Guidelines: Proposal must benefit the osteosarcoma patient and be a new project or distinct portion of an ongoing larger project for which results can be expected in 12 months. At the completion of 12 months, results must be made available to share - regardless of succeed or fail outcome. The recipient must be available to present work underway and completed at the FACTOR conference in 2019. Fund may not be used for the formation of new organization or used for planning stages of research or other initiative. Presentation of check and tour of facility by MIB Agents is requested.

Please fill out the form below, proposals will be submitted as a layman's summary and are limited to front and back of this page. Completed RFP will be available for the public to view on MIB Website and social media so the public can vote. Deadline for submission is April 20, 2018. Email to info@MIBagents.org

Name: John H. Healey, MD

Organization: Memorial Sloan Kettering Cancer Center

Email: Redacted Phone: Redacted

Initiative Name: Preclinical Development of Novel Therapies for Metastatic Osteosarcoma Amount Requested: $100,000

Desired Impact: Unique characteristics of lung metastases and individual patient tumors will be identified and analyzed. These unique characteristics of lung metastases of osteosarcoma can then be targeted in a patient-specific fashion with appropriate chemotherapeutic agents.

Projected Milestones: Isolation of exosomes & establishing PDX models (months 1-6); characterization of exosomes, premetastatic niche, and tumor initiating cells (months 6-9); genetic analysis and target identification (months 6-12)

I agree to Guidelines: Yes

Preclinical Development of Novel Therapies for Metastatic Osteosarcoma

Investigators: John H. Healey[1] [principal investigator], Vinagolu K. Rajasekhar[1], David C. Lyden[2]
Affiliations: (1) Orthopaedic Service, Dept of Surgery, Memorial Sloan Kettering Cancer Center, (2) Dept of Pediatrics, Memorial Sloan Kettering Cancer Center

BACKGROUND:

Patients with osteosarcoma (OGS) have an urgent need for new therapies. Metastases are most common in the lung. Although about 15% of patients are cured by surgical removal of the primary tumor alone, the microscopic metastases in the remainder are addressed by chemotherapy with failure in about 30% of patients with localized disease. Those presenting with overt lung metastases have poor survival (32%) [Chou A, *Cancer* (2009)115:5339-48]. Surgical resection of limited lung lesions can salvage the minority of patients. Metastases that develop during chemotherapy have a particularly bleak prognosis. There are no effective drug treatments for them.

Treatment for the last several decades for all patients has used three drug regimens of standard drugs (doxorubicin, platinum, methotrexate and sometimes ifosfamide). New strategies should address the unique biologic characteristics of OGS. (1) We hypothesize that metastases arise preferentially from tumor initiating cells (TICs): a distinct but a minor population in primary tumors and enriched within metastatic tumors. TICs have considerable plasticity, remaining dormant or altering their metabolism as needed to adapt to new and distant local environments. Characterizing and targeting these cells has not been investigated as a therapeutic strategy in osteosarcoma and may have potential to improve the present day patients' outcome. (2) The metastatic process is influenced by the action of cancer exosomes. Cancer exosomes, the lipid bilayer-enveloped vesicles that contain tissue-specific proteins and genetic material, are shed into the circulation in body fluids in the early stages of tumorigenesis, promoting tumor growth and establishing premetastatic niche in select target organs, which are in turn appeared to be determined by integrin types expressed on exosome surface. We have preliminarily confirmed this in many cancers, including metastatic osteosarcoma [*Nature* (2015)527:329].

RESEARCH PLAN:

Aim 1: Rapid characterization of osteosarcoma metastases and the premetastatic niche. We will identify unique characteristics of metastatic TICs and the metastatic tissue microenvironment *in vivo*. The first part of this aim leverages our expertise with PDX modeling of freshly harvested specimen of a patient's treatment-refractory osteosarcoma primary tumor in bone. We then propagate the specimen as patient-derived xenograft (PDX) in immunocompromised/humanized mice to increase the tumor volume in a humanized tumor microenvironment and thus the levels of TICs as we did with other cancer models [*Nature Commun* (2011)2:162; *Nature Commun* (2016)7:10442]. PDXs can be used to conduct precise co-clinical trials with a battery of potential therapeutic agents (the "mouse hospital" concept). The development of specifically humanized (patient-personalized) PDXs would further enhance the value of this approach. The second part of this aim focuses on the role of cancer exosomes in osteosarcoma metastasis. By isolating and characterizing exosomes from patients with potential to end up with metastatic osteosarcoma, we will investigate the potential for these exosomes as to where in the body establishes pre-metastatic niches in PDX models. If successful, we will have an exceptional opportunity to characterize a wealth of *in vivo* molecular and proteomic characteristics of the microenvironment before and after tumor formation. These *in vivo* biologic characteristics will be compared with that of patients to identify clinically relevant therapeutic targets.

Aim 2: Delineate the functional interactions between metastatic tumor cells and their microenvironment, and any exosome-associated factors mediating these interactions. We have previously identified horizontal genetic transfer and molecular signaling crosstalk between cancer-associated fibroblasts (CAFs) in the tumor stroma and breast TICs to foster therapy resistance [*Cancer Res* (2017)77:5438]. However, the specific pathways of genetic transfer and signaling crosstalk, as well as the role of exosomes in these interactions, have not yet been fully characterized. We will use our in-house expertise and ongoing collaborations in whole-genome single-cell copy number and gene expression profiling assays, chromosome instability characterizations, and adoptive immunotherapy approaches [*Mol Cancer Ther* (2017)16:2701; *Nature* (2018)553:467] to identify and verify any novel, functionally associated cell surface biomarkers in the treatment-refractory osteosarcoma TICs from primary and metastatic tumors.

Aim 3: Development of novel therapeutic approaches for metastatic osteosarcoma. In addition to our integrated systems biology related approaches [*Clin Orthop Relat Res* (2016) 474:178; *Cell Rep* (2017)20:1623], our center also performs integrated mutation profiling of actionable cancer targets (MSK-IMPACT) testing (approved by the FDA) to characterize mutations in about 458 cancer-related genes in clinically resected tumors. As a result of tumor heterogeneity in humans, a single primary tumor may give rise to metastatic clones with different genomic profiles; each clone may respond differently with respect to the uptake of the same candidate drugs, a problem often encountered in solid tumors. We plan to compare IMPACT data from the primary osteosarcoma patients to those of metastatic patients to prospectively identify possible and functionally conserved therapeutic targets. Our metastatic PDX model derived spheroids and organoids will allow us to perform *in vitro* miniscreens for identifying novel investigational therapeutics so that they can be loaded in patients own exosomes to deliver only at the metastasizing locations with any systemic toxicities often encountered in systemic chemotherapies. If the above approaches fail, we will still have the epitopes of the exosomes and novel surface proteins present only in the metastasis TICs determined and they could be employed in targeted/adoptive immunotherapy approaches pioneered by ongoing collaborators in our center.

Patients will benefit by identifying and targeting the unique tumor initiating cells and exosomes that characterize the metastatic lung tumor. By comparing the actionable (IMPACT) genetic changes in the primary and metastatic tumors, the unique characteristics of the individual patient tumors can then be targeted in a patient-specific fashion with appropriate chemotherapeutic agents.