DEVELOPING A NEW TARGETED THERAPY FOR PEDIATRIC RHABDOMYOSARCOMA: αvβ3 AND HER2 AS PROMISING TARGETS FOR BISPECIFIC CAR-T CELL THERAPY

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Background Even though rhabdomyosarcoma (RMS) is the most prevalent sarcoma in children, therapy relies on decades-old, multi-agent chemotherapy, radiation and surgery, which are ineffective in the salvage or metastatic disease setting, with only 30% of patients surviving three years[1]. Therefore, more effective treatments are desperately needed.

Chimeric antigen receptor T-cell (CAR-T) therapy has been highly successful for relapsed/refractory hematologic malignancies, but antigen downregulation by tumors have not commonly led to immunotherapy escape across several platforms [2–5]. We hypothesize that simultaneously targeting two RMS antigens with a bispecific CAR-T will provide a new treatment modality while reducing the likelihood of antigen escape. We previously established the efficacy of αvβ3 (αvβ3) and HER2 CAR-Ts against diffuse intrinsic pontine glioma/glioblastoma[6] and medulloblastoma[7], respectively. HER2 is known to be expressed on RMS[8], and one child with RMS experienced a temporary remission with HER2-CAR-T[9]. Therefore, targeting αvβ3 and HER2 using a bispecific CAR approach may be effective for RMS.

Methods Six alveolar and embryonal RMS cell lines were evaluated by flow cytometry for αvβ3 and HER2 expression. The in vitro kinetics of αvβ3 and HER2-CAR-T killing of RMS were assessed at varying effector-to-target ratios in an xCelligenance analyzer, with CD19-CAR and non-transduced T-cells as controls. Antigen-specificity was evaluated by pre-incubating targets with αvβ3/HER2 blocking or control antibodies or by targeting ITGB3 or ERBB2 CRISPR knock out tumor cells. CAR-Ts were then evaluated for changes in activation marker expression by flow cytometry.

Results (1) αvβ3 and/or HER2 are expressed on 6/6 RMS cell lines. Five express both antigens (figure 1A). (2) Separately, αvβ3 and HER2-CAR-Ts rapidly and robustly kill antigen-positive RMS in an antigen- and dose-dependent manner. Furthermore, αvβ3 and HER2-CAR-Ts are sensitive, achieving 70% cytolysis at 300 and 1100 targets/cell, respectively (figure 1B) suggesting a lower likelihood of disease escape by antigen down-regulation. (3) Cytolysis is antigen-specific (figure 2). (4) Co-culture of both CARs with RMS drives increased expression of the activation markers CD107a, CD137, CD25, CD69 and PD-1, demonstrating antigen-driven induction of a strong effector response (figure 3).

Conclusions Our data demonstrate that αvβ3 and HER2 are viable RMS targets for a bispecific CAR-T approach. αvβ3 and HER2 CAR-Ts independently control RMS well. They exhibited robust and low-level antigen-specific killing and effector cell activation in vitro, supporting further investigation into their control of RMS in vivo. αvβ3/HER2 bispecific CAR-Ts are being developed, which we expect to perform as well as or better than each CAR independently.

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References


Ethics Approval The study was approved by the University of Virginia’s Institutional Review Board, approval number 18842.
avß3.28z and HER2.28z CAR-Ts robustly kill antigen+ rhabdomyosarcoma targets even at low effector-to-target ratios. A) RMS cells were analyzed for expression of avß3 and HER2 by flow cytometry. Antigen levels were quantified using BD QuantiBrite beads. B) CAR-T cell cytolysis was measured via impedance assay in an xCelligence real time cell analyzer against RMS targets. Non-specific CD19 CAR-Ts (negative control) and mock (non-transduced) T-cells were used as controls.

avß3.28z and HER2.28z CAR-T killing of RMS cells is antigen-specific. A) RH18, B) RH30 and C) RH36 tumor cells were pre-incubated with avß3- or HER2- blocking antibodies or with control IgG antibodies. CAR-Ts were evaluated for tumor cell killing after 12 hours co-culture at 2.5:1 effector:target ratio.

Higher percentage of CAR-Ts express multiple activation markers with increased antigen expression on tumor cells. Cells were co-cultured for 24 hours at 2.5:1 effector:target ratio and then evaluated by flow cytometry for expression of CD25, CD137 and CD107a. Percentages of CD8+ and CD8- A) avß3.28z and B) HER2.28z CAR-Ts that were positive for 0-3 of the activation markers.