ATA3431: Allogeneic CD19/CD20 Bispecific CAR EBV T-cells for the Treatment of B-Cell Malignancies

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BACKGROUND

- Allogeneic CD19-targeted chimeric antigen receptor (CAR) Epstein-Barr virus (EBV) T cells have shown encouraging efficacy and safety profile with no supportive evidence for cytokine release syndrome or high-grade graft-versus-host disease (GvHD).[1]
- ATA3431 is a next-generation allogeneic CD20/CD19-targeted CAR containing the novel 1XX signaling domain, built on Atara's EBV T-cell platform.
- EBV T-cells represent a unique approach for generating off-the-shelf therapies and make up the first approved allogeneic T-cell immunotherapy with the EU approval of tabelecleucel. Unlike gene-edited approaches aimed to inactivate T-cell receptor function to reduce the risk for GvHD, EBV T cells maintain expression of native T-cell receptors (TCRs) that promote *in vivo* persistence,[2] while also demonstrating inherently low alloreactivity due to their recognition of defined viral antigens.
- The 1XX domain contains two mutations of tyrosine to phenylalanine in ITAM2 and ITAM3 of the CD3ζ signaling domain, inhibiting their phosphorylation and downstream signaling. This 1XX domain enhances CAR T-cell persistence by sustaining T-cell effector functions without eliminating or compromising their potency[3] and has been associated with favorable response rates, safety, and durability.[4]
- We have optimized ATA3431 to enrich for a less differentiated phenotype that is clinically correlated to durability.[5] Process improvements, prioritizing CAR transduction, expansion, and viability while maintaining a predominant memory phenotype, yielded product lots that showed *in vivo* expansion and potent anti-tumor efficacy in preclinical models.

METHODS

- We generated ATA3431, a CD20/CD19-targeting CAR containing 1XX the modified CD3ζ signaling domain, built on our EBV T-cell platform without endogenous TCR modification **(Figure 1)**. To serve as a benchmark comparator, we additionally produced autologous CD20/CD19 CAR T-cells using a 12-day process from CD3/CD28-activated T-cells.
- Functional assessments were conducted to characterize the phenotype, *in vitro* potency, proliferation, and *in vivo* anti-tumor efficacy and safety.

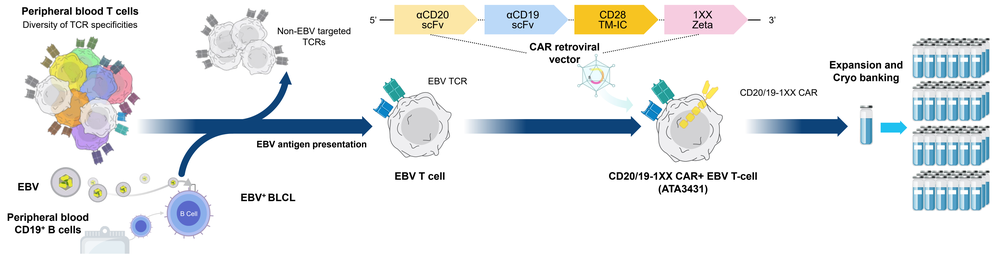
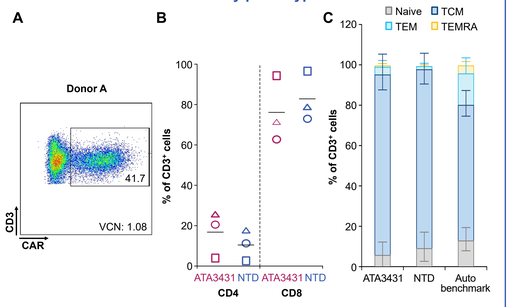


Figure 1. Peripheral blood mononuclear cells (PBMCs) are isolated following healthy donor leukapheresis. The B-cell fraction is transformed with EBV, generating an EBV$^+$ B-lymphoblastoid cell line (BLCL). PBMCs are stimulated with BLCLs prior to retroviral introduction of the CD20/CD19-targeting CAR with 1XX signaling domain. CD20/19-1XX CAR+ EBV T-cells (ATA3431) are expanded and then harvested for downstream use.

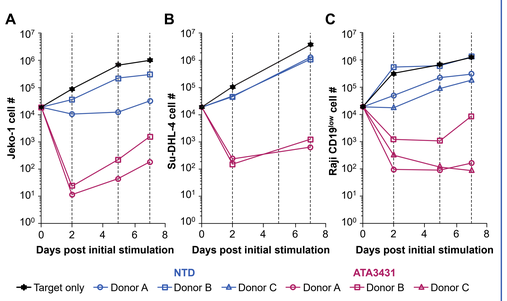
RESULTS

Figure 2. ATA3431 demonstrates stable CAR expression and maintains robust central memory phenotype



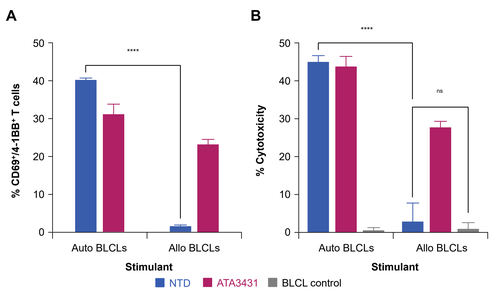
Flow cytometry analyses of CAR expression on ATA3431 products from 3 different donors demonstrated successful and stable transduction of EBV T cells with ATA3431 retroviral vector (A; representative figure). Vector copy number (VCN) analyses showed consistently lower than 5 vector copies per genome. CD4 and CD8 distribution in ATA3431 products demonstrated predominantly CD8$^+$ T cells (B). Flow cytometry analyses of memory markers CD45RO and CD62L showed ATA3431 products maintain a robust central memory population compared with autologous benchmark CAR T cells (C). Bars represent standard error of the mean.

Figure 3. ATA3431 demonstrates CD20- and/or CD19-specific functional activity against various non-Hodgkin lymphoma cell lines



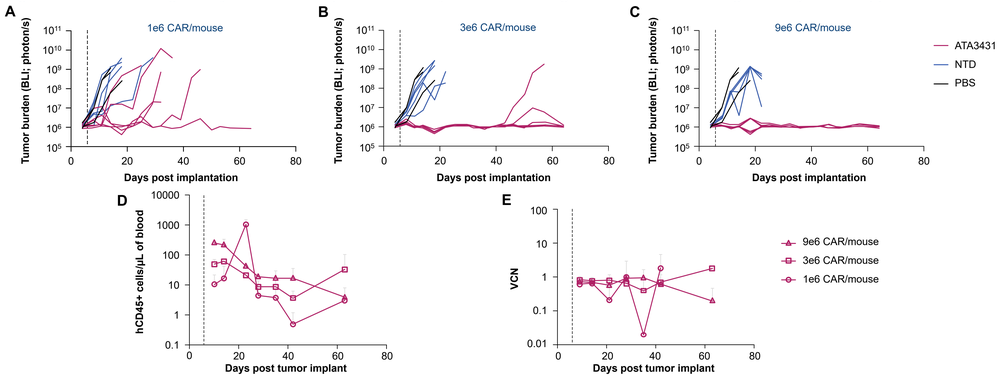
Specific functional activity of ATA3431 against CD20- and/or CD19-expressing target cell lines was measured in a serial stimulation assay using a 5:1 E:T ratio co-cultured with Jeko-1 (A), Su-DHL-4 (B), Raji CD19^{low} (C), wild type K562, K562-CD19, K562-CD20, and K562-CD20/CD19 (data not shown) targets. Enumeration of target cells following repeated serial stimulation (denoted by vertical dashed lines) demonstrated durable tumor killing.

Figure 4. ATA3431 demonstrates minimal alloreactivity against HLA-mismatched targets



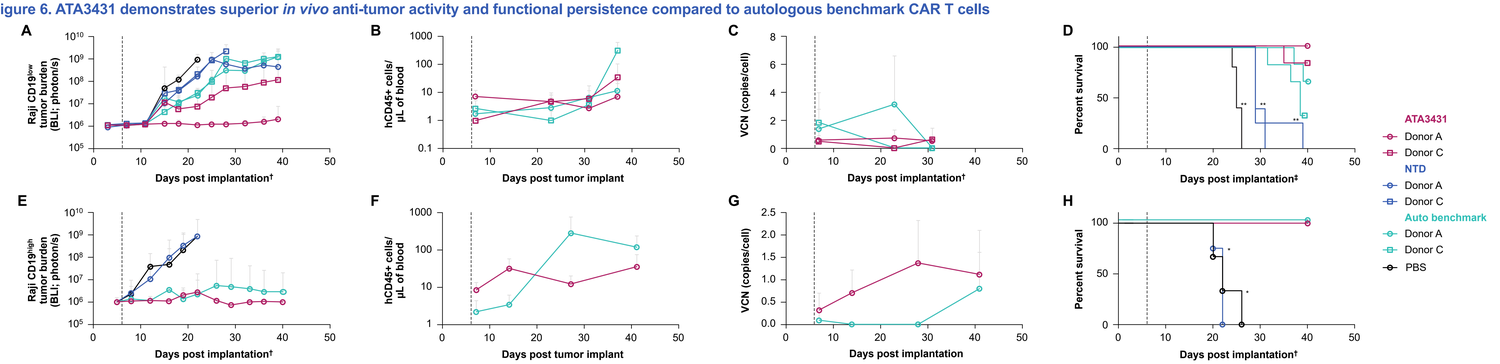
Alloreactive potential of ATA3431 was measured through the activation of T cells, CD69$^+$/4-1BB$^+$ T cells (A), and the cytolysis of HLA-matched (autologous [auto]) and mismatched (allogeneic [allo]) CD20$^+$/CD19$^+$ BLCL targets after 2 days in co-culture. The maintenance of ATA3431 EBV specificity was evaluated using flow cytometry. Autologous EBV$^+$/CD20$^+$/CD19$^+$ BLCLs were lysed by both ATA3431 and NTD EBV T cells via CD20, CD19, and/or HLA-restricted EBV recognition; but HLA-mismatched EBV$^+$/CD20$^+$/CD19$^+$ BLCLs were lysed only by ATA3431 via HLA-independent CD20/CD19 CAR-directed lysis (B). The target cell lysis was measured using xCELLigence RTCA. Error bars represents SD. ****P<0.0001 by two-way analysis of variance (ANOVA).

Figure 5. ATA3431 dosage study demonstrates potent tumor rejection after a single low dose without exogenous cytokine support



NOD scid gamma (NSG) mice were intravenously implanted with 0.5x10^6 Raji CD19^{low} cells on day 0. Mice were randomized into different groups by bioluminescence imaging (BLI) and treated with single intravenous (IV) injections of 1x10^6 (A), 3x10^6 (B), or 9x10^6 (C) freshly thawed ATA3431 or NTD EBV T-cells, as indicated by the vertical dashed lines. BLI radiance for each mouse (n=6) was measured post tumor implantation (A-C). The absolute count of circulating human T cells (D) and VCN (E) were measured. No treatment-related toxicities were observed following all dosages.

Figure 6. ATA3431 demonstrates superior *in vivo* anti-tumor activity and functional persistence compared to autologous benchmark CAR T cells



NSG mice were intravenously implanted with 0.5x10^6 Raji CD19^{low} cells (A-D) or with 0.5x10^6 Raji CD19^{high} cells (E-H) on day 0. Mice were randomized into different groups by BLI and treated with single IV injections of 3x10^6 freshly thawed ATA3431 or NTD EBV T-cells, as indicated by the vertical dashed lines. BLI radiance for each mouse (n=5) was measured post tumor implantation. The geometric mean of BLI (A/E), the mean absolute count of circulating human T cells (B/F), the mean VCN (C/G), and survival (D/H) for each group were measured. No treatment-related toxicities were observed following all dosages. Animals treated with autologous benchmark CAR T cells showed alloreactive expansion of non CAR T cells after 40 days. Error bars represents SD. *P<0.1; **P<0.01; †Geometric mean; one outlier from ATA3431 and NTD excluded. ‡Log rank tests were each tested against ATA3431.

SUMMARY

- **ATA3431 is an off-the-shelf allogeneic T-cell therapy targeting CD20 and/or CD19 via next-generation CAR (1XX) built on Atara's EBV T-cell platform (Figure 1)**
- **We have engineered allogeneic EBV T cells to stably express CD20/CD19-targeting CAR with a 1XX costimulatory domain with high efficiency (Figure 2)**
- **Inclusion of the novel 1XX signaling domain and optimization of the ATA3431 generation process yields predominantly central memory EBV CAR T cells (Figure 2)**
- **ATA3431 demonstrates durable CD20 and CD19 antigen-specific cytotoxic activity (Figure 3)**
- **ATA3431 demonstrates minimal alloreactivity against HLA mismatched targets and shows HLA-independent activity against CD20$^+$/CD19$^+$ targets *in vitro* (Figure 4)**
- **ATA3431 demonstrates potent tumor rejection with no signs of treatment-related toxicity in a lymphoma xenograft animal model without any exogenous cytokine support (Figure 5)**
- **ATA3431 shows more robust anti-tumor activity, survival, and persistence compared to autologous benchmark CAR T-cells with no observed toxicity or alloreactivity (Figure 6)**

In summary, this preclinical dataset supports advancing our next generation EBV T-cell dual-targeted CAR (ATA3431) to clinical evaluation. A Phase 1 study investigating Atara's next generation EBV T cell CD19 CAR is planned to open by the end of 2023.

REFERENCES

1. Curran KJ, *et al. Biol Blood Marrow Transpl* 2020;26(Suppl):S89. 2. Stenger D, *et al. Blood* 2020;136:1407–18. 3. Feucht J, *et al. Nat Med* 2019;25:82-88. 4. Park, JH *et al. Blood* 2022;140(Suppl 1):403–4. 5. Barba, P *et al. Blood* 2022; 140(Suppl 1):1056–9

ABBREVIATIONS

ANOVA = analysis of variance; BLCL = B-lymphoblastoid cell lines; BLI = bioluminescence imaging; CAR = chimeric antigen receptor; EBV = Epstein-Barr virus; GvHD = graft-vs-host Disease; HLA = human leukocyte antigen; ITAM = immunoreceptor tyrosine-based activation motif; IV = intravenous; NSG = NOD scid gamma; ns = non-significant; NTD = non-transduced; PBMCs = peripheral blood mononuclear cells; PBS = phosphate buffered saline; ScFv = single chain fragment variable; SD = standard deviation; TCM = T-cell central memory; TCR = T-cell receptor; TEM = T-cell effector memory; TEMRA = terminally differentiated effector memory; TM-IC = transmembrane-intracellular; VCN = vector copy number

Presented at the 65th Meeting of the American Society of Hematology (ASH 2023), December 9–12, 2023