CO-OPTING IL-8 TO ENHANCE EFFICACY OF B7H3 CAR T CELLS AGAINST PEDIATRIC SARCOMA

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Background The 5-year disease-free survival for children and young adults with metastatic sarcoma at diagnosis or recurrent disease after front-line therapy is 20–30%.^{1–2} Cellular immunotherapy using chimeric antigen receptor (CAR) T cells has shown dramatic benefits in leukemia, but only limited success in solid tumors.^{3–4} One limitation of CAR T cell therapy has been poor trafficking into solid tumors.^{5–7} Chemokines are small, secreted, cytokine-like molecules that mediate lymphocyte homing and migration.⁸ In this study, we discovered that both osteosarcoma (OS) and rhabdomyosarcoma (RMS) cells significantly increase expression of the chemokine IL-8 after clinically achievable doses of radiation, but not at rest. Given that CAR T cells do not express the receptor for IL-8, we created a construct with an IL-8 receptor (CXCR2) and a B7H3 CAR in T cells to improve CAR T homing and to create an effective new immunotherapy for patients with sarcoma.

Methods Multiple OS and RMS cell lines were irradiated at 10 Gy and IL-8 was measured by ELISA. We created retroviral constructs, B7H3 CAR-T2a-CXCR2 and B7H3 CAR. Peripheral blood T lymphocytes were stimulated with IL-2 and anti-CD3/28 antibodies for 48 hours prior to transduction with the retroviral vectors. Surface expression of the scFv (by L protein) and CXCR2 (mAb) were assessed using flow cytometry. In vitro cytotoxicity assays using sarcoma tumor spheroids were conducted using Incucyte. INF- γ and IL-2 production were measured by ELISA. NSG mice injected orthotopically with an IL-8 overexpressing RMS cell line were treated 4–7 days later with the B7H3 CAR-CXCR2 T cells or B7H3 T cells (control) and followed weekly with bioluminescent imaging.

Results Irradiated (10 Gy) sarcoma cells express 2–9x higher IL-8 than non-irradiated sarcoma. T cells were transduced with efficiencies of 60–90%. INF- γ production was equivalent between the B7H3 CAR-T2a-CXCR2 T cells and B7H3 CAR T cells, but IL-2 production was significantly higher in the dual expressing CAR T cells. In vitro cytotoxicity with sarcoma spheroids was measured by Incucyte and showed faster and greater killing by B7H3 CAR-T2a-CXCR2 T cells than B7H3 CAR T cells. Furthermore, when sarcoma tumor bearing mice were treated with B7H3 CAR-T2a-CXCR2 T cells, tumors resolved completely by 4–5 weeks and had long-lasting remission.

Conclusions Chemokine receptor expressing CAR T cells showed superior cytokine production and T cell activation/cytotoxicity compared to a CAR T construct alone. These findings lead to better efficacy in animal models and suggest a promising approach for pediatric sarcoma.

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Ethics Approval The animal experiments discussed in the abstract were approved by the University of Colorado IACUC, protocol #00251.

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