OFF-THE-SHELF EPSTEIN BARR VIRUS SPECIFIC T CELLS ARMORED WITH B7-H3-TARGETING CHIMERIC ANTIGEN RECEPTORS IS AN EFFECTIVE AND SAFE TUMOR- AGNOSTIC THERAPY FOR B7-H3 POSITIVE SOLID MALIGNANCIES

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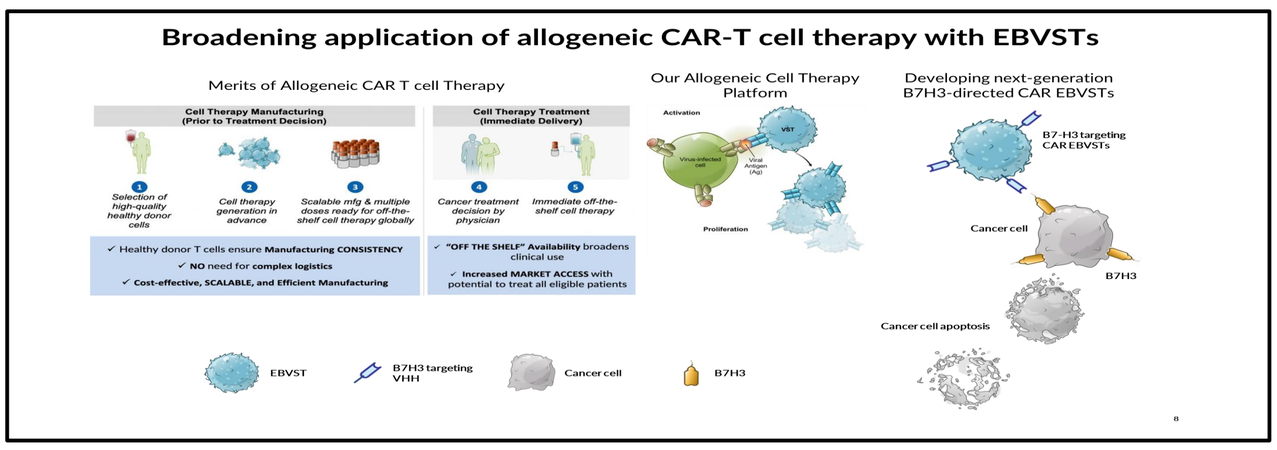
Abstract

The B7 homolog 3 protein (B7-H3) is over-expressed in many human tumors compared to normal tissues, making it an attractive target for chimeric antigen receptor (CAR) T cell therapy.

We expressed B7-H3 targeting CARs on Epstein-Barr Virus Specific T Cells (EBVSTs) as an off-the-shelf therapy against solid tumors. B7H3.CAR EBVSTs demonstrated potent in-vitro and in-vivo activity against multiple B7-H3 positive tumor and patient-derived xenografts models. Analysis of human healthy tissues and tumors microarrays indicated that B7-H3 expression, while elevated in patient tumors, was differentially low in healthy tissues. Consistent with this, mouse T cells expressing a murine equivalent of our B7H3.CAR displayed good on-target off-tumor safety in immunocompetent mice bearing syngeneic tumors. Further in-vitro evaluation revealed that while allogeneic human T, B and NK cells were unaffected by B7H3.CAR EBVSTs targeting, monocytes were readily targeted due to the upregulation of B7-H3. Consequent to depletion of human myeloid cells which are key mediators of cytokine release syndrome (CRS), a low incidence of CRS was observed in humanized mice after B7H3.CAR EBVST treatment. Lastly, we showed that B7H3.CAR EBVSTs can target myeloid-derived suppressor cells (MDSCs) that express B7-H3 and in doing so, alleviate immune suppression.

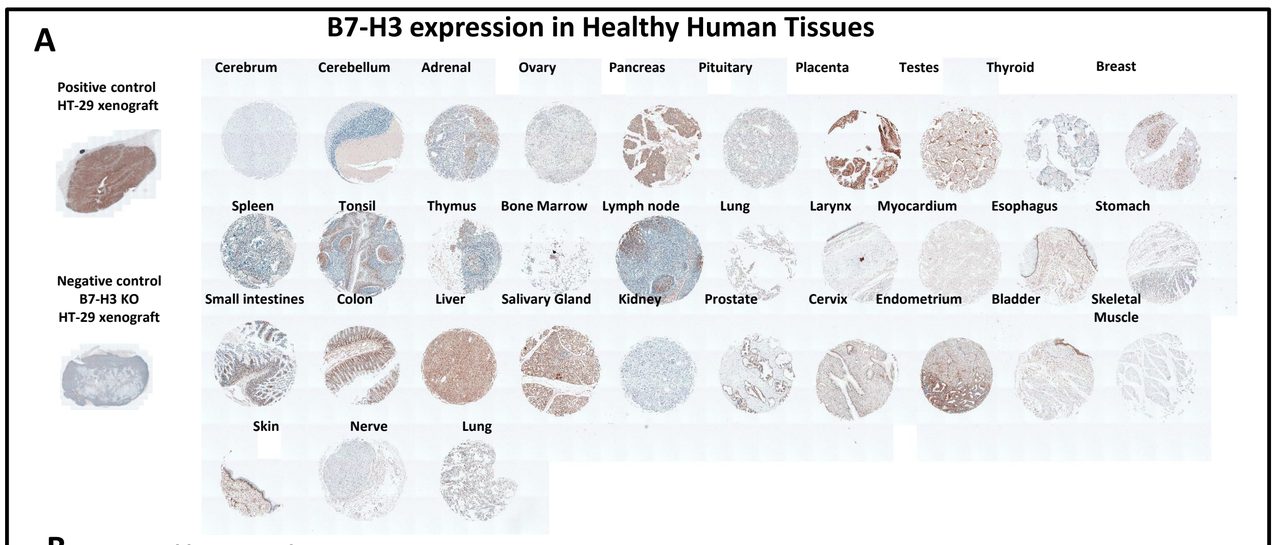
Our findings demonstrate that B7H3.CAR EBVSTs is an effective and safe off-the-shelf approach for targeting any B7-H3-positive solid tumor and overcome challenges that hindered the clinical development of CAR T cells.

Introduction



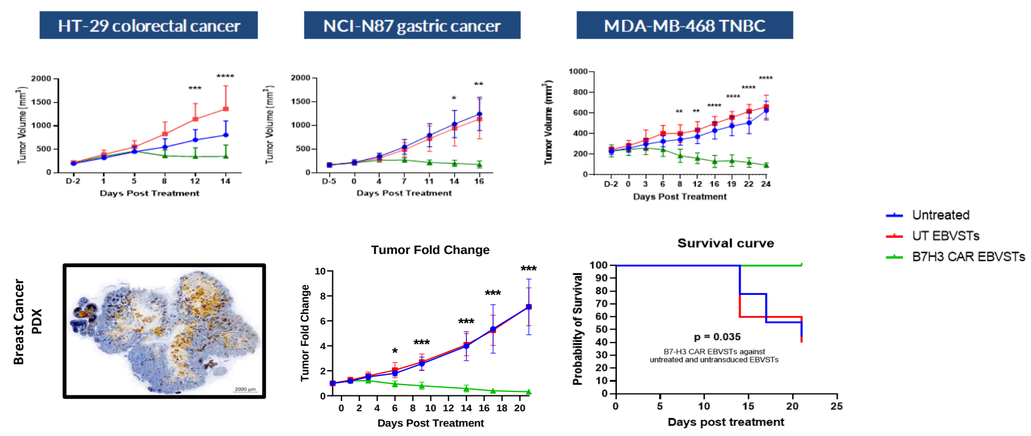
Results

1. B7-H3 expression in tumors and healthy tissues



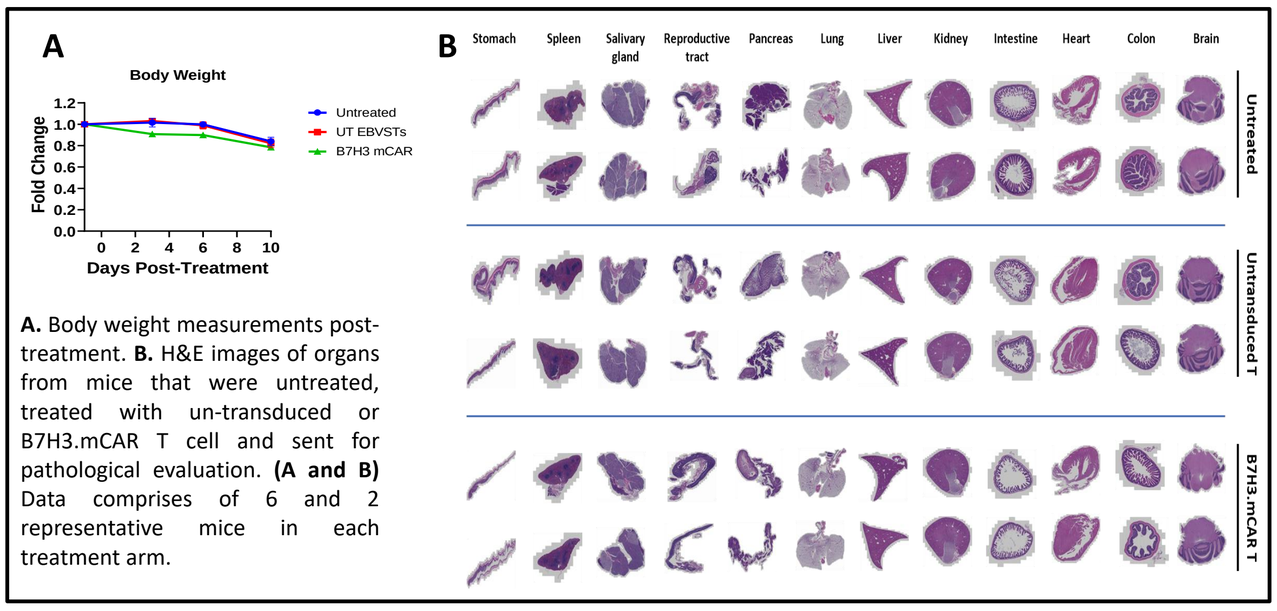
A. Grading and pattern of B7-H3 expression in IHC staining of human normal tissues. **B.** B7-H3 H-score for healthy versus tumor breast tissues. Each ID or point on the graph represents a patient and error bars denotes means ± SD where applicable. ****: p<0.0001

2. B7H3.CAR EBVSTs have good in-*vivo* activity against B7-H3-positive solid tumors



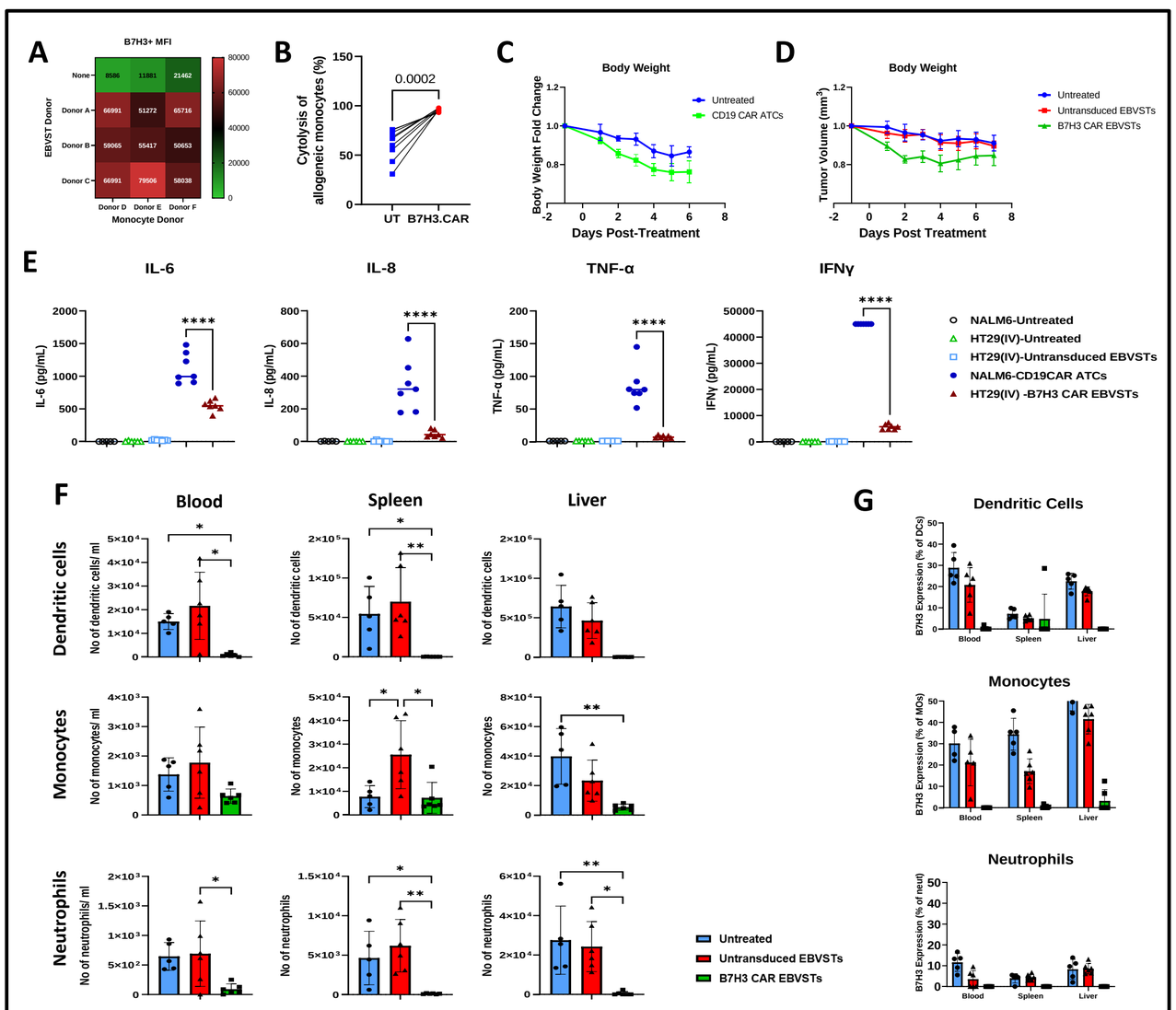
A. Tumor volumes of untreated, UT and B7H3.CAR EBVST treatment groups in the HT-29, NCI-N87 and MDA-MB-468 cell-line derived solid tumor models. **B. Right**, IHC staining of B7-H3 on breast cancer PDX. Brown color = positive B7-H3 staining. Blue color = cell nucleus. **Middle**, Tumor volume after treatment. **Left,** Kaplan-Meier survival curve. Data comprises of 6-7 **(A)** and 9-10 **(B)** mice in each treatment arm. Error bars denotes means ± SD where applicable. *: p<0.05; **: p<0.01, ***: p<0.001,]

3. B7-H3.mCAR T cells exhibit minimal toxicity in a syngeneic mouse model



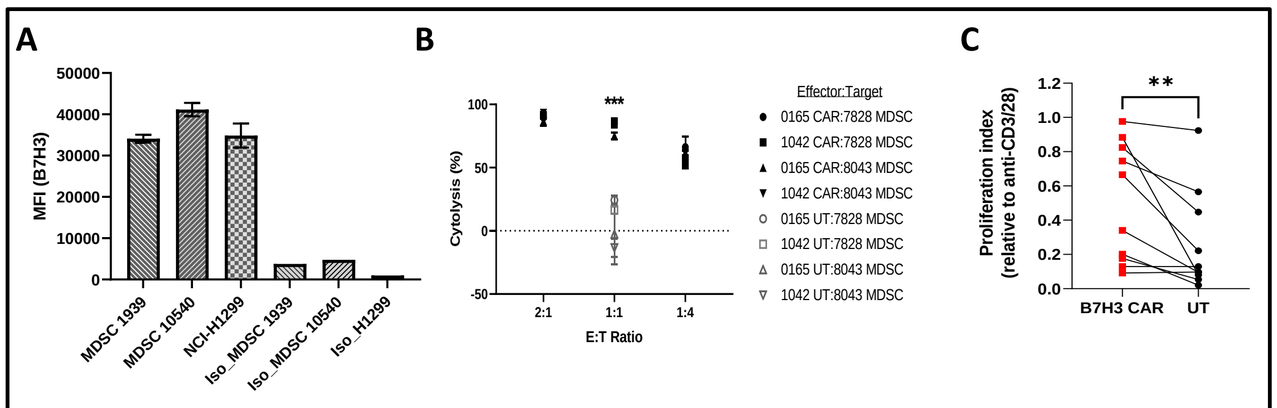
A. Body weight measurements post-treatment. **B.** H&E images of organs from mice that were untreated, treated with un-transduced or B7H3.mCAR T cell and sent for pathological evaluation. **(A and B)** Data comprises of 6 and 2 representative mice in each treatment arm.

4. B7H3.CAR EBVSTs target allogeneic myeloid cells and lower risk of CRS



A. B7-H3 levels on allogeneic monocytes from 3 independent donors after co-culture with EBVSTs from 3 other donors. **B.** UT or B7H3.CAR EBVST cytolysis of allogeneic monocytes after 24h of co-culture at a 1:1 Effector : Target ratio. **(C and D)** Body weight measurements of NALM-6-injected **(C)** or HT-29-injected **(D)** mice post-treatment. **E.** Serum levels of human cytokines at 3 days post-treatment quantified by multiplex bead immunoassay. **F.** Counts of dendritic cells (top), monocytes (middle) and neutrophils (bottom) in blood, spleen and liver. **G.** B7-H3 expression on dendritic cells (top), monocytes (middle) and neutrophils (bottom) remaining in blood,, spleen and liver after treatment. Data comprises of up to 6-9 mice in each treatment arm. *: p<0.05; **: p<0.01, ****: p<0.0001.

5. B7H3.CAR EBVSTs targets myeloid-derived suppressor cells



A. Median fluorescence intensities (MFI) of B7-H3 expressed on MDSCs from 2 healthy donors and the NCI-H1299 cell line. **B.** Cytotoxicity of UT or B7H3.CAR EBVSTs against allogeneic MDSCs. **C.** Proliferation of UT and B7H3.CAR EBVSTs after anti-CD3/CD28 stimulation in the presence of allogeneic MDSCs. Each data point represents proliferation from each effector: target donor pair. Proliferation index was calculated by normalising percentages of proliferated cells against that of the no MDSC control condition. Data were compiled from co-culture of effector EBVSTs generated from 2 donors and target MDSCs generated from a different pair of donors. Error bars denote means ± SD where applicable. **: p<0.01, ***: p<0.001

Conclusions

We adopted EBVSTs as universal donor T cells and armored them with a new generation nanobody-based CAR targeting B7-H3. Our study demonstrates the promising in vitro and in vivo efficacy of our B7H3.CAR EBVST in targeting various solid tumor types that express B7-H3. We further demonstrate that in addition to its favorable on-target off-tumor safety, our candidate carries a low risk of treatment-induced cytokine release syndrome. Lastly, our study reveals a novel function of B7H3.CAR EBVST, specifically its ability to target and reverse the immunosuppressive effects of myeloid-derived suppressor cells (MDSCs).