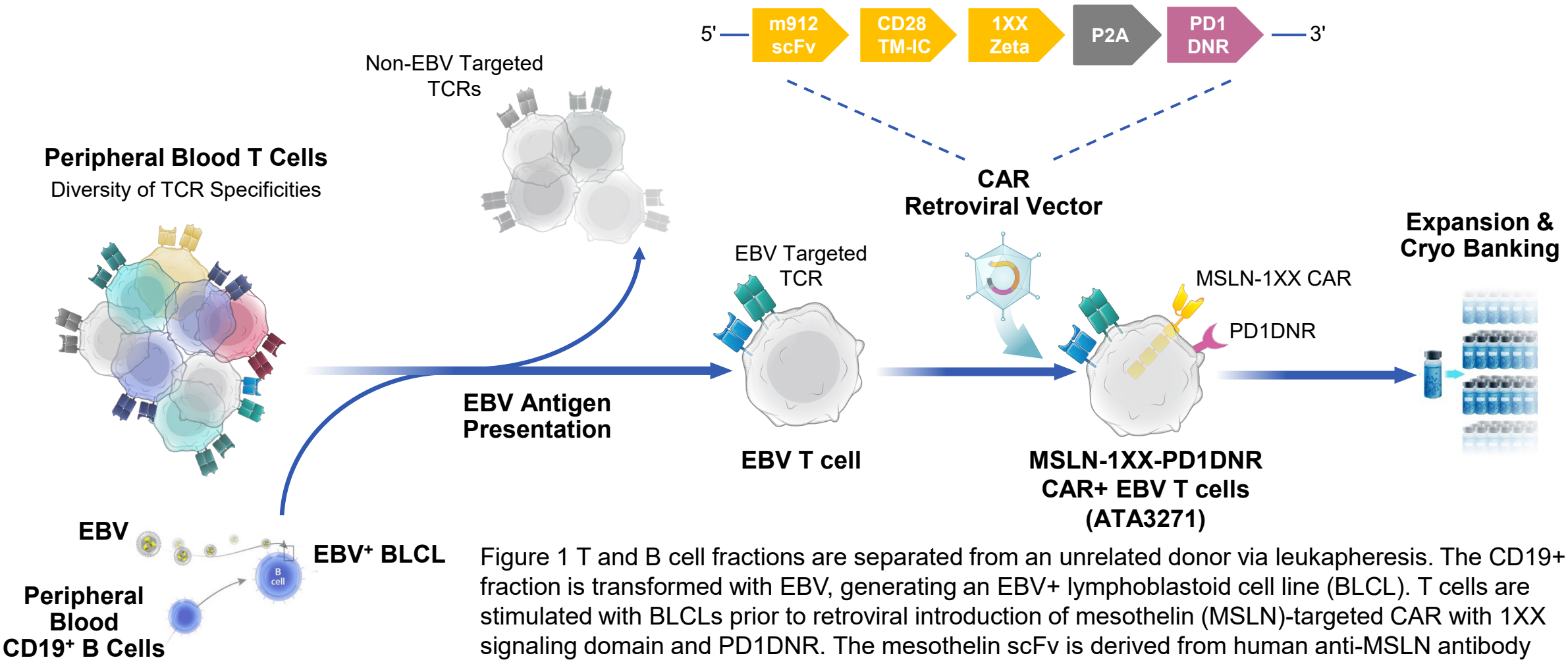


Figure 1 T and B cell fractions are separated from an unrelated donor via leukapheresis. The CD19+ fraction is transformed with EBV, generating an EBV+ lymphoblastoid cell line (BLCL). T cells are stimulated with BLCLs prior to retroviral introduction of mesothelin (MSLN)-targeted CAR with 1XX signaling 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.

Figure 2. ATA3271 Is Transduced to Express MSLN-1XX CAR and PD1DNR

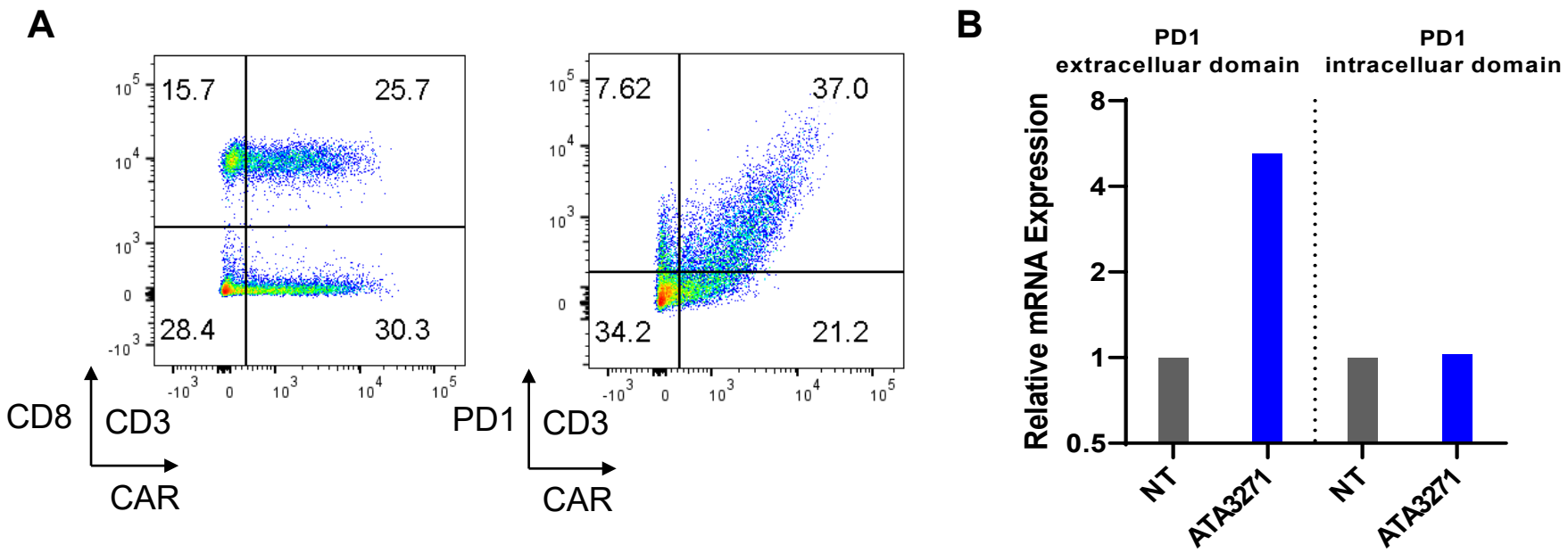
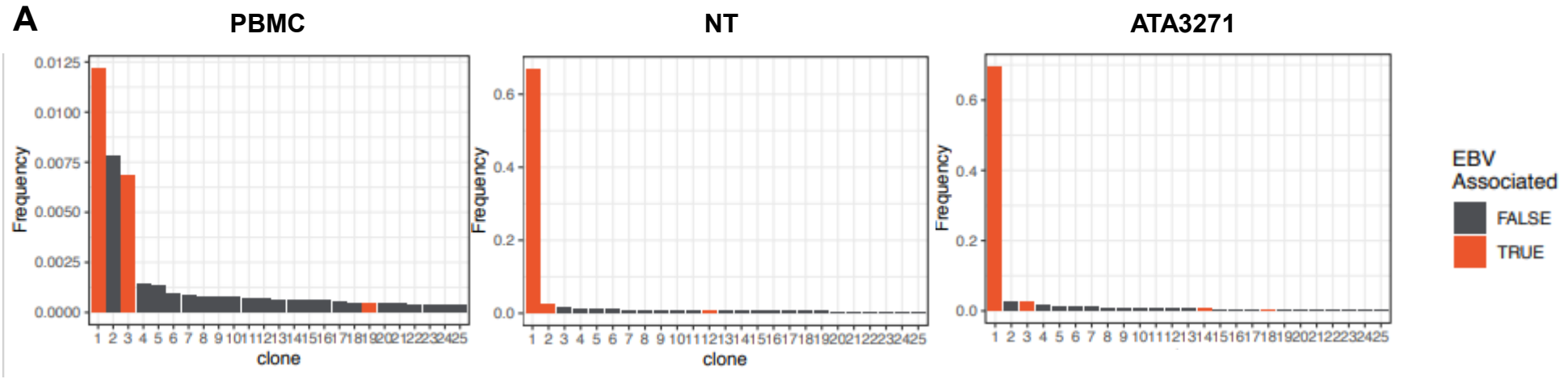
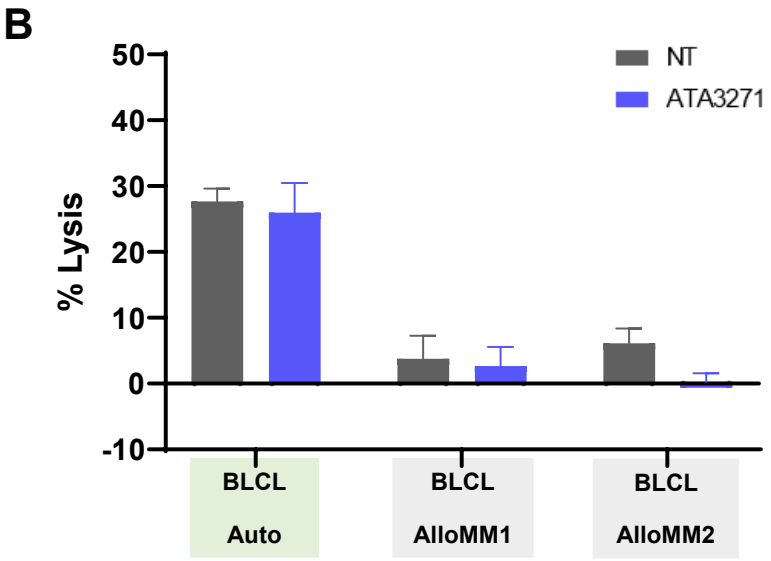


Figure 2 (A) Flow cytometric analysis after staining with CD4, CD8, CAR and PD1 demonstrates the successful transduction of EBV T cells with ATA3271 retroviral vector at high efficiency. (B) Relative mRNA expression of PD1 extracellular and PD1 intracellular domain shown as fold change, compared with that of non-transduced T cells (NT).

Figure 3. ATA3271 Exhibits High Frequency of EBV-associated Clones and Reduced Alloreactivity towards HLA Mismatched Targets





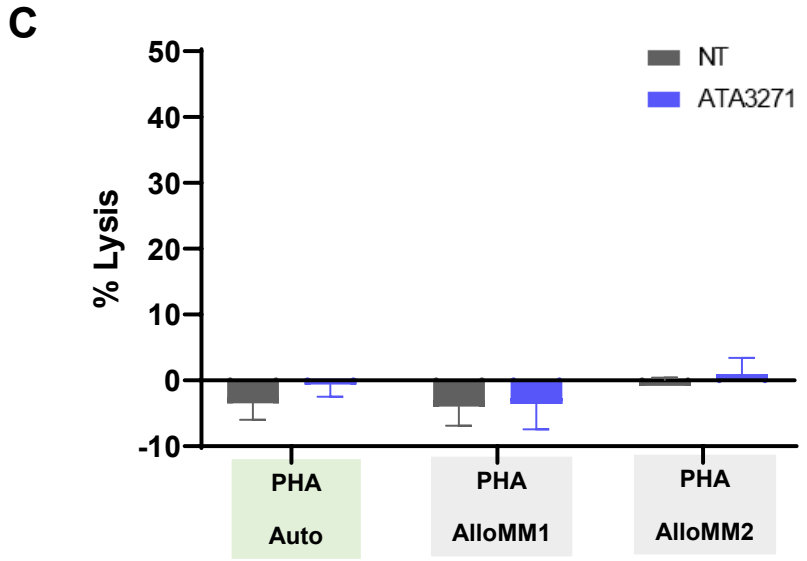


Figure 3 (A) Frequencies of top 25 clones in PBMC, NT and ATA3271 from the same donor by TCR sequencing illustrate the clonal expansion of EBV specific T cells during ATA3271 generation process. Alloreactivity was assessed in vitro by measuring the release of 51Cr from labeled (B) EBV+ and MSLN- HLA-matched (Auto) and mismatched (AlloMM) BLCLs and (C) EBV- and MSLN- HLA-matched (Auto) and mismatched (AlloMM) PHA blasts after 4 hours in coculture with ATA3271 at the effector : target (E:T) ratio of 25:1.

Figure 4. ATA3271 Demonstrates MSLN-specific Antitumor Activity

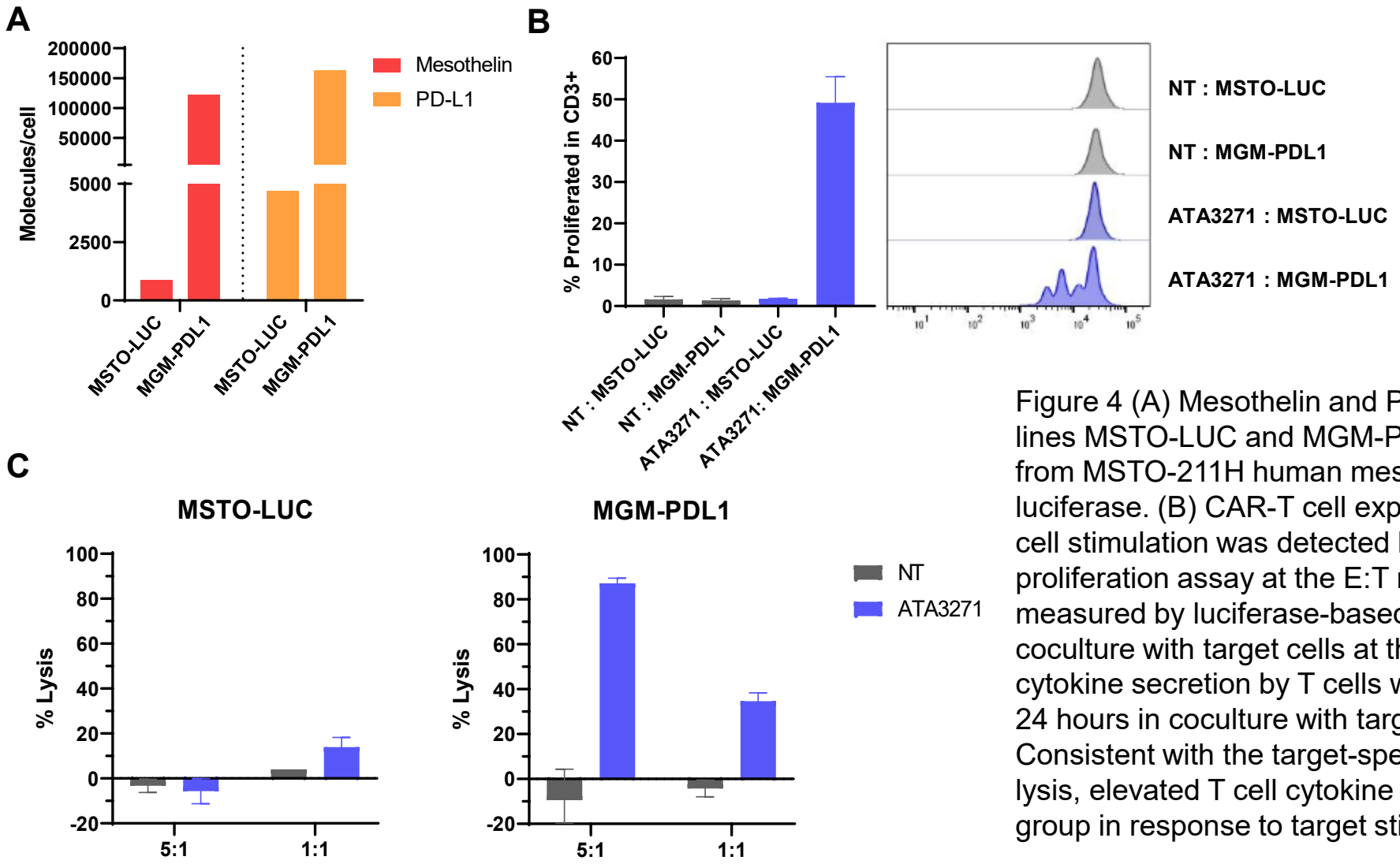


Figure 4 (A) Mesothelin and PD-L1 expression levels in target cell lines MSTO-LUC and MGM-PDL1. These two cell lines are derived from MSTO-211H human mesothelioma cell line and both express luciferase. (B) CAR-T cell expansion in response to MSLN+ target cell stimulation was detected by a 96-hour CTV-based cell proliferation assay at the E:T ratio of 2:1. (C) Tumor cell lysis was measured by luciferase-based cytotoxicity assay after 24 hours in coculture with target cells at the E:T ratios of 5:1 and 1:1. (D) cytokine secretion by T cells was measured by Luminex assay after 24 hours in coculture with target cells at the E:T ratio of 5:1. Consistent with the target-specific CAR-T cell expansion and tumor lysis, elevated T cell cytokine secretion was detected in ATA3271 group in response to target stimulation.

Figure 4 (cont). ATA3271 Demonstrates MSLN-specific Antitumor Activity

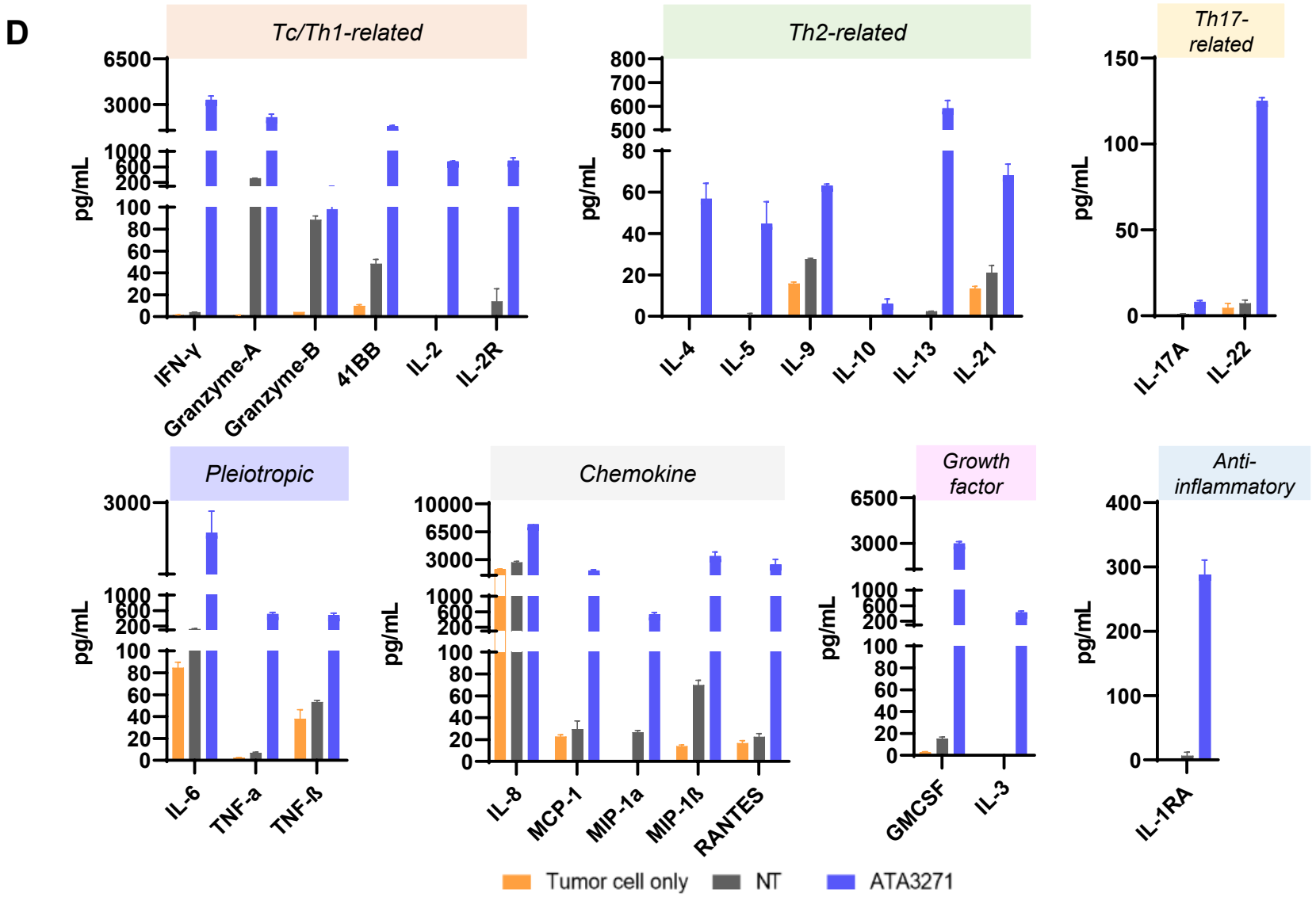


Figure 5. ATA3271 Shows Strong Proliferative Capacity and Persistent Antitumor Activity in Response to Repeated Tumor Cell Challenge without Cytokine Supplementation

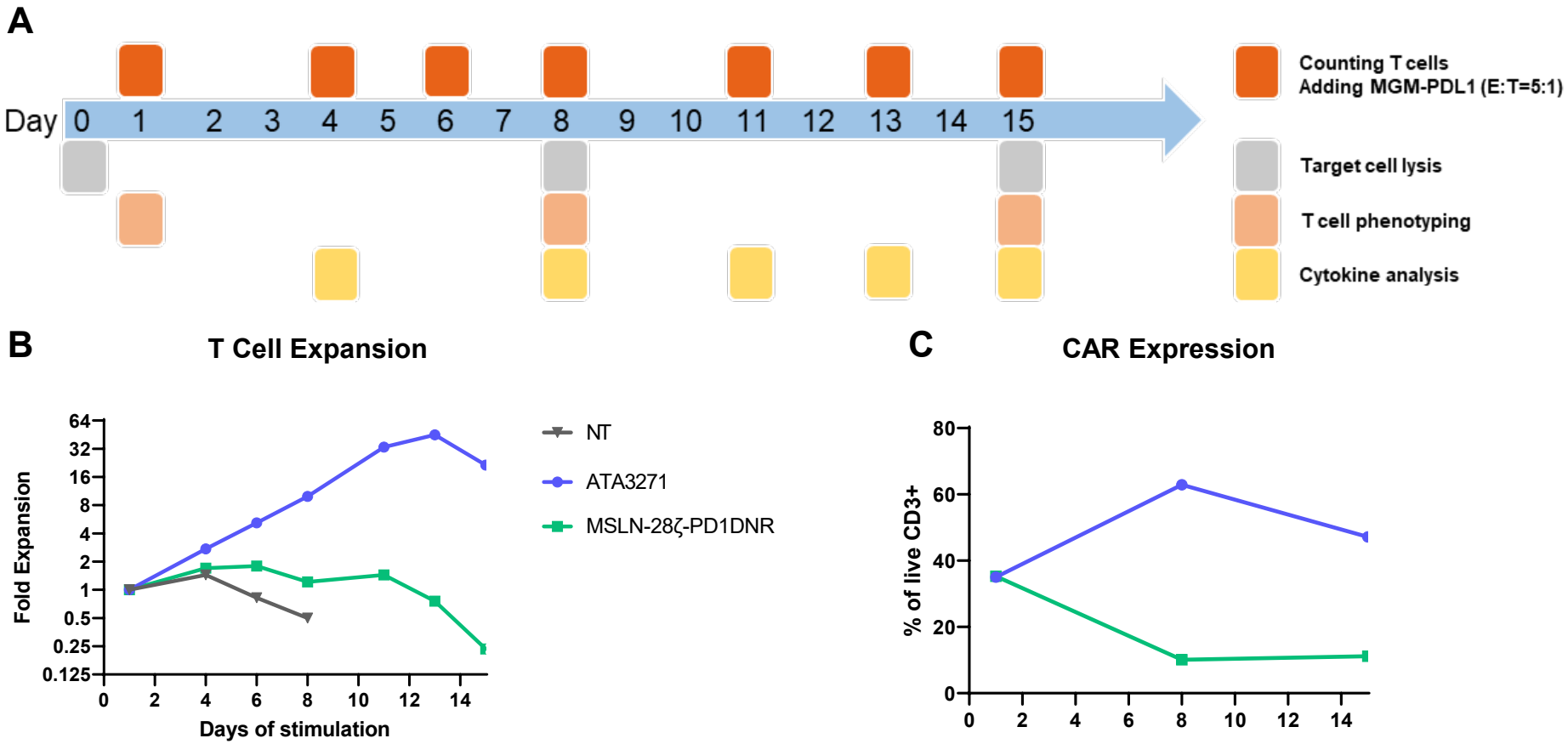


Figure 5 15-day tumor cell challenge assay comparing ATA3271 with anti-mesothelin CAR-T cells that express PD1DNR. (A) Experimental schedule of the tumor cell challenge assay. (B) T cell expansion, (C) percentage of CAR+ T cells and (D) T cell cytokine secretion levels during the repeated challenge. (E) Tumor cell lysis capability at 1st, 4th and 7th round of tumor cell challenge at the E:T ratio of 5:1.

Figure 5(cont). ATA3271 Shows Strong Proliferative Capacity and Persistent Antitumor Activity in Response to Repeated Tumor Cell Challenge without Cytokine Supplementation

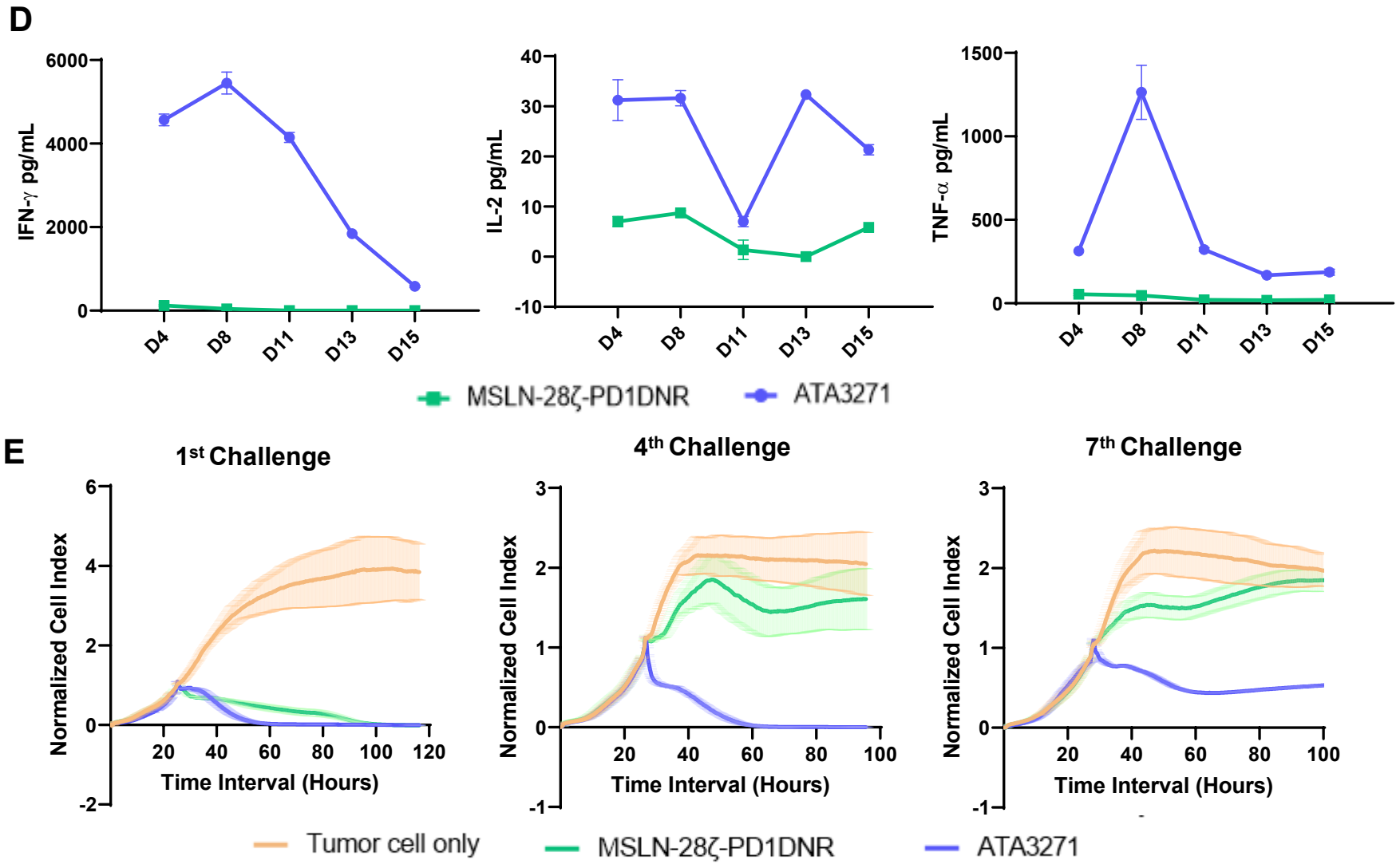


Figure 6. ATA3271 Demonstrates Robust Antitumor Response in Orthotopic Pleural Mesothelioma Model with *In Vivo* Expansion

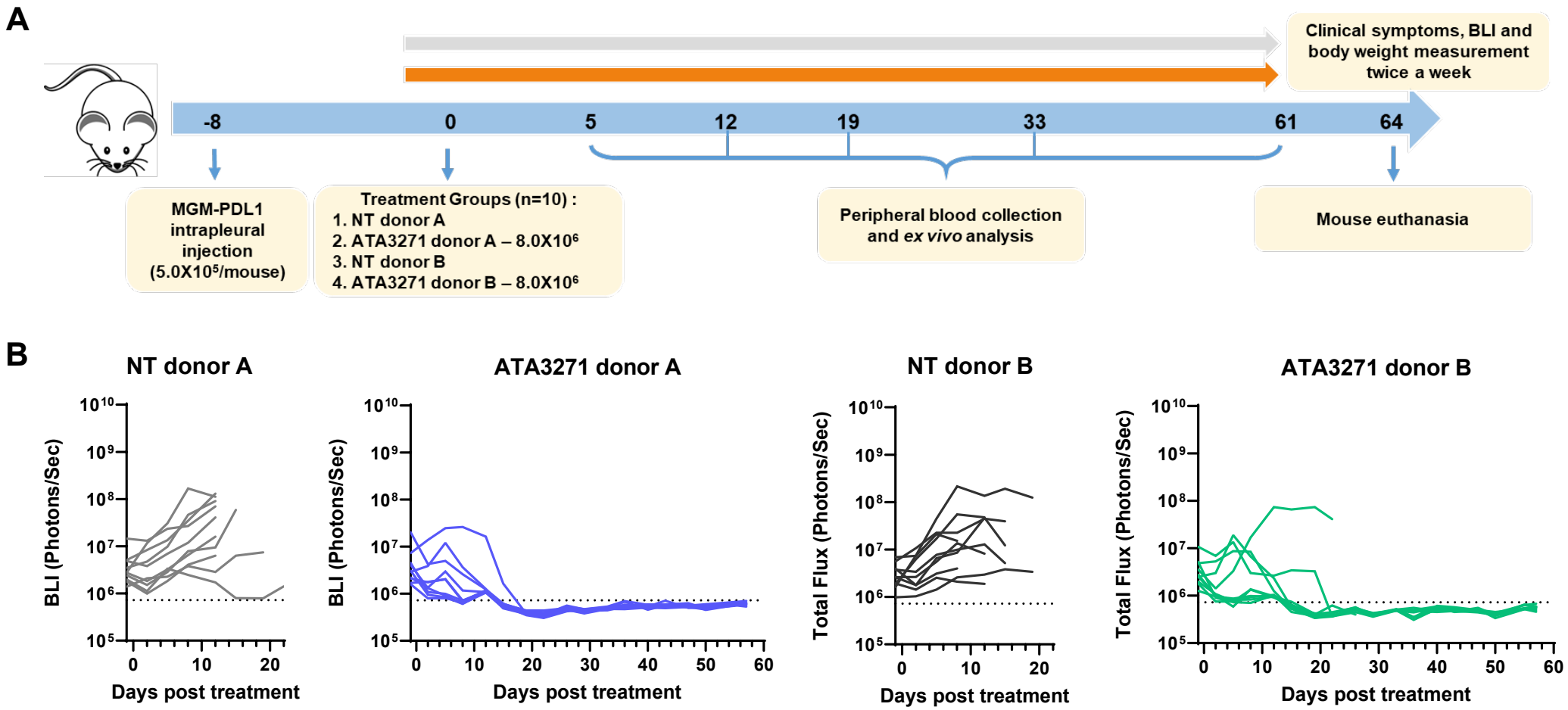


Figure 6 (cont). ATA3271 Demonstrates Robust Antitumor Response in Orthotopic Pleural Mesothelioma Model with *In Vivo* Expansion

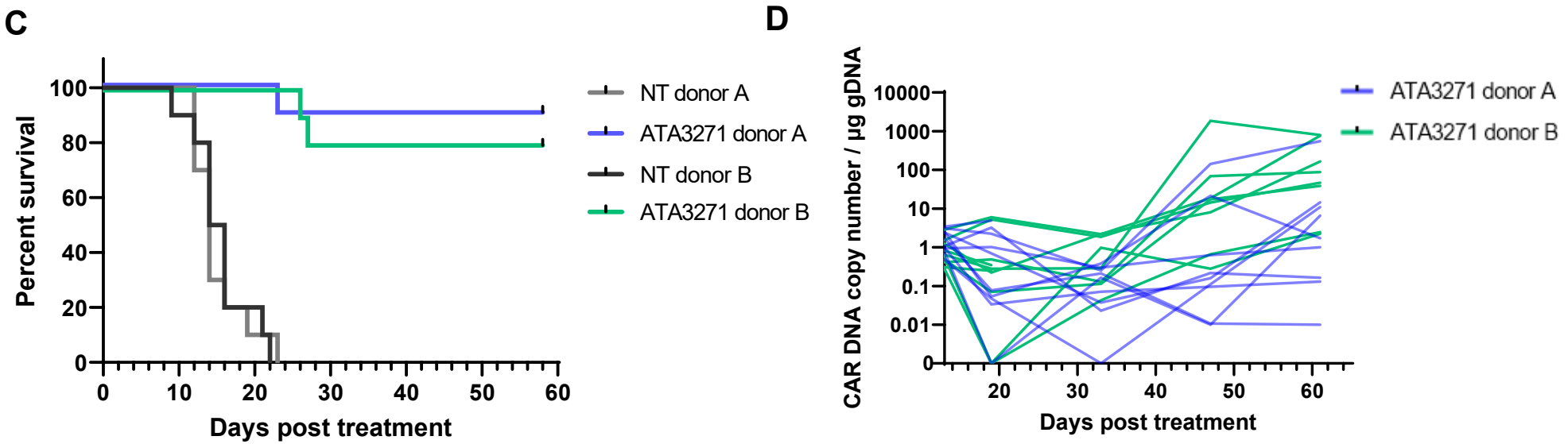


Figure 6 (A) NSG mice implanted with $0.5x10^6$ MGM-PDL1 cells through intrapleural injection on day 0. Mice were randomized into NT (n=10) or ATA3271 treatment groups (n=10) by BLI radiance on day 8 and treated with a single intrapleural injection of freshly thawed T cells ($8x10^6$ CAR-T cells for ATA3271 treatment groups). Mice were followed for another 64 days. (B) BLI radiance for each mouse was measured at various time points post treatment. (C) Overall survival rates of NT groups and ATA3271 groups were compared. (D) MSLN CAR DNA copy number in the peripheral blood of ATA3271 groups were analyzed at various time points by ddPCR.

Figure 7. ATA3271 *In Vivo* Antitumor Response Associates with CAR-T Enrichment, Preferred CD4+T Expansion, T Cell Differentiation, Minimum T Cell Exhaustion and Secretion of Antitumor Effectors

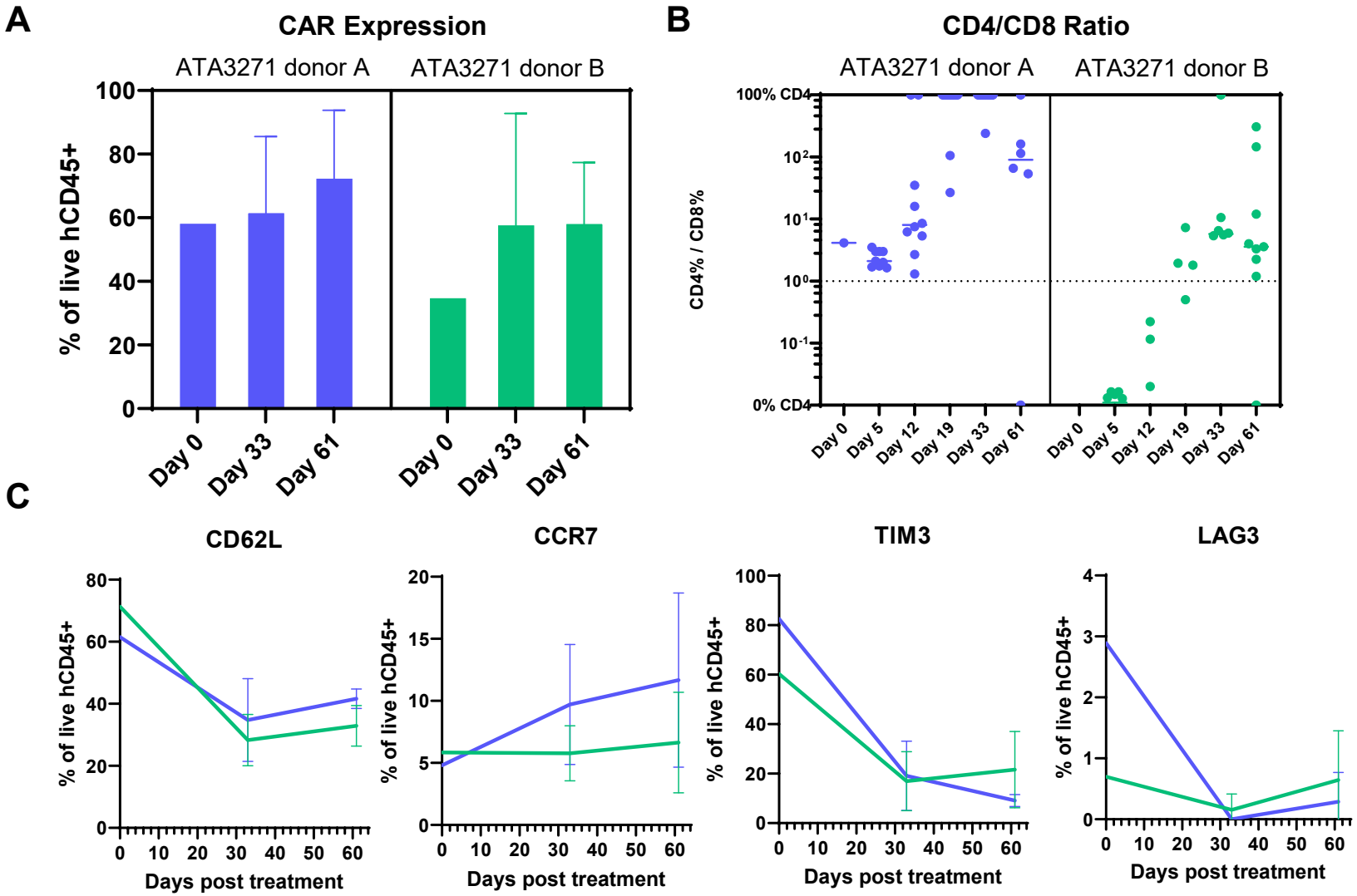


Figure 7 *Ex vivo* analyses for peripheral blood samples collected from the mice treated with ATA3271. (A) CAR expression detected by mesothelin CAR idiotype antibody staining at day 33 and 61 post treatment. (B) CD4/CD8 ratio at day 5, 12, 19, 33 and 61 post treatment. (C) Phenotypic analysis for T cell including CD62L, CCR7, TIM3 and LAG3 at day 19, 33 and 61 post treatment (n≥3). Measurements at day 0 represent T cells before injection. (D) Secretion level of T cell anti-tumor effectors (IFN-γ, TNF-α, granzyme A and granzyme B) at day 61 post treatment. These analyses provide evidence for sustained T cell persistence and functions.

Figure 7(cont). ATA3271 *In Vivo* Antitumor Response Associates with CAR-T Enrichment, Preferred CD4+T Expansion, T Cell Differentiation, Minimum T Cell Exhaustion and Secretion of Antitumor Effectors

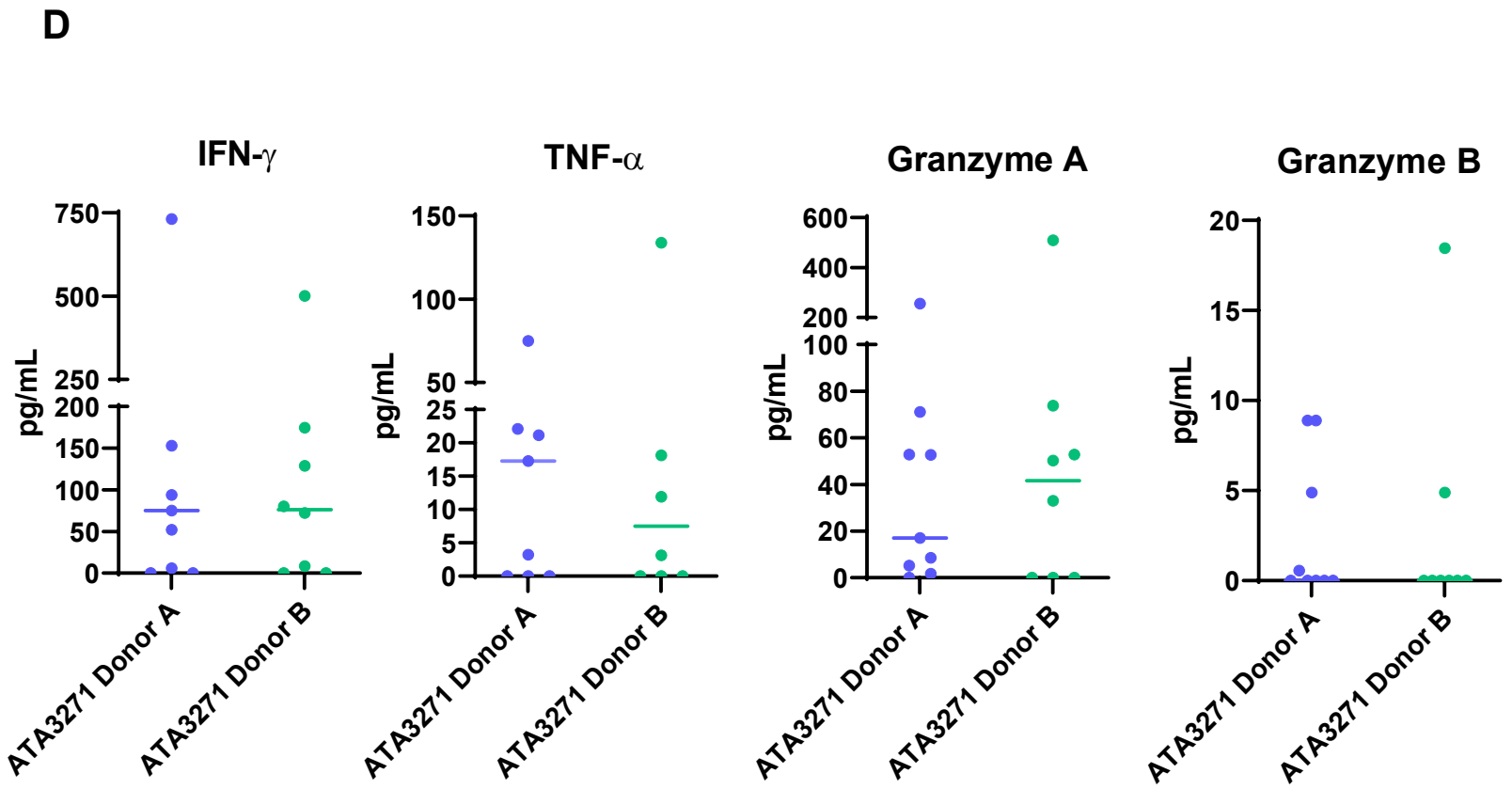
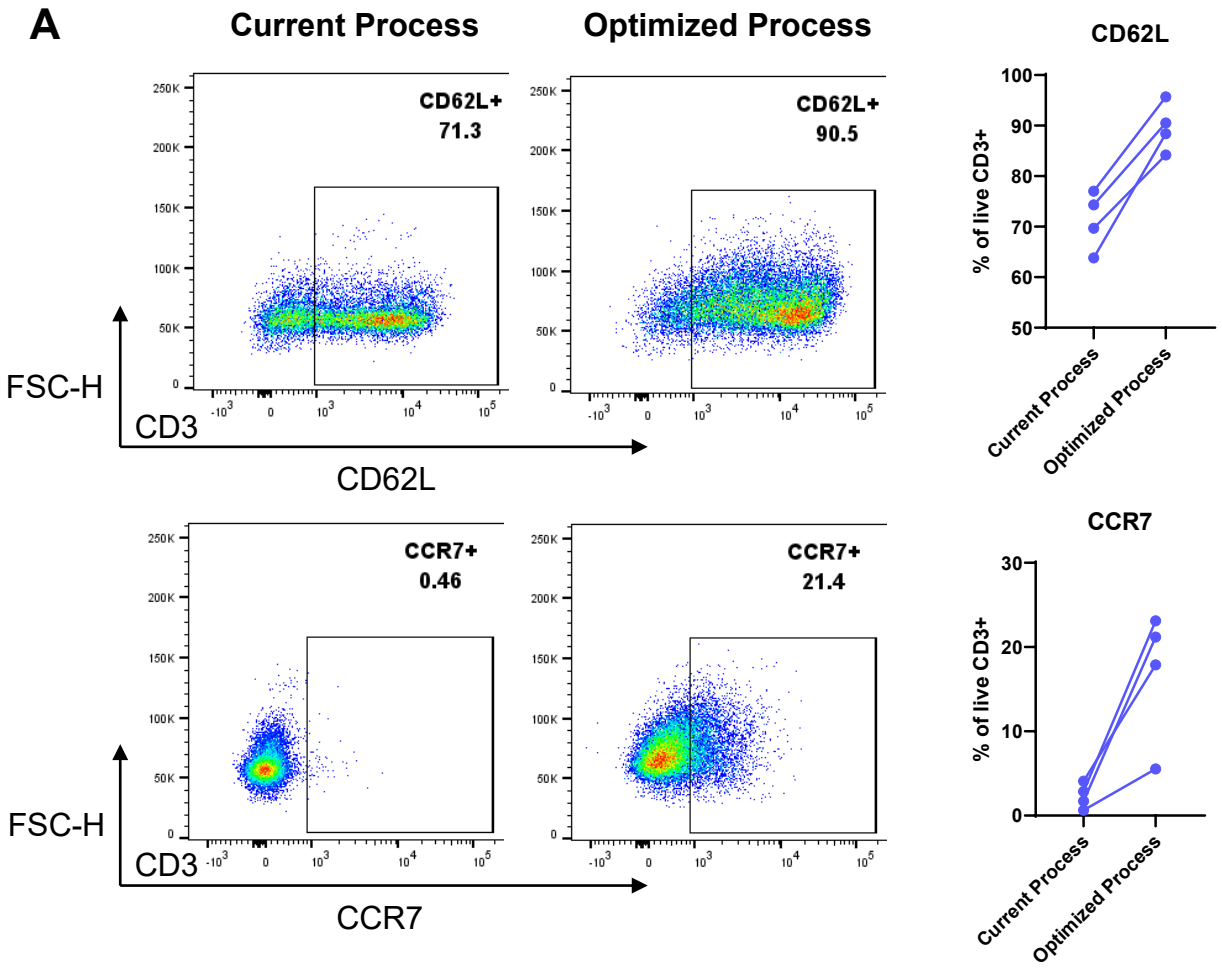


Figure 8. Optimized Process Yields ATA3271 cells with Less Differentiation and Enhanced Antitumor Response



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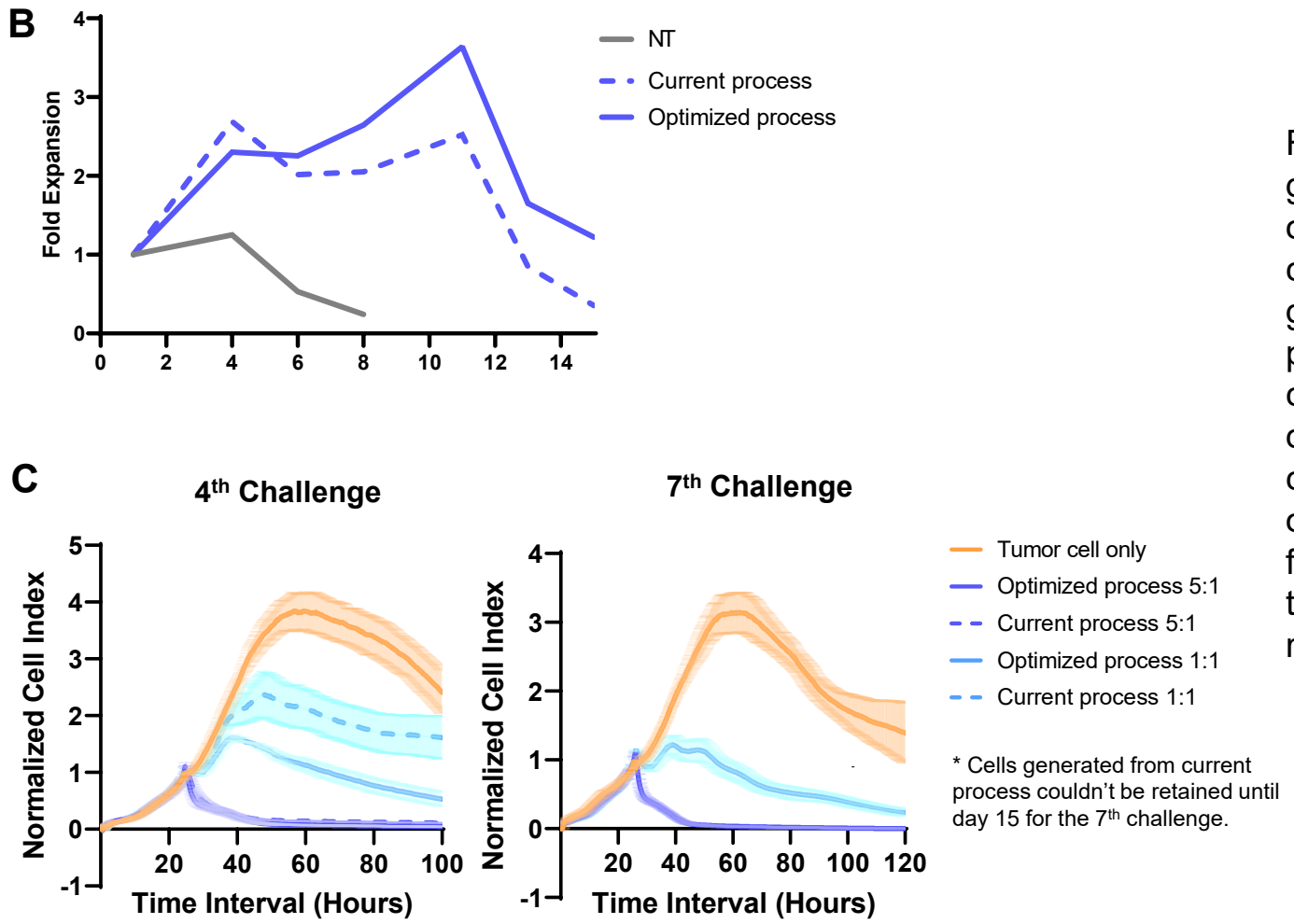

Figure 8 Comparison between ATA3271 cells generated from current process and optimized process. (A) Flow cytometric analysis shows ATA3271 generated from optimized process preserved more CD62L+ and CCR7+ T cells indicating less T cell differentiation in 4 donors. (B,C) In the 15-day tumor cell challenge assay, ATA3271 generated from optimized process expanded more than that from current process and retained better tumor lysing capability during the repeated challenge.

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 express MSLN CAR with 1XX signaling domain and PD1DNR (**Figures 2**)
- ATA3271 demonstrates clonal expansion of EBV-specific T cells and reduced alloreactivity against HLA mismatched targets *in vitro* (**Figure 3**)
- ATA3271 exhibits potent and durable antigen-specific antitumor activity *in vitro* and *in vivo* (**Figures 4, 5, 6, 7**)
- ATA3271 demonstrates better *in vitro* proliferative capability and better persistence of antitumor activity than MSLN CAR-T cells without 1XX (**Figure 5**)
- Optimized process preserves more memory T cells and enhances antitumor activity of ATA3271 **(Figure 8)**
- 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.