**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**

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**BACKGROUND**

Antisense CD19 chimeric antigen receptor (CAR) T cells have demonstrated impressive clinical responses in the treatment of advanced B-cell malignancies. Despite significant advancements, broad application of these therapies has been limited by a number of factors, including the need for patient-specific tumor targeting, CAR T-cell expansion to large numbers, and the requirement for in vivo gene editing. Development of off-the-shelf, allogeneic CAR T cells from healthy donors is a significant focus in the field and is anticipated to overcome these challenges. Allogeneic strategies generally utilize gene-editing techniques to eliminate T-cell receptors and HLA expression, enabling to prevent GvHD and increase host chimerism, respectively. Allogeneic platforms utilizing these genetic approaches and cytokines antagonizing T-cell receptor signaling are currently undergoing evaluate 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 eradicate TCR function and abrogate potential, CAR-engineered T cells maintain expression of their native TCR. This also maintains genetically intact HLA and retains sufficient persistence required for clinical efficacy. Transmembrane Induced Killing Activity (TICKA) is a newly introduced approach targeting EBV antigens associated with latent EBV and solid tumors. To date, it has been shown to be generally well tolerated with low-grade infections, EBV lymphoproliferative disease, and low adverse events in patients with EBV post-transplant lymphoproliferative disorders (PTLD). Off-the-shelf, allogeneic EBV-TICKA T cells are current underway 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 panel for controlling the second-generation CAR constructs, we have previously described EBV CAR T cells to facilitate CAR T-cell engineering targeting EBV antigens associated with latency EBV and solid tumors. To date, it has been shown to be generally well tolerated with low-grade infections, EBV lymphoproliferative disease, and low adverse events in patients with EBV post-transplant lymphoproliferative disorders (PTLD). Off-the-shelf, allogeneic EBV-TICKA T cells are current underway clinical development.

**EBV-SPECIFIC T CELLS PROVIDE A UNIQUE APPROACH FOR GENERATING T-CELL IMMUNOTHERAPIES THAT ARE AMENABLE FOR USE IN THE OFF-THE-SHELF, ALLOGENEIC SETTING.**

**METHODS**

**ALLOIMMUNE RESPONSES**

Allogeneic CAR engineered EBV-specific T cells were generated with lower EBV epitopes from EBV-immune donors. To determine the specificity and HLA restriction of these CAR-engineered T cells, cell-based assay was performed. To determine the functionality of these CAR-engineered T cells, a tumor cell-based assay was also performed.

**RESULTS**

EBV-specific T cells demonstrated cytotoxicity kinetics comparable to conventional CD19 CAR T cells. EBV specific T cells demonstrated in vivo antitumor activity in an aggressive disseminated lymphoma model.

**SUMMARY AND CONCLUSIONS**

EBV-targeted cytotoxic T cells (CTLs) are a clinically advanced off-the-shelf, allogeneic immunotherapy that is gaining prominence in expanding tumor antigen targeting through CAR transduction.

With high efficiency, engineered EBV CTLs to express second-generation CARs associated with either CD8 or CD28 signaling domain (Fig. 3-7).

- EBV-CAR T cells exhibit an enhanced central memory T phenotype and demonstrate antigen-specific activation and proliferation (Fig. 3).
- EBV-CAR T cells exhibit HLA-independent killing of both GBM and EBV expressing targets with comparable kinetics and efficacy to conventional CD19 T cells in vitro (Fig. 5).
- In conventional CD19-specific T cells, EBV-CAR T cells generated intercellular cytotoxicity against GvHD (Fig. 6).
- EBV-specific CAR 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 conditions with associated CAR constructs.

**REFERENCES**