Summaries of UW ICTR Translational Basic & Clinical Research Pilot Awards, 2023

Polarizing Cytotoxic NK Cells for Treatment of Metastatic Osteosarcoma of the Lung
Christian Capitini, MD, UW School of Medicine & Public Health**; Sandro Mecozzi, PhD,** UW School of Pharmacy
Collaborator: Jacques Galipeau, UW SMPH
Co-Funding: Comprehensive Cancer Center
There are limited treatment options for metastatic osteosarcoma (OS), which has a survival rate of 20% despite chemotherapy and surgery. A common site for metastases is the lung, but to date there are no treatments that are curative. Studies for bone marrow transplant (BMT) have not shown benefit for this disease but may provide a platform for infusing adoptive cellular therapy with natural killer (NK) cells. Unfortunately, the OS tumor microenvironment (TME) is immunosuppressive to NK cells in part due to overproduction of the anti-inflammatory cytokine TGF-β. This project will study immunotherapeutics that can potentially enhance the cytotoxicity of NK cells systemically (FIST15) or locoregionally (SIS3) for lung metastases as a means of overcoming the OS TME. Success of either project aim will be a significant advancement to the field of adoptive cellular therapy, and could lead to novel adjuvant therapies for NK cells and ultimately children with metastatic OS.

A human iPSC Disease Modelling Platform for Down Syndrome-related Diabetes
Barak Blum, PhD; Valentina Lo Sardo, PhD, UW School of Medicine & Public Health
Collaborator: Anita Bhattacharyya, UW SMPH
Individuals with Down Syndrome (trisomy of human chromosome 21) are highly susceptible to a unique
form of diabetes, that is both early onset and autoimmune, but is not associated with the genetic variants
typically linked autoimmune (type-1) diabetes in the general population. It is hypothesized that aberrant development of the insulin-producing pancreatic β cells in individual Down Syndrome is at least partly responsible for this unique disease manifestation. Nonetheless, studying the development and causes of Down Syndrome-related diabetes remains difficult due to the lack of reliable animal models and scarcity of patient samples. Recently, Dr. Anita Bhattacharyya has established sets of isogenic trisomy 21 human induced pluripotent stem cell (iPSC) that are identical to each other apart for the presence of an extra copy of chromosome 21. We propose to use these iPSC lines to generate human stem cell-derived pancreatic β (SC-β) cells as an *in vitro* disease model for Down Syndrome-related diabetes. We will further investigate β cell development, maturation, function, response to stress, and immunogenicity of these trisomy 21 SC-β cells. Completion of this proposal will result in a novel SC-β cells disease modelling platform to study the etiology of Down Syndrome-related diabetes in humans, with the goal of preventing this common, yet severely understudied co-morbidity of Down Syndrome.

Dissecting the Role of *Il11* on heart regeneration
Junsu Kang, PhD; Ahmed Mahmoud, PhD, UW School of Medicine & Public Health
Co-Funding: Stem Cell & Regenerative Medicine Center
Foremost goals of regenerative medicine for MI patients are to replenish lost cardiomyocytes (CMs), avoid scar-associated pathology, and restore cardiac function. Although heart regeneration is limited in adult mammals, adult zebrafish and neonatal mice naturally regenerate damaged hearts by triggering regenerative programs via interaction of non-cardiomyocytes and CMs. Identification of essential regenerative factors to stimulate myocardial hyperplasia in regenerative species can hold great potential for addressing human cardiovascular disease. *Interleukin11* (*il11*) is a pleiotropic cytokine exhibiting diverse effects in various tissues, including the heart. In preliminary studies, we identified that *il11a*, one of zebrafish homologs of *Il11*, plays critical roles in zebrafish heart

regeneration. Importantly, *il11a* overexpression (OE) in uninjured hearts can activate CM proliferation and key regenerative events to stimulate myocardial hyperplasia and to vascularize adult cardiac muscle in the absence of injury, implicating *il11* for the potential mitogenic factors of mature hearts. Despite the promising regenerative effects of *il11a*, we also found that long-term treatment of *il11a* can cause fibrosis through the activation of epicardium to generate new myofibroblast. We will determine the mechanisms of *Il11* on neonatal, juvenile, and adult mouse hearts by dissecting their roles in CM proliferation and cardiac fibrosis. Understanding *Il11* biology behind heart regeneration is crucial to develop more efficacious and appropriate therapeutic strategies for heart repair.

Targeted Radionuclide Therapy for Treatment of Leptomeningeal Disease
Zachary Morris, MD, PhD; Mahua Dey, MD, UW School of Medicine & Public Health
Collaborator: Paul Clark, UW SMPH
Co-Funding: Comprehensive Cancer Center
Leptomeningeal disease (LMD) is a challenging form of cancer metastasis involving dissemination of tumor cells via the cerebrospinal fluid (CSF). patients diagnosed with LMD have a very poor prognosis. Currently, external beam radiotherapy (EBRT) targeting the whole brain and/or spine is the only treatment option for most patients with LMD, but this carries considerable risk for toxicities and offers limited efficacy. Targeted radionuclide therapies (TRT) are a growing class of cancer therapies that are injected intravenously and allow selective delivery of radiation to tumor sites throughout the body. Nonetheless, most TRT agents do not cross the blood brain barrier. To overcome this and test an alternative approach to craniospinal EBRT for LMD, we propose to evaluate the application of a TRT delivered directly to the CSF for the treatment of LMD. However, no form of TRT has been tested against disease that is spread via the CSF. For this pilot, we will define a maximum tolerated doses of intrathecally injected 225Ac-NM600 and evaluate acute and late toxicities, comparing mice treated with NM600 or craniospinal EBRT. We will evaluate the efficacy of administering NM600 to the CSF, with the goal of extending survival and achieving complete eradication of LMD in preclinical models of metastatic medulloblastoma and melanoma. We expect that findings from these studies will demonstrate feasibility, safety, and efficacy for intrathecal administration of a TRT agent for the treatment of LMD.

Role of Tumor Microenvironment in the Progression of Chronic Myelomonocytic Leukemia with NRAS and ASXL1 Mutations
Kaylan Nadiminti, MBBS, Jing Zhang, PhD, UW School of Medicine & Public Health
Collaborators: Huy Dinh, Yubin Feng, UW SMPH; Mrinal Patnaik, Mayo Clinic
Co-Funding: Comprehensive Cancer Center
Chronic myelomonocytic leukemia (CMML) is a devastating malignancy with limited treatment options. It
affects mostly geriatric patients who are generally ineligible for intensive chemotherapies and curative
therapies such as allogeneic transplantation. ASXL1 and RAS pathway mutations, particularly NRAS
mutations, are prevalent in CMML. *ASXL1* mutations predict inferior overall survival and are significantly
associated with *NRAS* mutations. Corroborating the human data, Dr Zhang's lab recently reported that *NrasG12D/+; Asxl1-/-* (NA) mice developed CMML with accelerated progression. In this proposal, we will comprehensively characterize the tumor microenvironment (TME) in NA (NA-CMML and NA-sAML) mice (Aim 1) as well as in NA human samples (Aim 2). The proposed studies will capitalize on our novel NA mouse models and unique collection of NA-CMML and RAS;ASXL1-

sAML patient samples. Our results could identify the altered TME cells as potential therapeutic targets/biomarkers for the future development of immunotherapies.

Heavy Metal Toxicants, Microbiome, and Pet Ownership: A Pilot Study
Amy Schulz, PhD, UW School of Medicine & Public Health; **Freya Mowat, PhD**, UW School of Veterinary Medicine
Collaborator: Ajay Sethi, Noah Stafford, UW SMPH
The human gut microbiome impacts multiple health outcomes and is susceptible to influences from environmental factors. Two such potential home environmental factors are (1) exposure to heavy metals (e.g. Pb), which can negatively impact the gut microbiome, and (2) cohabitation with companion animals, which can positively impact the microbiome. Nonetheless, the degree to which companion animals contribute to household metal exposures and the subsequent deleterious effects, and to microbiome diversity/abundance and resultant health promoting effects, has not been studied. The current proposal will leverage data collected in the Survey of the Health of Wisconsin, including human urinary lead concentration and gut microbiome, to investigate the relationship between heavy metals (Pb) and the microbiome in yard soil and indoor dust specimens. Results from this study will help to inform whether companion animal cohabitation may be related to transfer of environmental toxicants and microbiome from outside to inside the home and whether these impact human metal and microbiome exposures. Moreover, the study seeks to understand potential impacts, both positive and negative, of companion animals on human health.

Identifying Biomarkers for Patient-Specific Treatment of Hip Osteoarthritis
Andrea Spiker, MD, Connie Chamberlain, PhD, UW School of Medicine & Public Health
Collaborators: Ray Vanderby, UW SMPH; Peng Jiang, Cleveland State University
Femoroacetabular impingement syndrome (FAIS) is a common cause of hip pain and dysfunction and one of the leading etiologies of early hip osteoarthritis (OA). If FAIS is detected early, hip arthroscopy can be performed to repair the torn labrum, treat loose cartilage, and remove excess impinging bone, thereby arresting the progression of OA. However, many patients with FAIS are beyond the surgical window when hip arthroscopy prevents the OA sequelae, as cartilage damage is present at the time of diagnosis. While currently available drugs may be of symptomatic benefit, they will inadequately prevent OA progression. Understanding the pathologic transcriptomic changes of FAIS-induced OA would better identify therapeutic targets to prevent OA progression. The goal of this proposal is to utilize an FAIS-based, human model to detail transcriptomic regulation of FAIS-induced OA and to identify serum-derived biomarkers that will facilitate strategically focused treatments. The data-driven gene prioritization computational approach using human tissue collected at the time of hip arthroscopy offers an unprecedented opportunity to understand mechanisms associated with FAIS-induced OA tied to serum biomarkers. Successful results will lead to more robust computational studies whereby transcriptional regulators will be correlated with repurposed drugs to optimize currently available treatments for FAIS patients.

Relating Socioeconomic Status and the Built Environment to Health Outcomes though the Human Gut Microbiome
Garret Suen, PhD; UW College of Agriculture and Life Science; **Ajay Sethi, PhD**, UW School of Medicine & Public Health
Greater attention is being given to the roles of structural factors and the built environment (zooming out) as well as mechanistic pathways (zooming in) in understanding how social disparities impact health. For example, the composition of the gut microbiome has been found to be correlated with a wide range of numerous chronic morbidities and poor health outcomes including colonization with antibiotic-resistant pathogens. Few studies have considered how neighborhood-level conditions, as

well as individual aspects of socioeconomic status (SES), impact the composition of the human gut microbiota. Recently, we leveraged a cohort of 721 adult participants of the Survey of the Health of Wisconsin (SHOW) Microbiome Study and found in a cross-sectional study that gut microbiome composition and the presence of antibiotic-resistant pathogens are correlated to community-level SES. Specifically, we showed that low SES correlates with decreased gut microbial diversity and a higher prevalence of colonization by multi-drug resistant microbial pathogens. Here, we propose to extend our study to include an examination of how the built-environment microbiome (BEM), which is known to influence overall health, is related to the correlation we have observed between SES and the human gut microbiome. We expect our findings will help understand environmental influences on the microbiota of more vulnerable populations, providing a framework for interventions to mitigate the SES health gap.

Development and Pilot Validation of a High Sensitive MRM-based Assay for PSMA Detection in Metastatic Prostate Cancer

Shuang (George) Zhao, MD; UW School of Medicine & Public Health; **Yanlong Zhu, PhD**, UW School of Medicine & Public Health

Collaborators: Allan Brasier, UW SMPH

Prostate-specific membrane antigen (PSMA) is a transmembrane protein, upregulated and shed into the plasma in advanced prostate cancers. PSMA is the target of the recently FDA-approved radiopharmaceutical PSMA Lu-177 (Pluvicto). Currently, eligibility for Pluvicto is determined using PSMA PET scans, which cost ~$8K, and only provide qualitative information. Physicians therefore are left to make treatment decisions based on judgement alone. There is a clear unmet need for a PSMA biomarker to predict the benefit for Pluvicto, which costs ~$45K per dose, with patients typically receiving 4-6 doses. Administration of Pluvicto to patients who will not benefit is not only a waste of health care resources, but also deprive patients the opportunity for more effective treatment. To address this problem, we will optimize and test a selective multiple reaction monitoring (MRM) assay for the detection of PSMA in plasma. As an antibody-independent, specific and highly reproducible assay, MRMs have significant, but not fully realized, translational potential for application in the clinic.