ATA3219: A Potent Next-Generation Allogeneic Off-the-Shelf CD19-CAR T Therapy without the Need for Gene-Editing

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DISCLOSURES

- This study was funded by Atara Biotherapeutics

- C Pham, T Spindler, E Hwang, A Brito, Y Bulliard and B Aftab are employees and shareholders of Atara Biotherapeutics, Inc.
- C Pham and B Aftab are co-inventors on related methods for expanding antigen-specific T cells with rights assigned to Atara Biotherapeutics.

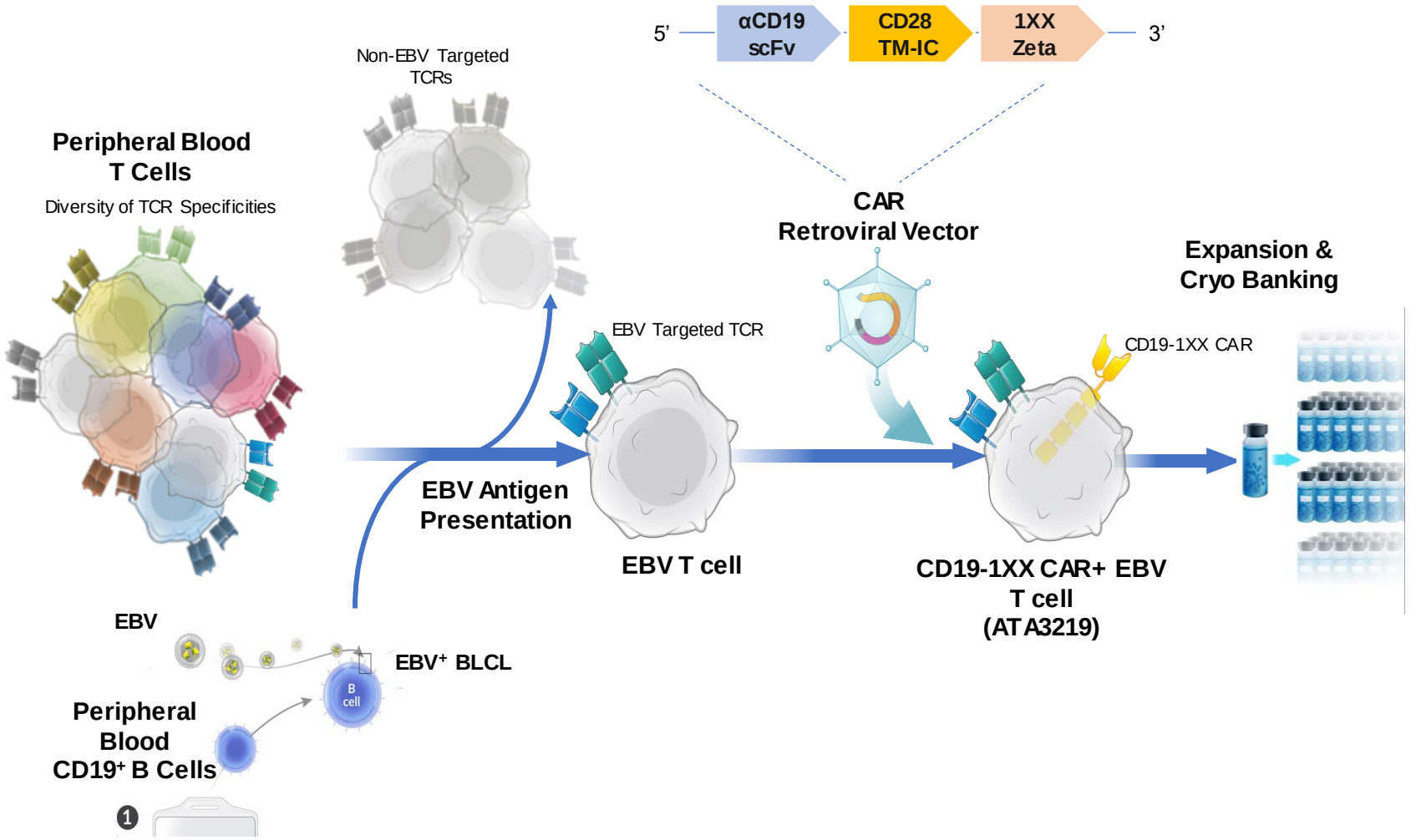
BACKGROUND

- 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 responses in association with CD19 targeting [Curran et al. TCT 2020]. Here we describe the first preclinical evaluation of ATA3219, a next-generation allogeneic CAR EBV T-cell therapy targeting CD19 and designed for the treatment of B-cell lymphomas.
- We generated EBV T cells engineered with a CD19-targeted CAR containing a modified CD3ζ signaling domain, 1XX (CD19-1XX CAR+ EBV-CTLs). CD19-1XX CAR+ EBV-CTLs demonstrate high CAR expression, polyfunctionality, expansion and in vitro potency through HLA-independent killing of CD19+ targets. Furthermore, CD19-1XX CAR+ EBV-CTLs demonstrate highly potent antitumor activity in an established disseminated tumor model of acute lymphoblastic leukemia and is associated with long-term persistence of the allogeneic CAR T. No treatment-related toxicities were observed in this animal model.

Curran KJ, Sauter CS, Kernan CS, et al. Durable remission following "off-the-shelf" chimeric antigen receptor (CAR) T-cells in patients with relapse/refractory (R/R) B-cell malignancies. 2020 Transplantation & Cellular Therapy Meetings. Orlando.

Generation of EBV T cells expressing CD19-1XX CAR

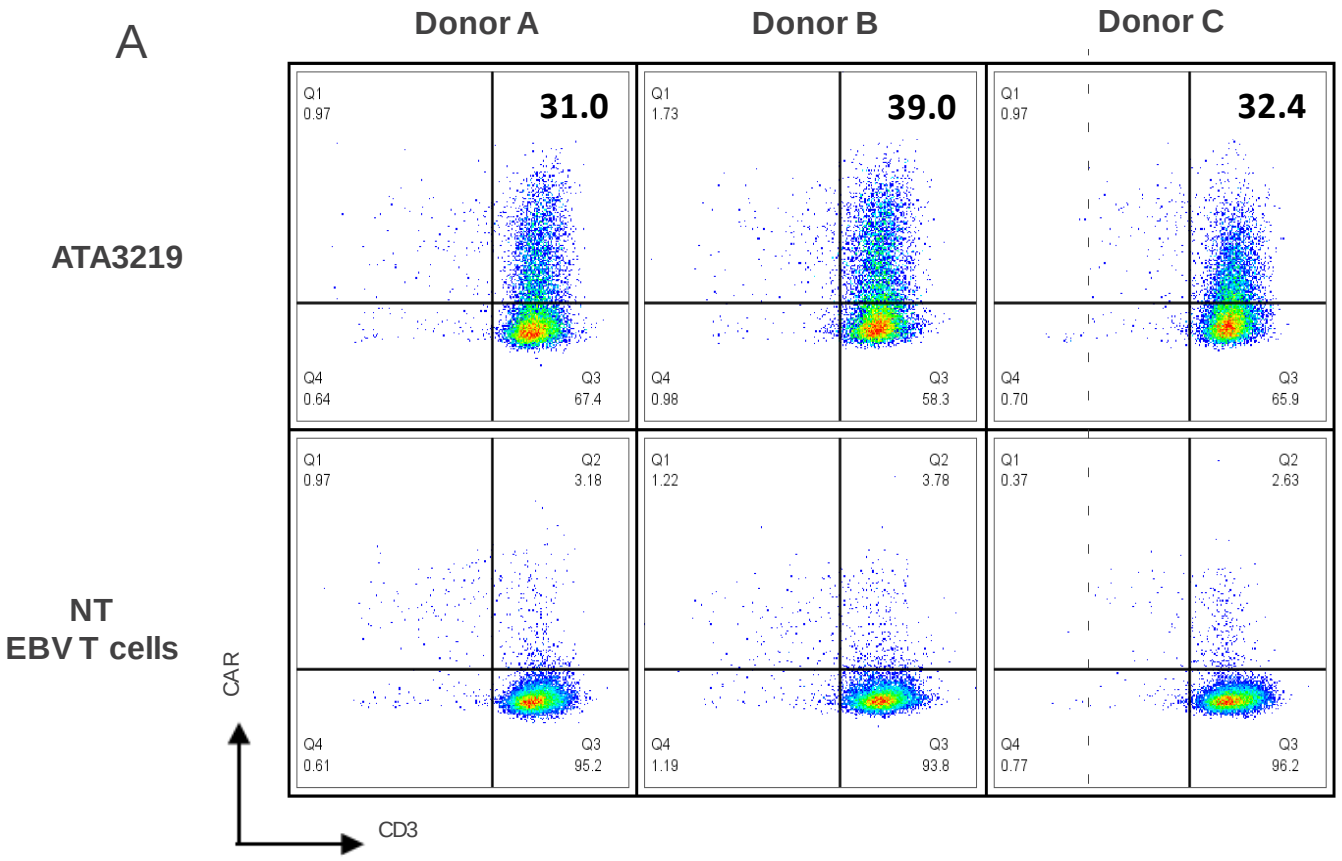
Figure 1. T and B cell fractions are separated from a healthy donor leukapheresis. The B-cell fraction is transformed with EBV, generating an EBV+ lymphoblastoid cell line (BLCL). T cells are stimulated with BLCLs prior to retroviral introduction of the CD19-targeted CAR with a 1XX co-stimulatory domain. Continued expansion of CD19-1XX CAR+ EBV T cells (ATA3219) occurs prior to harvest and cryopreservation for downstream use.



ATA3219 is transduced with high efficiency to express CAR

Figure 2. Flow cytometric analyses for CD3 and CAR on post thaw ATA3219 and Non-transduced (NT) EBV T cell material demonstrated the successful and stable transduction of EBV T cells with ATA3219 retroviral vector at high efficiencies **(A)**. Vector copy number analyses showed consistently less than 5 vector copies per genome **(B)**.

A

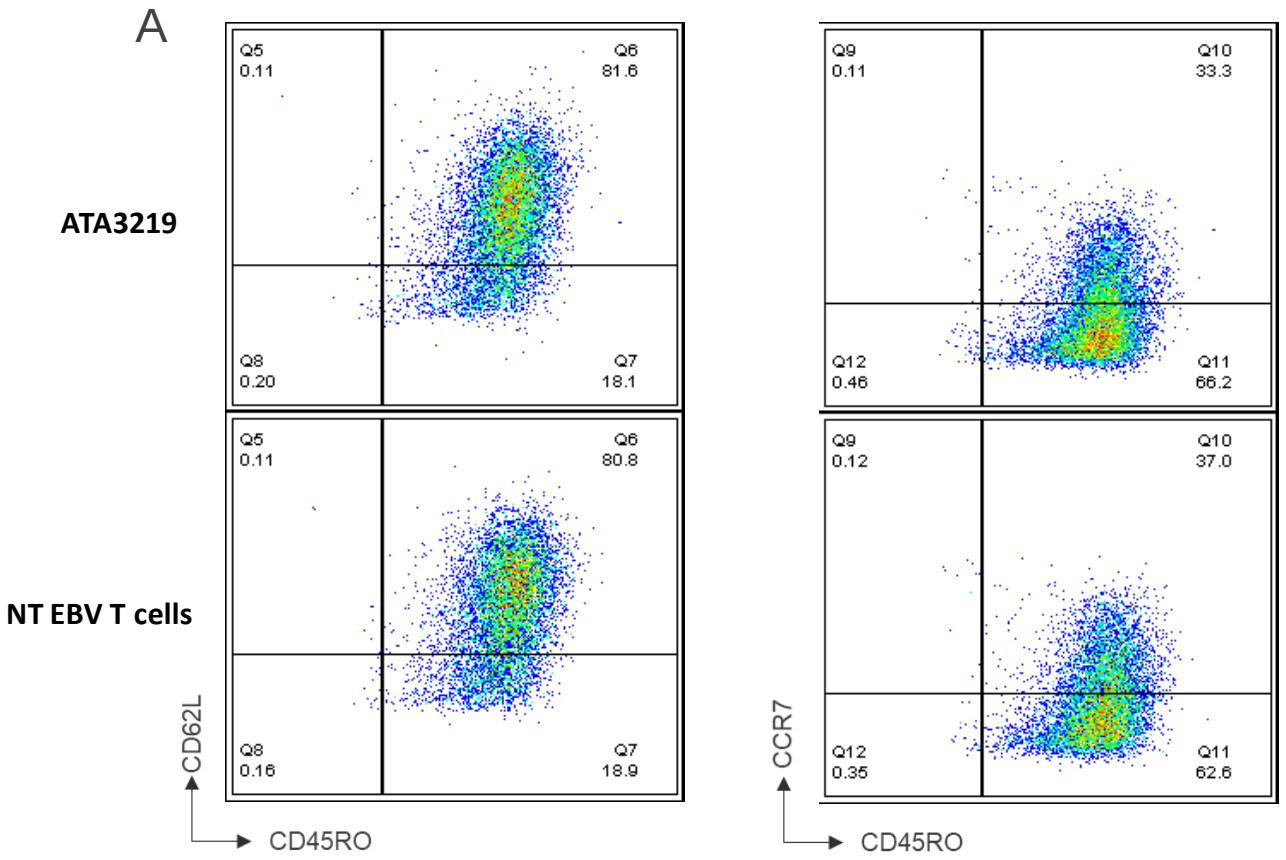


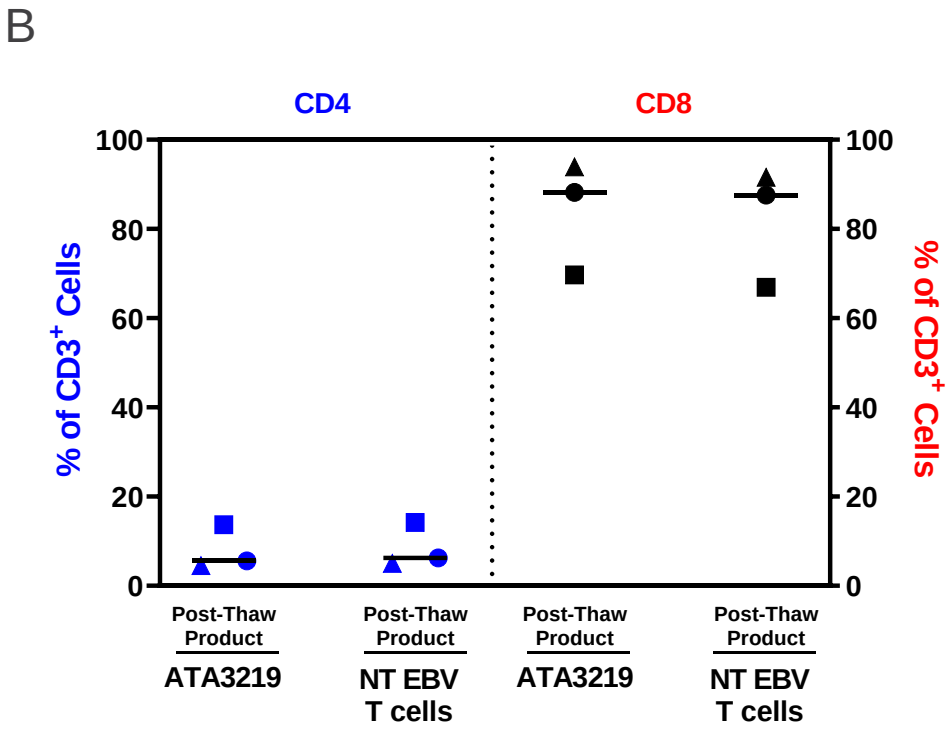
B

	Vector Copy Number (VCN)		
	Donor A	**Donor B**	**Donor C**
ATA3219	1.13	1.26	1.51

ATA3219 retains robust memory phenotype and is predominantly CD8 T cells

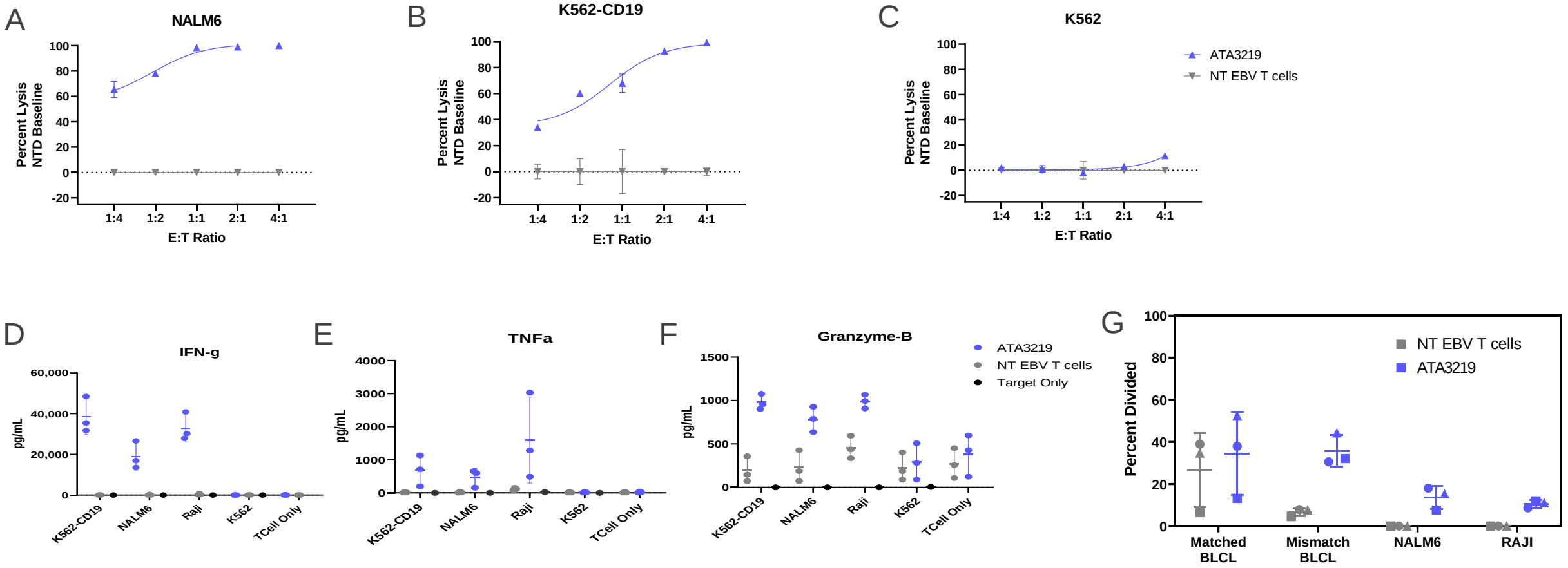
Figure 3. Flow cytometric profiling of ATA3219 for CD45RO, CD62L and CCR7 showed the maintenance of robust central memory and effector memory T cell phenotypes **(A)**. CD4/CD8 distribution analyses of ATA3219 across 3 different donors showed both ATA3219 and NT EBV T Cells to be predominantly CD8+ **(B)**.





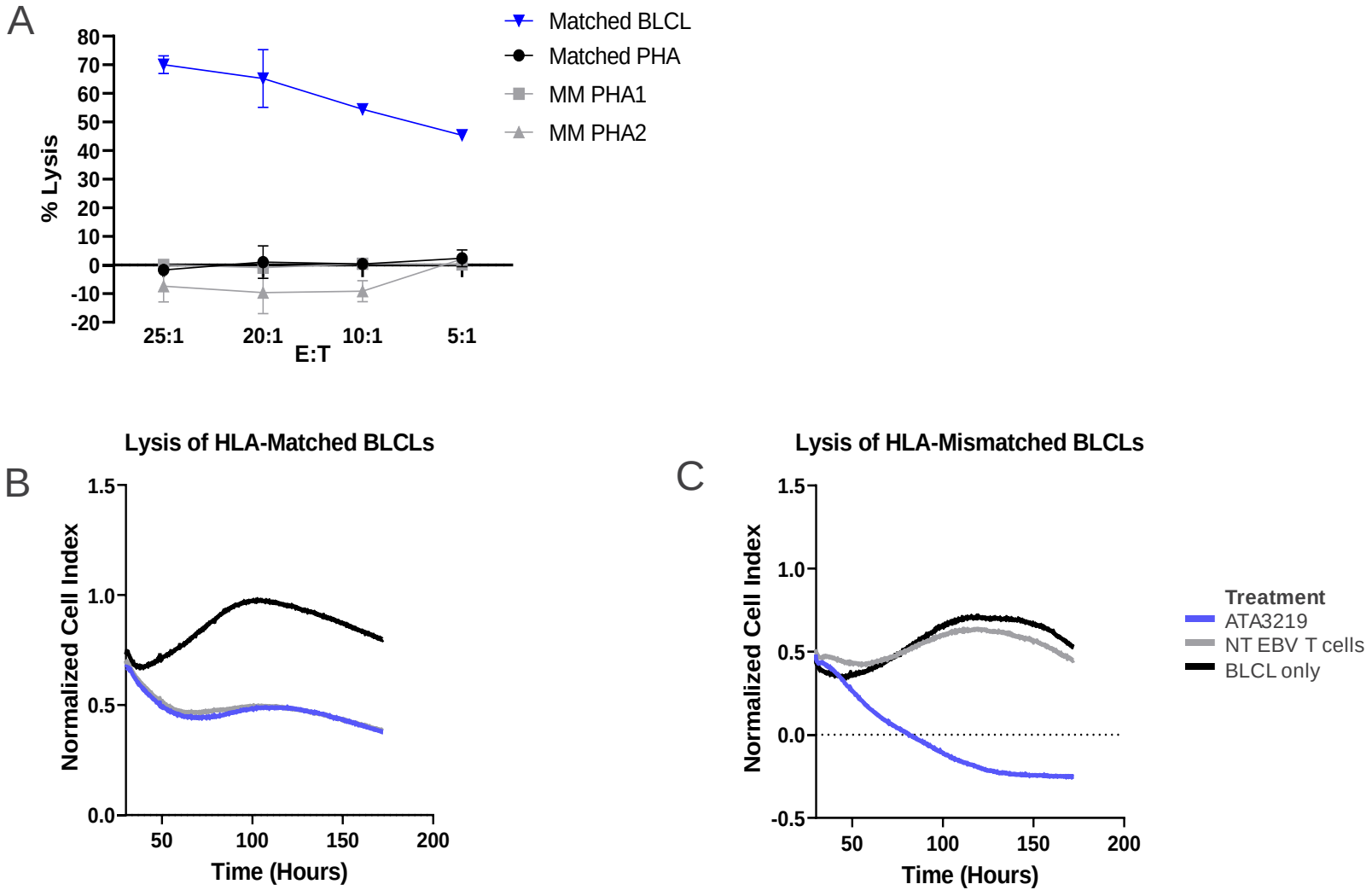
ATA3219 demonstrates CD19-specific functional activity against CD19-expressing target lines

Figure 4. Assessment of specific functional activity against CD19-expressing target lines. Potency cytolysis results were taken after a 48-hour co-culture at the indicated E:T ratios using NALM-6 **(A)**, K562-CD19 **(B)** and K562 **(C)** targets. Post thaw functional assessment of ATA3219 exhibited antigen-specific cytolytic activity against CD19-expressing target cell lines in a dose-dependent manner compared to NT EBV-CTL. ATA3219 demonstrates antigen-specific inflammatory cytokine secretion with higher levels of) IFNγ **(D)**, TNFα **(E)** and Granzyme-B **(F)** in response to CD19-expressing target lines compared to non-transduced controls. A higher percentage of antigen-associated proliferating ATA3219 cells was measured against CD19 targets compared to fewer dividing non-transduced EBV-CTLs **(G).**



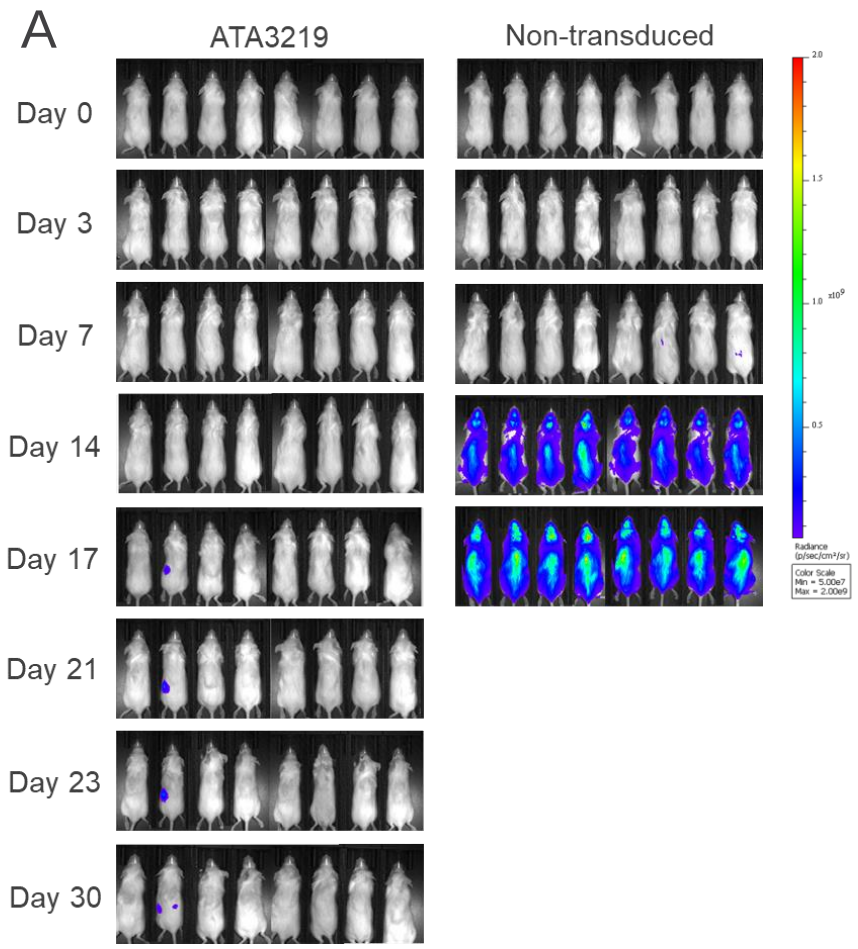
ATA3219 demonstrates reduced alloreactivity towards HLA mismatched targets and potential for off-the-shelf use

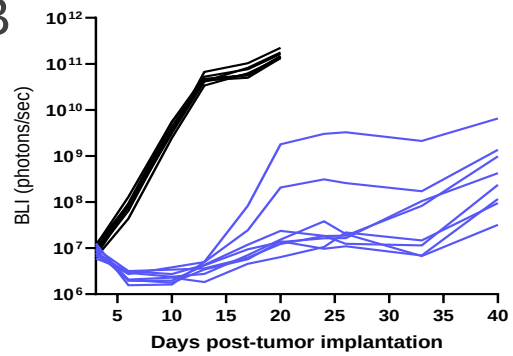
Figure 5. Alloreactive potential was assessed *in vitro* by measuring the release of ^{51}Cr from labeled HLA-matched and mismatched (MM) PHA blasts after 4 hours in coculture with ATA3219 **(A)**. In functional assessments of surrogate alloreactivity using the xCELLigence RTCA, matched BLCLs were lysed by both ATA3219 and NT EBV T cells **(B)**, but only complete HLA-mismatched BLCLs were fully lysed by ATA3219 via CD19 CAR directed lysis **(C)**.

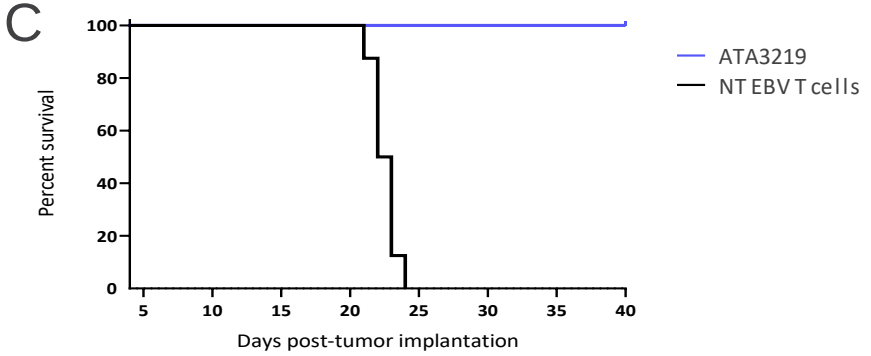


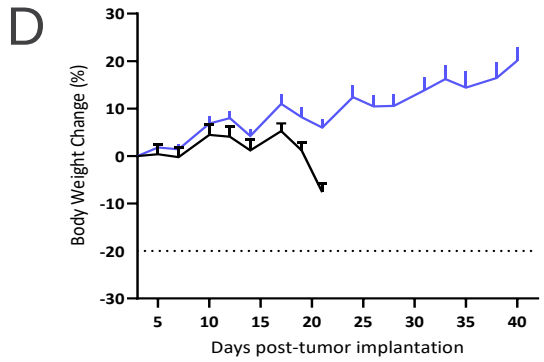
ATA3219 demonstrates potent *in vivo* antitumor activity, persistence and significant survival benefit without evidence of allotoxicity

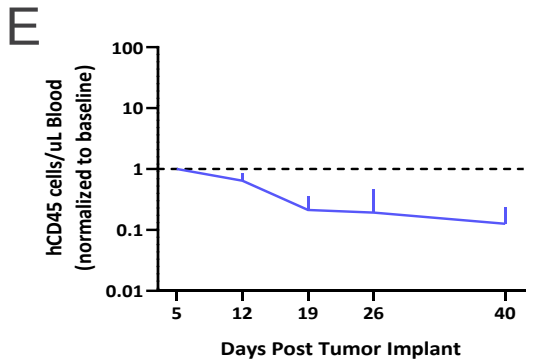
Figure 6. NSG mice were intravenously implanted with 0.5x10^6 NALM-6 cells on day 0. Mice were randomized into different groups by bioluminescence imaging (BLI) on Day 0 and treated with a single intravenous injection of freshly thawed T cells. Mice were followed for 40 days. BLI radiance for each mouse was measured at various time points post tumor **(A)**. Individual mouse BLI (**B**), survival **(C)**, and average body weight change **(D)** of the NT EBV T cell group and the ATA3219 treatment group were compared. Human T cell count **(E)** and CD19 CAR vector copy number **(F)** in the peripheral blood of ATA3219 group were analyzed at various time points by flow cytometry and ddPCR, respectively.

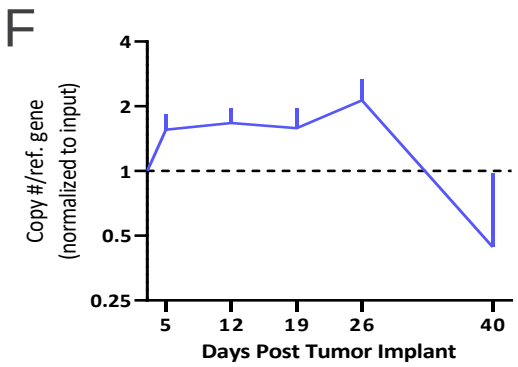












CONCLUSIONS

- **ATA3219 is an off-the-shelf allogeneic T-cell therapy targeting CD19 via next generation CAR (1XX) built on our EBV T-cell platform.**
- **We have engineered allogeneic EBV T cells to stably express CD19 CAR with a 1XX costimulatory domain with high efficiency (Fig 2).**
- **ATA3219 maintains a predominant memory phenotype (Fig 3A), polyfunctional activation profile (Fig 4D-F) and proliferative potential (Fig 4G).**
- **ATA3219 demonstrates durable CD19 antigen-specific and HLA-independent activity *in vitro* (Fig 4A-C).**
- **ATA3219 demonstrates reduced alloreactivity against HLA mismatched targets *in vitro* (Fig 5).**
- **ATA3219 shows robust antitumor activity (Figs 6A,B), superior survival (Fig 6C), persistence (Fig 6E,F) and no observed toxicity or alloreactivity (6D).**
- **In summary, ATA3219 shows potent antitumor activity both *in vitro* and *in vivo*, with no evidence of allo-toxicity *in vivo* and represents a promising approach for the treatment of CD19-positive cancers.**