Desired Impact: This project will define how resistance occurs in osteosarcoma in response to treatment to targeted therapy. It will help identify the patients most likely to benefit from the targeted therapy. Defining the mechanism of resistance will define potential weaknesses in the osteosarcoma and how best to combine the targeted therapy with other treatments to overcome resistance. The results of the project will provide the clinical foundation for integrating these agents to the treatment and improve the outcomes for children diagnosed with osteosarcoma.

Projected Milestones: At the time of starting the project, we will have completed DNA barcode libraries in the patient derive tumor models. The first 6 months will be necessary to complete the treatment experiments in the barcoded patient derived tumors models. The second months will involve analysis of the DNA, RNA, and protein changes associated with development of resistance to the targeted therapy.
Osteosarcoma is the most common primary malignancy of bone in children and young adults. Over the past three decades, these results have remained stagnant when compared across 6 different clinical trials involving multiple international consortiums. There is a clear need to effectively integrate novel agents to improve survival outcomes in patients with osteosarcoma.

Recurrence of tumor can result from acquired resistance but methods have not existed to elucidate whether this represents an intrinsically resistant, previously undetectable sub-population of cells or acquisition of new properties driven by time and potentially enhanced by chemotherapy effects. To improve delivery of chemotherapy to the tumor, drugs can be attached to antibodies forming antibody-drug conjugates (ADC). Our laboratory has tested two ADC’s, ABBV-085 and m276-PBD, with broad activity in osteosarcoma patient derived xenografts (PDX) models. ABBV-085 is an ADC with the antibody directed against a protein located on the outer membrane of the osteosarcoma cell (LRRC15). LRRC15 is found on the membrane of the cell in a number of cancers, with high levels on the surface of most osteosarcoma PDXs, but with low levels in normal tissue. This makes LRCC15 an attractive target for treating osteosarcoma with low levels of toxic effects to the patients.

ABBV-085 contains a drug (MMAE) that inhibits the microtubules which are necessary for the tumor cell to grow and divide. ABBV-085 significantly inhibited tumor growth and improved event-free survival in mice injected with tumors derived from patient samples with high levels of LRRC15, but with limited disease control in PDX models with low levels of LRRC15. One model experienced a maintained complete response with complete eradication of the entire tumor (manuscript in preparation).

m276-PBD is an ADC targeting another membrane protein (CD276). m276-PBD contains the drug pyrrolobenzodiazepine (PBD). PBD directly damages the DNA, inhibiting the tumor cells to copy its DNA necessary for the cell to divide and grow. CD276, also known as B7-H3, is a protein that inhibits the immune system’s ability to attack the osteosarcoma. High levels can be found on cancer cells and the blood vessels feeding the tumor with low levels on normal tissue. CD276 is found on greater than 90% of osteosarcomas and increased levels is associated with decreased overall survival. In our PDX models, m276-PBD demonstrated significant improvement in event-free survival in 5/5 models tested, with all models demonstrating maintained complete responses (being presented at the AACR Molecular Targets Meeting).

**Innovation:** The innovation of this proposal is the use of DNA barcoding to delineate the mechanisms of resistance in osteosarcoma PDX models. DNA barcoding is a method for tracking single cells by inserting unique pieces of DNA into each individual cell to allow tracking which cells survive and how cells change before and after treatment. Because DNA barcoding, allows us to track individual cells, we can detect changes in the DNA, RNA and proteins of individual cells of the osteosarcoma patient derived models in response to these targeted treatments.

**Project Aim:** This project will seek to determine how osteosarcoma becomes resistant to treatment with the targeted therapies, ABBV-085 and m276-PBD.

**Research Design:** We have already developed multiple libraries of barcoded tumors from patient derived osteosarcomas. We are currently in the process of confirming that these libraries contain unique and identifiable DNA barcodes. Each patient derived tumor will have 10 million unique barcodes allowing us to detect very rare mutations that may lead to resistance. We plan on testing 6 different PDX models,
with 10 mice in each group. We will treat these barcoded tumors with ABBV-085 and m276-PBD and then wait for the tumors to grow back following treatment. We will define the mechanisms of resistance in the outgrowths by evaluating the DNA, RNA, and proteins in the resistant tumors. We will specifically look for changes in the proteins targeted by ABBV-085 and m276-PBD, LRCC15 and CD276, respectively.

**Expected Outcomes:** This project will detect if resistance to the treatment occurs by changes in the targeted proteins or changes inside the cells leading to resistance to the drug attached to the antibody. DNA barcoding will allow us to detect if the resistance occurs because of rare cell present in the original tumor, or if resistance occurs in response to changes in the cells in response to treatment.

**Research Findings:** Understanding the mechanisms of resistance to targeted therapy is important to ensure that we can select the patients most likely to benefit from these targeted therapies. Defining the mechanisms of resistance will provide guidance on potential targets for combining with these targeted agents. In the future, we plan on testing how best to combine these treatments.