Desired Impact: Clinically, identifying patients with Myc driven osteosarcoma at diagnosis is extremely feasible and rapid (within 48-72 hours), and our approaches can be translated into real-time therapeutic interventions by identifying, risk-stratifying, and targeting critical tumor features and molecular signatures, thus providing precision treatment for thus extremely high-risk group of osteosarcoma patients.

Projected Milestones: During the first 6 months of the proposal we will execute Aim1 and through high throughput approaches identify candidate small molecules targeting Myc-driven osteosarcoma models. We will test these small molecules with additional in vitro studies to further understand their effects on osteosarcoma biology. We will then quickly move top candidates to in vivo studies. Throughout the whole funding period we will using our models to assess therapeutic regimens we have identified though our preliminary studies (Aim2). These include agents targeting both the intrinsic Myc-driven tumor properties (e.g.,RNA splicing, transcriptional regulators) as well as extrinsic, tumor micro-environmental effects (e.g. macrophage activators, CD40 agonists, etc..) driven by enhance Myc-expression. We will also streamline in candidates from Aim1. We anticipate that by the end of this funding period we will have additional significant insights into the biology of Myc-driven osteosarcoma as well as pre-clinical indications for candidate novel therapeuetic regimens for the treatment of this high-risk group of osteosarcoma patients.
**Project Aims:** While defining genetic mutations are rare for osteosarcoma (OS), broader alterations in the DNA are quite frequent, which can lead to an increase, or decrease in certain regions of the DNA inside each OS cell. These DNA changes are referred to as copy number alterations (CNA). One CNA alteration present in about 30-40% of all OS patients leads to an amplification, or increase in the gene c-MYC (referred to as Myc)\(^1,2\). Myc is a potent cancer-causing gene that regulates the expression of many other genes in the cancer cell that are thought to contribute to the biology of the tumor. Prior reports, as well as unpublished institutional data, have demonstrated that the presence of extra copies and enhanced presence of Myc has been associated with extremely poor outcomes for OS patients\(^3\). Unfortunately, directly targeting Myc has been a tremendous challenge, thus signifying the need to gain a more comprehensive understanding of the **complete Myc-driven tumor** biology, including both internal tumor and external environmental features, in order to identify effective and actionable treatment options. We have used complementary resources, including publically available genomic databases and innovative, homologous human and mouse tumor models we have developed and preliminarily characterized towards dissecting and targeting Myc-driven OS\(^4-8\). Our preliminary studies have enabled us to identify Myc-driven, tumor-defining targetable features. In addition, we will expand our therapeutic potential and use our novel Myc-defined models to identify small molecules targeting Myc-driven OS cells. We propose the following Aims:

*Specific Aim 1: Perform small molecule targeting in Myc-driven OS cells.*

*Specific Aim 2: Targeting features of Myc-driven tumors using homologous OS models*

**Impact:** This work addresses an unmet need in a clinically significant portion of OS patients with very poor prognosis that harbor enhanced expression of Myc. The approach is *innovative* because it integrates comprehensive molecular and small molecule approaches in homologous model systems to identify, prioritize, and translate candidate therapeutic modalities. The *long-term goal will change the treatment paradigm* by enabling upfront targeted therapy and enhanced patient outcomes for this high-risk subgroup of OS patients.

**Research Design:**

*Specific Aim 1: Perform small molecule targeting in Myc-driven OS cells.*

The screen will be performed in collaboration with the Texas Screening Alliance for Cancer Therapeutics (TxSACT). The TxSACT Drug Library contains 6685 unique FDA approved and investigational drugs. Our
screen will focus on the use of a total of 9 cell lines, including 3 human Myc-driven and 4 mouse lines—2 from Myc-driven and 2 from our novel Myc-overexpressing model. We will also screen one human and murine non-Myc driven lines for comparative purposes and a normal, non-tumor bone cell line.

**Specific Aim 2: Targeting features of Myc-driven tumors using homologous OS models**

Our Myc-driven mouse tumor cells will be transplanted into the bone of mice with normal immune system. Two murine Myc-driven and two non-driven cell lines will be used. All murine lines will be labeled so they can be monitored while the tumors are growing in the primary injected site and for evidence of metastasis, or disease that has spread to other locations in the body. Our initial studies will focus on the therapeutic targets identified from candidate genetic susceptibilities identified from preliminary studies (e.g., spliceosome inhibition\(^9,10\), β-catenin inhibition\(^5\)), our TARGET database analysis (AZD4673 (CDK9 inhibitor\(^11,12\))) and those from Aim1. We will perform single agent and combinations with the following immune altering therapies that we have identified from analysis of human and our murine models as candidate treatments for Myc-driven tumors: (1) MTP-PE\(^13,14\), (2) CD40 agonist\(^15,16\), (3) 2',3'-cGAMP sodium salt (STING agonist\(^17\)). Appropriate control cohorts will be used as well.

**Expected Outcomes:** For Aim 1, we anticipate selecting 8-10 effective Myc-specific therapeutics for therapeutic evaluation in mouse OS models that have broad single agent activity in at least 2 of the 3 human and 3 of the 4 murine Myc-driven cell line models and display strong cytotoxicity to tumor cells with lesser effects on normal bone cells. We will prioritize clinically relevant agents for their potential treatment of OS. The goal will be to select as diverse a collection of candidate drugs as possible. We will take into account, but not exclude potential redundancy of drugs since many target classes of drugs possess valuable off-target effects. For Aim2, using our MYC-driven tumor models, we expect that targeting critical OS cell pathways (e.g., WNT signaling) and enhancing immune responses suppressed by MYC will combine and demonstrate significant anti-tumor activity.

**Research Findings:** Our analysis of genetic data from Myc-driven human tumors and our novel mouse model of Myc-driven OS has identified candidate alterations that are potential therapeutic avenues for the treatment of this high-risk subgroup of OS patients. Specifically, we have identified that Myc tumors have enhanced activity of critical tumor cell signaling pathways and diminished specific anti-tumor immune features, including decreased levels of macrophages. We intend to exploit these findings in the experimental design outlined above.