Chemokine-induced homing of CAR-expressing immune cells to sarcoma
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Introduction
Advances in pediatric oncology care have occurred for some forms of cancer, however, new therapies for sarcoma have been sparse. The 5-year disease-free survival is 20-30% for patients with metastatic osteosarcoma at diagnosis or recurrent disease.1 Cellular immunotherapy using chimeric antigen receptor (CAR) T cells has dramatic benefits in leukemia,2 but have been unsuccessful in solid tumors, including sarcoma. One of the major limitations has been the inability of CAR modified T cells to ‘find’ the tumor, thus improved cell homing is likely to be beneficial. Chemokines are small, secreted, cytokine-like molecules that mediate lymphocyte homing and migration. To date, few studies have investigated the effect of treatment on chemokine production. It may be possible to use traditional therapy to induce chemokine production to co-opt CAR T cell homing into solid tumors.

Hypothesis
We hypothesize that sub-therapeutic radiation can induce a unique chemokine response that in combination with a CAR in T cells, can improve homing to and killing of sarcoma tumors.

Specific Aims
The overall objective is to investigate the role of sub-therapeutic radiation-induced chemokine response as a unique homing target for CAR T cell therapy.
Specific Aim 1: Identify tumor-derived chemokines that can be co-opted to facilitate T cell migration into sarcomas.
Specific Aim 2: Clone and use retroviruses to transduce T cells with a chemokine receptor.
Specific Aim 3: Create T that co-express a chemokine receptor and a B7H3 CAR targeting sarcoma.

Methods
We first tested chemokine expression by both osteosarcoma and rhabdomyosarcoma cell lines with and without irradiation using a human chemokine array (31 different chemokines). Next, we quantified IL-8 expression using an ELISA. This was then tested in a patient-derived xenograft implanted into the pretibial space of NSG mice. The affected limb containing the tumor was irradiated at 10 Gy and IL-8 gene expression was measured at different time points by qRT-PCR. A knockout IL-8 RMS cell line has been created (Figure 4).

Results
To confirm the chemokine response from irradiated tumors, we performed a chemokine array on RNA from osteosarcoma (OS; Figure 1) and rhabdomyosarcoma (RMS; not pictured). The Chemokine Array showed increased IL-8 expression after irradiation in both osteosarcoma (OS; Figure 1) and rhabdomyosarcoma (RMS; not pictured).

Specific Aim 1:
Chemokine Array showed increased IL-8 expression after irradiation in both osteosarcoma (OS; Figure 1) and rhabdomyosarcoma (RMS; not pictured).

Specific Aim 2:
The IL-8 receptor CXCR2 was cloned into a retroviral vector (along and in combination with CXCR2) using gateway cloning techniques. Retroviral production to co-opt CAR T cell homing into solid tumors.

Specific Aim 3:
B7-H3 (CD276) is a tumor-associated ligand selectively expressed on multiple tumor types, including OS and RMS. We have combined B7H3 CAR with the IL-8 receptor in a retroviral vector and transduced T cells (Figure 6). We have combined B7H3 CAR with the IL-8 receptor in a retroviral vector and transduced T cells (Figure 5). An Incucyte cytotoxicity assay (Figure 7) was done and showed increased killing of sarcoma by dual CXCR2 + B7H3 CAR-expressing T cells over B7H3 CAR alone (T cells). B7-H3 is highly expressed by sarcomas.

Conclusions
IL-8 is induced to high levels after subtherapeutic doses of irradiation by OS and RMS tumors in vitro and in vivo. B7-H3 is highly expressed by sarcomas. T cells can be successfully transduced by retrovirus containing CXCR, B7H3 CAR and dual CXCR2 + B7H3 CAR. CXCR + B7H3 CAR-expressing T cells kill sarcoma better than B7H3 CAR-expressing T cells.

Next Steps
In vivo studies testing these CAR T cells are ongoing. Next steps are to expand the mouse trial and include mice with IL8 knockout tumors.

We were just awarded a V foundation grant in collaboration with CSU to test these B7H3 CAR T cells in dog osteosarcoma (a common malignancy in certain breeds).

References