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Functional Demonstration of CD19 Chimeric Antigen Receptor (CAR) Engineered Epstein-Barr Virus (EBV) Specific T Cells: An Off-the-Shelf, Allogeneic CAR T-Cell Immunotherapy Platform

Rhine R. Shen, Christina D. Pham, Michelle Min Wu, Philippe Foubert, Tassja J. Spindler, Daniel J. Munson, Blake T. Aftab

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

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Background

Autologous CD19 chimeric antigen receptor (CAR) T cells have demonstrated impressive clinical responses in the treatment of advanced B cell malignancies. Despite this significant advancement, broad application has been limited due to technical and operational challenges with the autologous approach. Development of off-the-shelf, allogeneic CAR T cells from healthy donors are a significant focus in the field and are anticipated to overcome these obstacles. Current allogeneic strategies generally utilize gene-editing techniques to eliminate T cell receptors and HLA expression, aiming to prevent GvHD and minimize host rejection, respectively. Allogeneic platforms utilizing these genetic approaches are currently undergoing safety evaluation in the clinic.

Virus-specific T cells represent a unique approach for generating T-cell immunotherapies that are amenable for use in the off-the-shelf, allogeneic setting. Unlike gene edited approaches aiming to eliminate TCR function and alloreactive potential, EBV-targeted T cells maintain expression of their native TCRs that are restricted to EBV antigens and have inherently low allo-specificity. Similarly, EBV-targeted T cells also maintain genetically intact HLA and retain sufficient persistence required for clinical efficacy. Tabelecleucel (tab-cel®) is an investigational off-the-shelf, allogeneic T-cell immunotherapy targeting EBV antigens associated with select lymphomas and solid tumors. Tab-cel® has been shown to be generally well tolerated with low incidence of GvHD, no cytokine release syndrome and demonstrated efficacy in patients with EBV+ post-transplant lymphoproliferative disorders (PTLD)[1]. Off-the-shelf, allogeneic EBV-targeted T cells are currently in phase 3 clinical development.

Introduction of CAR transgenes into these EBV-specific T cells provides an appealing approach for developing off-the-shelf, allogeneic CAR T immunotherapies. Using a novel process for combining retroviral transduction with an EBV-specific CTL expansion workflow, we generated EBV CTLs expressing second-generation CAR constructs based on the HD27 mAb anti-CD19 binding domain, as an archetypic tool CAR. Here we evaluate the feasibility for further developing EBV CAR T cells as an off-the-shelf, allogeneic CAR T immunotherapy platform.

Generation of EBV T cells expressing 2nd generation CD19CARs

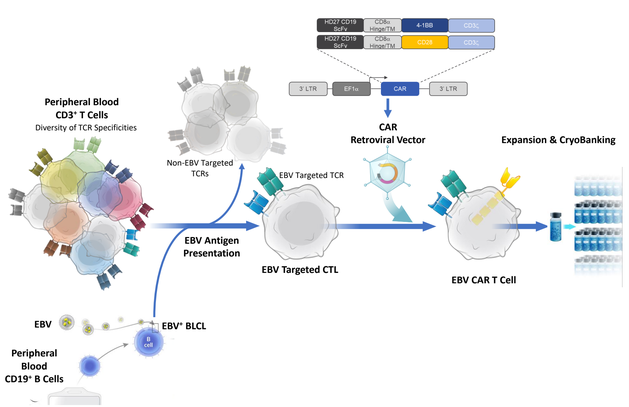

Figure 1 CD3+ and CD19+ cell fractions are separated from a normal healthy donor leukaphereses. The CD19+ fraction is transformed with EBV, generating an EBV+ lymphoblastoid cell line (BLCL). CD3+ T cells are stimulated with BLCLs prior to retroviral introduction of CD19.CD28 or CD19.41BB CAR. The CD19 ScFv is derived from the HD27 anti-CD19 mAb. Continued expansion of EBV.CD19.CAR cells occurs with BLCL stimulation prior to harvest and cryopreservation for later use.

EBV-CTLs maintain central memory phenotype and are compatible with high efficiency CAR transductions

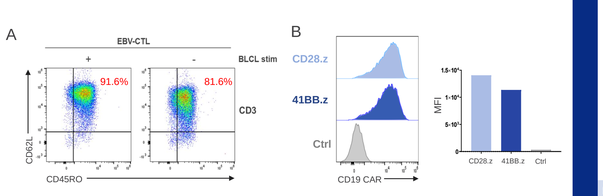

Figure 2A Central memory profile of EBV CTLs following BLCL stimulation. Flow cytometric analysis performed after staining with CD3, CD62L, and CD45RO **B.** Expression of CD19.CD28 and CD19.41BB CAR in EBV T cells following post transduction BLCL stimulation. Corresponding mean fluorescent intensities are plotted in bar graphs.

EBV-CD19CAR T cells exhibit robust central memory and activated phenotypes upon antigen stimulation

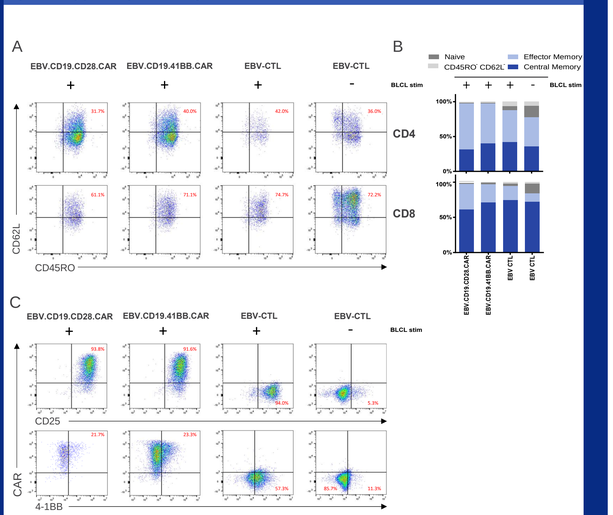

Figure 3 Flow cytometric profiling of CAR-transduced EBV CTLs for **(A)** memory **(C)** activation and costimulatory phenotypes as determined by staining for CD3, CD4, CD8, CD45RO, CD62L, CD25, and 4-1BB. Data are shown after gating on live CD3+/CD4+ and CD3+/CD8+ cells as indicated. Numbers indicate the percentage of cells in each gate. **(B)** Distribution of memory subsets for conditions shown in panel A are depicted in bar graphs as parts of the whole.

Conventional CD19CAR T cells exhibit enhanced alloreactive proliferation that is eliminated in the EBV-CD19CAR T process

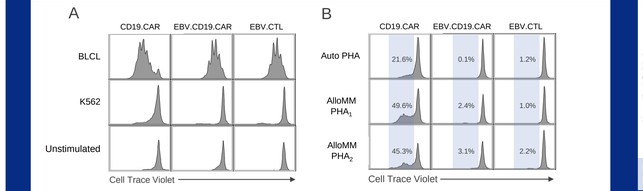

Figure 4 Proliferative capacity of EBV.CD19.CAR T cells as demonstrated by Cell Trace Violet dilution assay upon coculture with the indicated cell lines. **A** Profiles following coculture with CD19+ EBV+ autologous BLCLs (positive control), CD19- EBV- K562 cells (negative control), and isolated unstimulated T cells (negative control) are shown. **B** Cocultures with autologous (Auto) and two different HLA-mismatched PHA-stimulated PBMCs (AlloMM PHA_1 and AlloMM PHA_2) are shown. Alloreactive proliferation is highlighted and quantified in **B**.

EBV-CD19CAR T cells demonstrate HLA-independent CD19-specific cytotoxicity and low allo-cytotoxicity

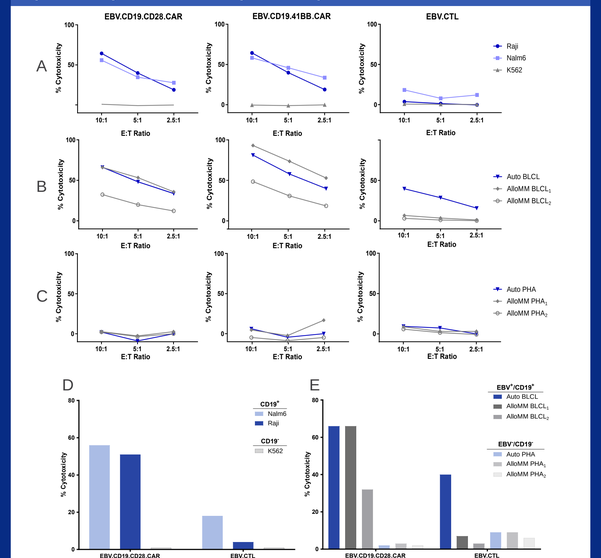

Figure 5 Cytotoxicity measured by LDH release from **A** CD19+ (NALM6 and Raji) and CD19- K562 tumor cell lines, **B** EBV+ and CD19+ HLA-matched and mismatched BLCLs and **C** CD19- and EBV- HLA-matched and mismatched PHA blasts after 4 hours in coculture with EBV.CAR19.CAR T cells or control EBV CTLs at the indicated E:T ratios. **D & E** Summaries of **A, B, C** at 10:1 E:T as bar graphs.

EBV-CD19CAR T cells demonstrate cytotoxicity kinetics comparable to conventional CD19 CAR T cells

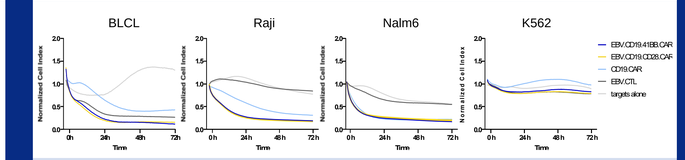

Figure 6 Kinetics of EBV.CD19.CAR-mediated cytotoxicity of CD19+ (BLCL, Raji, and Nalm6) and CD19- (K562) cells lines measured by the real-time xCELLigence cell analyzer over 3 days. Mean of cell index from triplicate cocultures are shown.

EBV-CD19CAR T cells demonstrate *in vivo* antitumor activity in an aggressive disseminated lymphoma model

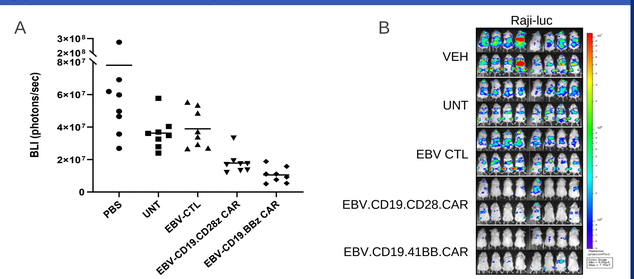

Figure 7 NSG mice implanted IV with 0.5x10^6 Raji-luc cells on Day 0. Mice were randomized by BLI radiance on day 5 and treated with a single bolus injection of freshly thawed T cells, as indicated. Mice were followed for 7 days post-injection. Resulting BLI radiance for each mouse on day 12 was quantified as plotted in panel **A** and depicted in panel **B.**
VEH = Vehicle; UNT = Untransduced Cells

Summary and Conclusions

- **EBV-targeted cytotoxic T cells (CTLs) are a clinically advanced off-the-shelf, allogeneic T-cell immunotherapy that are highly amenable to expanding tumor antigen targeting through CAR transduction.**
- **With high efficiency, we engineered EBV-CTLs to express second-generation CD19CAR associated with either a CD28 or 4-1BB signaling domain** (Fig 2-3).
- **EBV-CD19CAR T cells exhibit an enriched central memory T phenotype and demonstrated antigen-specific activation and cytotoxicity** (Fig 3-5).
- **EBV-CD19CAR T cells facilitate HLA-independent killing of both CD19 or EBV expressing targets with comparable kinetics and efficacy to conventional CD19CAR T cells *in vitro*** (Fig 5-6).
- **Relative to conventional CD19CAR T cells, EBV-CD19CAR T cells eliminated alloreactivity against CD19 and EBV negative HLA mismatched targets *in vitro*** (Fig 4-5).
- **EBV-CD19CAR T cells were confirmed to inhibit tumor growth of established lymphoma, *in vivo*** (Fig 7).
- **EBV-specific CAR T cells represent an attractive off-the-shelf, allogeneic CAR T immunotherapy platform and will be taken forward to develop clinical candidates with optimized CAR constructs.**

References

1.Prockop S, et al. HemaSphere. 2018. 2(S1):155. Abstract PF401. 23rd Congress of the European Hematology Association Stockholm, Sweden, June 14-17, 2018.