**Name:** Alex Y. Huang, M.D., Ph.D.

**Organization:** Case Western Reserve University / Angie Fowler AYA Cancer Institute

**Initiative Name:** New Immune-mediated Therapies for Lung OS

**Amount Requested:** 100000

**Desired Impact:** Developing multiple novel paradigm-shifting immunotherapies for lung Osteosarcoma in the next 12 months to target immune tolerating tumor microenvironment by leveraging 3 near IND-ready small molecule therapeutics.

**Projected Milestones:** Milestone 1: Complete FDA IND-enabling pre-clinical toxicity profile of BG34-200 and GMP-grade compound production scale-up protocol in 8-12 months. Milestone 2: Complete pre-clinical efficacy study of Vactosertib in Osteosarcoma-bearing mice and describe associated changes in immune cellular components in 6-15 months. Milestone 3: Demonstrate in vivo effect of carbonic anhydrase IX inhibitor (VD11-4-2) in pre-clinical Osteosarcoma models for IND filing and clinical trial design in 10-18 months.

**CLINICAL CHALLENGE:** Osteosarcoma (OS) is a highly aggressive malignant primary bone cancer with a high propensity for lung metastasis. OS frequently originates from primitive mesenchymal bone-forming cells in the long bones during periods of rapid bone growth. Consequently, OS represents the most prevalent bone cancers affecting children and adolescent and young adults (AYA), with ~400-600 cases a year and accounts roughly half of all new cases of OS diagnosed in the United States. Despite aggressive combination chemotherapy and surgery, the outcome for metastatic OS remains dismal, and the overall survival in children and AYA patients with metastatic OS has not improved significantly over the past 3 decades. A high proportion of OS patients develop metastatic disease at distant sites either at the time of diagnosis (one in five patients; 20%) or after initiation of multimodal therapy including combination chemotherapy and surgery (in ~30% of patients). The lung accounts for >80% of all OS metastatic sites. Unfortunately, almost all of the patients who develop surgically unresectable pulmonary metastatic OS (pOS) invariably succumb to this devastating disease. Therefore, pOS represent a disease with urgent unmet needs. As OS contains extremely complex and heterogeneous chromosomal and genetic alterations, recent advances in molecular precision medicine therapy approaches to target OS-specific mutations will likely be challenging in the immediate future. Immunotherapy is the new kid on the block as a potential new addition in the armamentarium for pOS treatment. Immunotherapy targets existing common immune pathways through which different cancers thrive by interacting with the host immune system. Such immune-mediated factors influencing tumor immunogenicity may or may not be directly related to known characteristic genetic alterations intrinsic to any particular cancer histology. Our multi-disciplinary investigator team at the Angie Fowler AYA Cancer Institute / UH Rainbow Babies & Children's Hospital in Cleveland, Ohio is highly resolved to bring novel immune-based therapeutic options to pediatric and AYA patients suffering from this devastating disease by providing novel "outside-of-the-box" and paradigm-shifting immunotherapeutic approaches. As described below, we are now on the verge of brining several such readily available therapies to the clinic for patients with pOS, and, with the help of MIB Agents everywhere, we aim to make major advancements in this front within the next 12 months.

**BARRIERS AND NEW OPPORTUNITIES:** Accumulating data clearly suggest that tumor microenvironment (TME) plays a pivotal role in cancer pathogenesis. Ongoing pre-clinical research in our laboratory has discovered two sets of such critical "enablers" of pOS survival in the lung tissue: A) pulmonary macrophages (MACs); and B) abundance of immune-suppressive TGF-$\beta$ cytokine signaling and low pH milieu due to activities of carbonic anhydrase IX (CA IX). *Our novel concept was that pOS thrives because of these molecular and cellular "enablers", and that disrupting each of these cellular or molecular functions using readily available immune-modulating drugs will be highly effective in treating established pOS while preserving immune function of MACs for infectious control.*

**PRELIMINARY DATA:** Our lab identified tumor-expressed Vascular Cell Adhesion Molecule-1 (VCAM-1) on OS cells facilitates OS metastasis by interacting with $\alpha4\beta1$ integrin on MACs to create an immune tolerant environment. Depletion of pulmonary MACs both prevents pOS formation and also ameliorates already-established pOS disease in more than 60% of the time. However, since presence of lung MACs is important for infectious control, we sought to functionally interfere their communication with pOS rather than depleting them all together. We tested the efficacy of using an antibody against the $\alpha4$ portion of the $\alpha4\beta1$ integrin on MACs, so that communication with VCAM-1 on pOS can be disrupted. Indeed, we are able to cure 60% of mice with established pOS by giving the anti-$\alpha4$ antibody via intranasal / inhaled routes. These data are extremely exciting, because for the first time we have been able to identify a set of molecular targets (VCAM-1/$\alpha4\beta1$) that are responsible for lung metastasis mediated by the immune "enablers" in the lung microenvironment. What's more exciting about this finding is that *antibody against human $\alpha4$ (Natalizumab or Tysabri$^{TM}$, Biogen) has already been FDA-approved* for the treatment of multiple sclerosis and inflammatory bowel disease. I was extremely grateful to be invited to share these exciting data at recent FACTOR 2019 Osteosarcoma Conference in Miami, Florida in January. Since then, this trial has been listed on ClinicalTrials.gov (NCT03811886) and we are at final stages of obtaining Biogen sponsorship and IND application with the FDA for the trial, which is set to open later in fall, 2019.

**PROPOSED RESEARCH:** Pertinent to the current OutSmarting Osteosarcoma Grant application, we have generated new preliminary data to support the development of 3 new clinical trials, all of which will be focused on Osteosarcoma immunotherapy. **First,** working closely with Dr. Mei Zhang in Biomedical Engineer Department at Case Western Reserve University, we have identified an Oat-derived beta-glucan with 3->4 O-

glucose linkages (BG34) macromolecular of a particular molecular weight (200kD) as possessing excellent immune stimulatory capacity via its effect on turning immune tolerant macrophages into an immune-stimulating, anti-tumor macrophages. Using the same mouse pOS model as above, we showed that intranasal administration of this water-soluble BG34-200 compound resulted in 75% pOS disappearance in established tumor-bearing mice. We are now finalizing the scale-up process for making GMP-grade BG34-200 with a goal to obtain preclinical safety data for FDA IND application within 12 months. **Second,** our ongoing preliminary data and other published results suggest that the immune-suppressive cytokine, TGF-β, are produced in large quantity by pOS and surrounding cells in TME, rendering any attempts by the immune system to fight off pOS ineffective. We are now working with MedPacto who has developed a highly effective, non-toxic, and orally-available small molecule inhibitor (Vactosertib or TEW-7197) of TGFβ Type 1 Receptor (ALKV) already in clinical trials of multiple adult cancers including Multiple Myeloma at UH Seidman Cancer Center here in Cleveland. The company has expressed a very high interest in sponsoring a clinical trial targeting pOS in pediatric and AYA population once pre-clinical efficacy can be fully demonstrated. **Third,** we have recently discovered that pOS create a highly acidic TME by expressing the membrane-bound enzyme, carbonic anhydrase IX (CA IX), which converts water and CO2 into bicarbonate and hydrogen ions (lower pH). The low pH environment renders immune cells dysfunctional. We have available a CA IX-specific small molecule inhibitor (VD11-4-2) through collaboration with ThermoPharma to test its safety and efficacy in pre-clinical OS models for IND filing and clinical trial design.

**MAJOR AIMS OF THIS PROPOSAL:** Based on these exciting pre-clinical leads, available pharmacologic agents and partnering institutions, I aim to secure funding from 2019 OutSmarting Osteosarcoma / MIB Agents to achieve meaningful, immunotherapy clinical trial-enabling pre-clinical studies in 3 areas: **1)** Perform *in vivo* treatment efficacy validation and complete safety and toxicity profiling studies using BG34-200 in 2 other pOS mouse models to establish generalizability of this approach as well as to gather data for IND filing with the FDA. A clinical protocol will be drafted and submitted through our institutional Protocol Review and Monitoring Committee (PRMC) in the next 12 months; **2)** Gather *in vivo* efficacy data of treating pre-clinical pOS model using TGFbR1 inhibitor, Vactosertib, with either immune checkpoint blockade or NK cell therapy. These results will inform the creation of another clinical trial design, submission for PRMC approval and IND filing with the FDA in the next 8-12 months; and **3)** Investigate anti-tumor efficacy and immune reactivation in pOS by treating pre-clinical pOS models with CA IX-specific inhibitor VD11-4-4 in the next 12 months in order to provide strong scientific rationale for future 3rd OS clinical trial.

**SIGNIFICANCE / INNOVATION / EXPECTED OUTCOME:** Our ongoing multi-pronged approach to finding novel therapeutic options for pOS is based on strong scientific rationales, demonstrated efficacy data, and unique opportunities to leverage existing ready-to-go pharmacologic agents and engaging industry partners. Coupled these factors with institutional knowledge in immuno-oncology translational research pipeline and infrastructure at Angie Fowler AYA Cancer Institute and Case Comprehensive Cancer Center, we are uniquely poised to quickly evaluate and offer these therapies for pediatric and AYA OS patients in the very near future. A demonstration of our ability to do this is our ongoing proposed trial use of Natalizumab (Tysabri™) for the immunotherapeutic treatment of refractory or unresectable pOS (NCT03811886). For the current proposal, 1 of the 3 potential trials (Vactosertib) is already in clinical trials for cancer, with the other two agents near IND filing status. Much of the needed work for IND filing can be accomplished in the next 12 months with the help of OutSmarting Osteosarcoma Funding. Our chances of success in this highly impactful venture is further sustained by a focused institutional commitment to build a sarcoma immunotherapy translational and research program in AYA oncology at the Case Comprehensive Cancer Center and the Angie Fowler AYA Cancer Institute in Cleveland, Ohio, recognized leaders in AYA Oncology Innovation. We will also leverage our extensive collaborations to offer this new therapeutic approach to other local and national. Dr. Huang will leverage his leadership in Cancer Immunotherapy initiative and co-leadership in Hematopoiesis & Immune Cancer Biology Program in the Cancer Center to collaboratively learn new biology about how such therapy works on a cellular and molecular level, thereby creating additional opportunities for future exploration of improved novel or combination approaches. Furthermore, Dr. Huang will continue to champion the cause of AYA sarcoma research and therapy needs through his involvement as a member of the National Moonshot Initiative Blue Ribbon Panel Working Group on Cancer Immunotherapy & Prevention.