ATA3219: Allogeneic CD19 CAR EBV T Cells for the Treatment of B-Cell Driven Autoimmune Diseases

Alfonso Brito, Ania Habibi, Seung Cha, Morgan Charbonneau, Philippe Foubert, Christina Pham, Cokey Nguyen

Atara Biotherapeutics, Inc., Thousand Oaks, CA

Abstract #4089635

BACKGROUND

- Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 has been highly effective in treating B-cell malignancies and has additionally led to improved outcomes in patients with systemic lupus erythematosus (SLE) through the elimination of pathogenic B cells.[1]
- Logistical hurdles associated with autologous CAR T-cell therapy present a challenge in scaling appropriately to treat patients affected by autoimmunity. The development of an allogeneic, off-the-shelf cell therapy is one strategy aimed at bypassing challenges associated with manufacturing.
- Epstein-Barr virus-specific (EBV) T cells are the most clinically advanced form of allogeneic T-cell therapy and has been shown to be well-tolerated.[2] EBV T cells maintain expression of native TCRs with inherently low alloreactivity due to their recognition of defined viral antigens.
- ATA3219 is an allogeneic, CD19-directed EBV T-cell therapy being developed for the treatment of SLE patients with lupus nephritis (LN).
- ATA3219 is comprised of EBV T cells that are modified to express a CD19-specific CAR with a 1XX signaling domain. The 1XX signaling domain, generated by two inactivating mutations to the CD3ζ immunoreceptor tyrosine-based activation motif (ITAM) regions, modulates T-cell inflammation, differentiation and exhaustion.[3]

METHODS

- We generated ATA3219 which is a CD19-targeted CAR containing a modified CD3ζ signaling domain, 1XX, built on our EBV T-cell platform without modification of the endogenous TCR. To serve as a benchmark comparator, we additionally produced autologous CD19 CAR T cells using a clinically relevant 12-day process from CD3/CD28-activated T cells.
- Functional assessments were conducted to characterize the phenotype and assess CAR-mediated cytolytic activity and cytokine response against CD19+ targets, including B-cell depletion of peripheral blood mononuclear cells (PBMCs) from SLE and multiple sclerosis (MS) patients.

CONCLUSIONS

- **ATA3219 is an allogeneic EBV T-cell therapy targeting CD19 that is optimized towards memory cell types that sustain T-cell effector function without compromising potency.**
- **ATA3219 stably expresses CD19 CAR with a 1XX co-stimulatory domain and retains a predominant central memory immunophenotype.**
- **ATA3219 demonstrates minimal alloreactivity against HLA-mismatched targets and shows HLA-independent activity against CD19+ targets.**
- **ATA3219 shows durable CD19 antigen-specific cytotoxic activity against CD19+ targets *in vitro* and *in vivo* compared with auto benchmark CD19 CAR T cells.**
- **ATA3219 induces less of an inflammatory cytokine profile than auto benchmark CD19 CAR T cells.**
- **ATA3219 shows complete CAR-mediated B-cell depletion against third-party patient SLE and MS PBMCs.**
- **The FDA has cleared Atara's Investigational New Drug application to initiate a Phase I study testing ATA3219 for the treatment of patients with LN. The study is expected to initiate in 2H 2024.**
- **In summary, ATA3219 mediates robust CD19+ B-cell depletion while inducing a reduced inflammatory cytokine response compared with auto benchmark CD19 CAR T cells. These results support advancing CD19 towards clinical evaluation in patients with B cell-driven autoimmune diseases.**

DISCLOSURES

This study was funded by Atara Biotherapeutics.

A Brito, A Habibi, S Cha, M Charbonneau, P Foubert, C Pham and C Nguyen are employees and shareholders of Atara Biotherapeutics, Inc.

REFERENCES

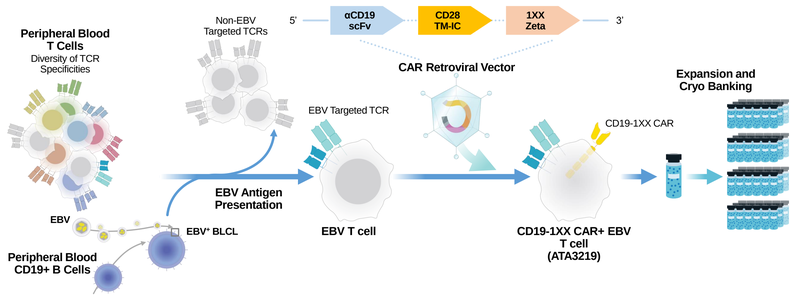
1. Müller F, *et al. N Engl J Med* 2024;390:687–700.
2. European Medicines Agency. Ebvallo (overview, authorisation details, product information, and assessment history). 2023. https://www.ema.europa.eu/en/medicines/human/EPAR/ebvallo (accessed April 2024).
3. Feucht J, *et al. Nat Med* 2019;25:82–88. .

ABBREVIATIONS

BLCL = B lymphoblastoid cell line; BLI = bioluminescence imaging; CAR = chimeric antigen receptor; EBV = Epstein-Barr virus; HLA = human leukocyte antigen; IFN-γ = interferon gamma; IL-4 = interleukin 4; IL-5 = interleukin 5; IL-6 = interleukin 6; ITAM = immunoreceptor tyrosine-based activation motif; LN = lupus nephritis; MS = multiple sclerosis; NTD = non-transduced; PBMC = peripheral blood mononuclear cell; PHAb, PHA blasts; ScFv = single chain variable fragment; SLE = systemic lupus erythematosus; TCR = T-cell receptor; Th2 = T helper 2; TM-IC = transmembrane-intracellular; TNF-α = tumor necrosis factor alpha; VCN = vector copy number.

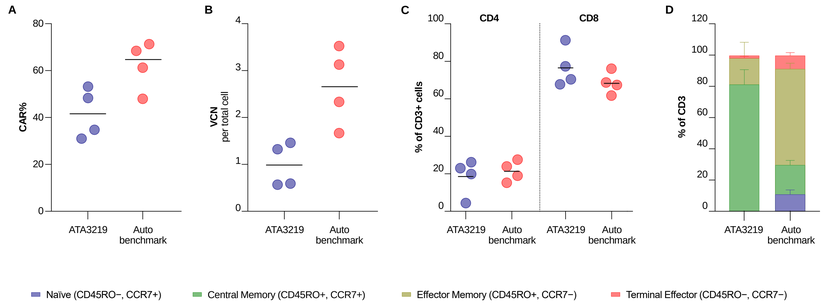
Generation of EBV T cells expressing CD19-1XX CAR

Figure 1. PBMCs are isolated following healthy donor leukapheresis. The B-cell fraction is transformed with EBV, generating an EBV$^+$ B lymphoblastoid cell line (BLCL). T cells are stimulated with BLCLs prior to introduction of the CD19-targeted CAR with a 1XX signaling domain. CD19-1XX CAR+ EBV T cells (ATA3219) are expanded and harvested for downstream use.



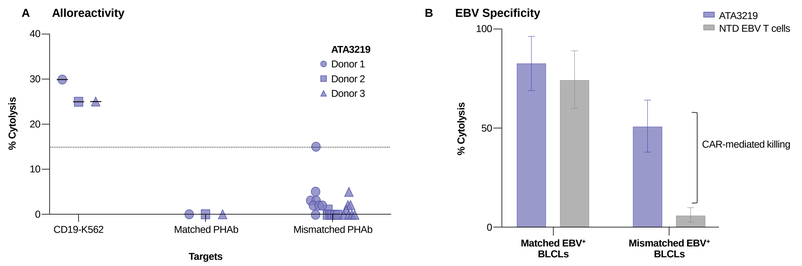
Stable CAR expression and robust central memory phenotype

Figure 2. ATA3219 product lots demonstrated stable CAR transduction of EBV T cells (A) with less than 5 vector copies per genome (B). CD4 and CD8 distribution showed ATA3219 to be predominantly CD8+ (C) and maintained a robust central memory population compared with autologous benchmark CAR T cells (D).



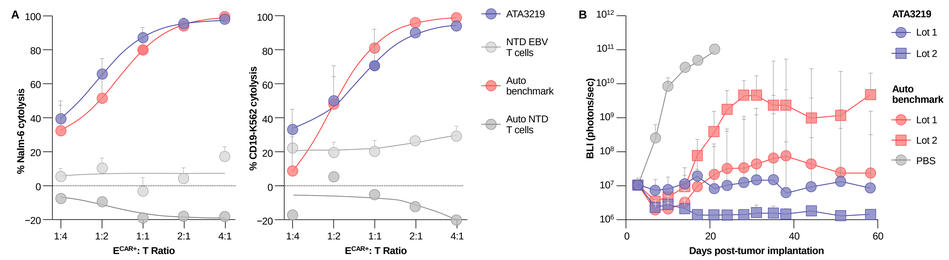
Minimal alloreactivity against HLA-mismatched targets while maintaining EBV specificity

Figure 3. Minimal alloreactive cytolysis was measured in a co-culture using ATA3219 (n=3 lots) with labeled human leukocyte antigen (HLA)-matched PHA blasts (PHAb, n=1) or HLA-mismatched PHAb (n=8) compared to CD19-K562 targets (A). Autologous EBV$^+$ BLCLs were lysed by both ATA3219 and NTD EBV T cells via CD19 and/or HLA-restricted EBV recognition, but only complete HLA-mismatched EBV$^+$ BLCLs were lysed by ATA3219 via HLA-independent CD19 CAR-directed lysis (B).



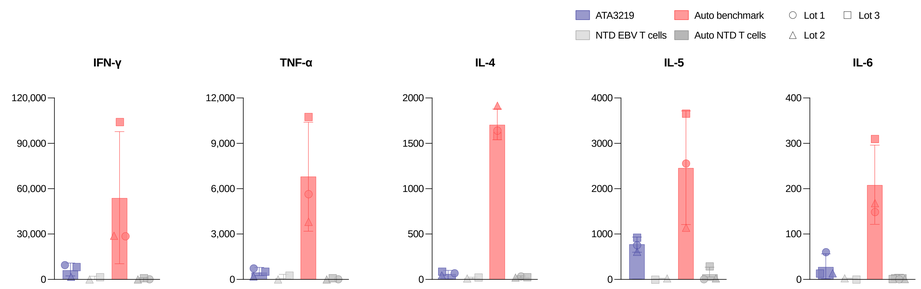
Robust CAR-specific functional activity against CD19+ target lines compared with auto benchmark CD19 CAR T cells

Figure 4. Multiple product lots of ATA3219 (n=4 lots) and auto benchmark CAR T cells (n=2 lots) exhibited comparable cytolytic activity against CD19+ target cell lines in a dose-dependent manner after a 24- or 48-hour co-culture at the indicated E:T ratios compared with NTD T cell controls (A). NSG mice (n=5–8) were implanted with 0.5×10^6 Nalm-6 cells and treated with a single injection of thawed T cells. Bioluminescence imaging (BLI) confirmed superior elimination of CD19+ cells following ATA3219 treatment compared with auto benchmark CAR T cells (B).



Reduced inflammatory cytokine release compared with auto benchmark CD19 CAR T cells

Figure 5. ATA3219 and auto benchmark CAR T cells generated from the same 3 donors were co-cultured with CD19+ Nalm-6 cells at a 3:1 E:T ratio for 24 hours. Supernatants were harvested and cytokine release was measured using a bead-based multiplex assay. ATA3219 displayed a less inflammatory profile with reduced secretion of pro-inflammatory cytokines IFN-γ and TNF-α, T helper 2 (Th2) cytokines IL-4 and IL-5, as well as IL-6.



Robust CAR-mediated B-cell depletion and CAR-specific cytokine release against third-party SLE and MS patient PBMCs *in vitro*

Figure 6. ATA3219 (n=4 lots) demonstrated robust B-cell cytolysis over 72 hours (left) in response to CD19+ B cells from third-party SLE patient PBMCs (A, n=3) and third-party MS patient PBMCs (B, n=3) at an E:T ratio of 1:1. Cytokine release was measured at 48 hours and showed elevated levels of IFN-γ and TNF-α following incubation of ATA3219 with SLE and MS PBMCs compared with NTD EBV T-cells, demonstrating CAR-mediated responses following exposure to CD19 on SLE and MS B cells (right).