Targeting immune checkpoint blockade module B7-H3 using CAR T cells as adjuvant therapy in chordoma

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Disclosures: None

INTRODUCTION: Chordoma is the most common primary malignant tumor of the spine with no approved therapy. The mainstay treatment approach remains en bloc resection despite high local and distal recurrence rates with a 5-year survival rate of 73%–86% and a 10-year survival rate of 49%–71%. Lack of curative therapy for chordoma has increased interest in immunotherapy. Our group has developed and implemented genetically engineered T cells with CAR that targets B7-H3, an immune checkpoint blockade inhibitor that is highly expressed on both differentiated chordoma cells and chordoma cancer initiating cells (CICs). Expression on CICs make B7-H3 an attractive target based on the widely accepted theory that a therapy must eradicate both differentiated cancer cells and CICs to be effective. Previous studies have shown that B7-H3 targeted CAR T cells are effective in killing chordoma cells both in vitro and in xenograft mouse models. In this study we hypothesized that B7-H3 CAR T cells can effectively kill B7-H3-expressing chordoma cells.

METHODS: CH22 and MUG-CC1 chordoma cells were given one subclinical dose of irradiation (10Gy) 24 h prior to B7-H3 targeted CAR T cells. CH22 or MUG-CC1 chordoma tumors were NSG mice via right hind limb injection, given one dose of focused IR to the tumor region followed by to B7-H3 targeted CAR T cell treatment once tumors reached 2 cm in diameter. Tumor volume was measured using Bioluminescence imaging. B7-H3-expressing cells were quantified with flow cytometry and analyzed with Flowjo

RESULTS: In this study we show that one subclinical dose of irradiation (10 Gy) combined with B7-H3 CAR T cell is effective in killing CH22 or MUG-CC1 cells 1:4 and 1:1 E:T ratios in vitro, (85.5 ± 0.5 %, p<0.001 and 83.1% ± 1.0, p<0.001, at 1:1 E:T ratio respectively. We observed a major pathological response in all mice treated with combined IR and CAR T cells. Further in vitro analyses showed that IR treatment increases B7-H3 expression in bulk chordoma tumor cells and cancer stem cells (ALDH+CD133+). All mice in the untreated and CD19 control group died before 100 days following tumor injection. All mice treated with B7H3 CAR T cells alone or in combination with IR survived for >100 days following tumor injection.

DISCUSSION: The results of this study suggest that B7-H3 targeted CAR T cell treatment is effective in killing chordoma cells in vitro and in vivo. Additionally focused IR treatment 24h prior to CAR T cell treatment increases target antigens, B7-H3, thus enhancing CAR T cell killing efficiency.

CLINICAL RELEVANCE: High effector to target ratios is associated with cytokine release syndrome (CRS), neurotoxicity and other life-threatening reactions resulting from generalized immune activation. To navigate a delicate balance between achieving robust antitumor activity and avoiding severe off-target toxic effects, we combine a stimulus single dose irradiation to increase the killing efficacy of B7-H3 CAR T cells thus requiring lower concentrations of CAR T cells to achieve tumor elimination.
Figure 1. B7-H3 Expression in clinical chordoma samples (n=68, 75 samples). B7-H3 expression is plotted by age, sex, as a function of survival. Figure 2. B7-H3 CAR-T cells are cytotoxic to chordoma cells and a radiosensitizer of chordoma cells. CH22 and MUG-CC1 cells were treated with 10 Gy IR and co-cultured with B7-H3 CAR T cells. *** indicates $p<0.001$. Figure 3. IR and B7-H3 CAR-T combinatorial therapy is more effective than either treatment alone in inhibiting growth of xenografts of CH22 tumors in NSG mice.