NEW INVESTIGATOR AWARDS

Emanuele Azzoni, PhD
University of Milan-Bicocca – Monza, Italy
$100,000.00 – ***Generating a New Model of Juvenile Myelomonocytic Leukemia to Study its Cellular Origins and to Identify Therapeutic Vulnerabilities***

Juvenile myelomonocytic leukemia (JMML) is a serious chronic blood cancer affecting young children of age 4 or less. It occurs in about 1 in one million children, and as such it represents the most common non-acute pediatric blood cancer. Without treatment, this disease is rapidly fatal. Currently, the only cure is stem cell transplantation, also known as bone marrow transplant. However, compatible donors are not always available, and there is still a high risk of relapse (approximately 50%). Research has studied the genetics of this disease, but this has not yet translated in new cures for the patients. Unfortunately, there is still a lot that we don't know about how this disease arises and progresses. JMML has a pre-natal origin, meaning it can already begin during gestation, and this makes it very difficult to study. This is especially true because blood cells in the fetus are fundamentally different from those in the adult. For these reasons, it is particularly important to create disease models that can allow researchers to study how leukemic cells arise, and learn how to kill them. In this project, we will generate a new model of JMML which will enable us to identify its cellular origins; in other words, we will identify the cells that will eventually become leukemic. We will search for the factors that can permit this malignant transformation, and we will use this information to search for the "Achilles heel" that could make JMML cells more vulnerable to therapies.

George Burslem, PhD
University of Pennsylvania – Philadelphia, Pennsylvania
$100,000.00 – ***Protein Degradation Induced Epigenetic Reprograming as a Therapeutic Strategy for MLL-r Leukemias***

Many cancers result from a genetic mutation causing an "always on" protein. Current treatments are based on the de-activation of the proteins by blocking that protein's active site. Herein I propose an alternative approach in which proteins are permanently degraded rather than temporarily deactivated, which may prove to be a more favorable form of therapy. To do this, I will take advantage of the cell's own natural ability to degrade proteins when they are in excess or no longer needed. I will design and prepare compounds which recruit the native protein degradation machinery to the target proteins by creating a bridge between protein degradation components and the target protein. We propose to prepare compounds capable of the degradation of target proteins involved in leukemia. Specifically, we focus on PRMT1, which is a protein important in acute myeloid leukemia (AML), especially in children. We will assess these compounds for the ability to degrade target proteins in cell-based models. It is conceivable that by employing protein degradation, it may be possible to completely remove all disease-causing protein and restore cancerous cells to their normal pathway. The ultimate goal is to produce a drug that may be useful for the treatment of AML while simultaneously increasing our understanding of what causes cancer.

Matt Christopher, MD, PhD
Washington University – St. Louis, Missouri
$100,000.00 – ***Resensitizing Relapsed AML Cells to the Graft-versus-Leukemia Effect after Allogeneic Hematologic Stem Cell Transplantation (HCT)***

One of the most effective treatments for acute myeloid leukemia is allogeneic hematopoietic stem cell transplantation (formerly called "bone marrow transplant.") About 3000 patients receive these treatments each year. Unfortunately, transplants only cure patients about 40% of the time, and the main reason why transplantation fails is that leukemia relapses after the transplant. A few years ago, our group performed a study of patients who relapsed after stem cell transplant. We wanted to discover what mutations or other genetic changes might happen that allows the leukemia cells to relapse. We observed a number of changes in the expression of genes that are involved in the immune system. This was intriguing, because one of the ways that transplants are believed to work is through

immune pressure from the donor immune system that suppresses leukemia and the recipient. One of the important mechanisms by which donor immune cells are able to kill AML cells is by recognizing molecule called MHC class II (MHC-II) that is found on the AML cells. We discover that in 1/3 to 1/2 of cases of AML relapse after transplant, MHC-II expression has been completely lost on the relapsing AML cells. We hypothesize that without MHC-II, AML cells are no longer recognized by donor immune cells and this leads to transplant. In this grant proposal, we will uncover mechanisms by which AML cells lose MHC-II expression. In addition, we will test a new drug, a long-acting form of interferon gamma.

Leslie Crews, PhD
University of California, San Diego – La Jolla, California
$100,000.00 – ***The Role of Inflammation-Responsive R-spondin Receptor Activation in Multiple Myeloma Stem Cell Generation***

Multiple myeloma (MM) is the second most common blood cancer in the United States with over 32,000 new cases estimated in 2021. With a five-year survival rate of approximately 50%, more specific and effective prognostic tools and therapies for this disease are a vital unmet medical need for the >125,000 MM patients in the U.S. Myeloma remains incurable as a result of drug-resistant dormant cancer stem cells in the bone marrow that contribute to disease progression mediated by inflammatory cues. Currently, no clinical therapies target cancer stem cells in MM, in part because the identifying features of myeloma stem cells in the bone marrow remain elusive. The overall goal of this project is to explore a novel myeloma stem cell biomarker that may improve our ability to selectively identify and inhibit cancer stem cell generation in MM. The proposed biomarker, known as leucine-rich repeat-containing G protein-coupled receptor-4 (LGR4), is present on the surface of some myeloma cells but not normal B cell types. We predict that LGR4 could be exploited in the targeted detection and eradication of malignant myeloma stem cells. In this context, this New Investigator grant proposal aims to determine the extent to which inflammation-responsive activation or inhibition of LGR4 expression controls emergence of drug-resistant myeloma stem cells. The proposed research will set the stage for future clinical translation of more selective therapies for MM.

Ernesto Diaz-Flores, PhD
University of California, San Francisco – San Francisco, California
$100,000.00 – ***Validating Targeted Therapies with Curative Potential against High-Risk Childhood Leukemia***

Acute lymphoblastic leukemia is the leading cause of cancer mortality in children and young adults. Notably, almost 10% of children and adolescents cancer patients present germline predisposition mutations. Li-Fraumeni Syndrome (germline mutations in TP53), was reported the most common cancer predisposition syndrome in the Pediatric Cancer Genome Project. Low hypodiploid B-cell lymphoblastic leukemia (LH B-ALL), an aggressive leukemia characterized by mutations in TP53 in more than 90% of patients, of which nearly 50% represent Li-Fraumeni syndrome results in 70% mortality compared to 15% for B-ALL patients overall. Aiming to turn this aggressive disease into one with a much-improved prognosis, we investigated therapeutic targets specific to hypodiploid leukemia with p53 mutations. Importantly, TP53, the most commonly mutated gene in cancer is especially prevalent at relapse after aggressive therapies. We exposed LH-B-ALL patient samples to APR-246, a drug targeting the p53, already in use in late phase clinical trials. These studies revealed complete induction of cell death in LH-B-ALL by APR-246. Moreover, gene expression analyses indicated that cells expressing mutant TP53 present overexpressed surface proteins. I hypothesize that hypodiploid leukemia with mutant TP53 can be selectively identified and targeted. I, thus, propose two therapeutic opportunities (pharmacological and immunotherapy) to reverse the dim prognosis for patients with LH-B-ALL harboring p53 mutations.

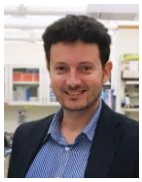
Cihangir Duy, M.S., PhD
The Research Institute of Fox Chase Cancer Center – Philadelphia, Pennsylvania
$100,000.00 – ***Targeting LSD1 in Senescence-like Resilient AML Cells***

Despite many advances in AML therapy and initial response to treatment, most patients will eventually relapse and succumb to the disease. There is a significant need to better understand the mechanisms that enable relapse of AML for developing novel therapeutic interventions. We recently discovered that AML cells enter a senescence-like state in response to chemotherapy. This process facilitates survival of AML cells by allowing them to endure chemotherapy in a transiently dormant state while retaining the potential to repopulate leukemia. It is important to target these senescence-like dormant AML cells as they represent a pool of leukemia cells

capable of initiating relapse. Hence, it is critical to identify key factors and understand the biology underlying the senescence-like leukemia cells. We identified a role of LSD1 in the senescence-like cells and will determine the functional consequence of LSD1 deficiency. Completion of this proposal will contribute to a better understanding of mechanisms regulating the senescence-like resilient phenotype in AML, which can provide a basis for future therapeutic interventions.

Ann-Kathrin Eisfeld, MD
The Ohio State University – Columbus, Ohio
$100,000.00 – ***Understanding Poor Survival and Treatment Resistance in Young Black NPM1-mutated Patients with Acute Myeloid Leukemia***

Acute myeloid leukemia (AML) is an aggressive blood cancer, of which only ~25% of patients are currently cured despite modern therapies. It became clear over the past years that the cancer cells of leukemia patients respond differently to available treatments based on differences in the molecular underpinnings of the cells. We recently performed the thus far largest study that examined the outcomes of Black patients diagnosed with AML compared to White patients with similar characteristics. Notably, both in a population-based study and in the setting of clinical trials, Black patients fared worse with ~40% higher chances to die from their disease compared to White patients. Unexpectedly, the survival discrepancy was highest in the molecular "favorable" risk group of patients harboring gene mutations in a gene called NPM1, thereby suggesting differences in disease biology that affect treatment response, and specifically the ability of standard chemotherapy to eradicate the leukemic clone based on the constitutional contributors associated with a patient's race. Our proposal is designed to further understand the underlying mechanisms for the survival discrepancy and treatment response specifically in the "favorable" risk group, based on the overarching hypothesis that race-associated genetic features cause differential molecular/pathologic responses to treatment in Black and White AML patients.



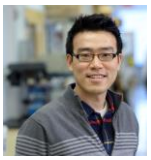
Pietro Genovese, PhD
Dana-Farber Cancer Institute – Boston, Massachusetts
$100,000.00 – ***Generation of "Stealth" Tyrosine Kinase Receptors for an Immunotherapy Resistant Hematopoiesis***

Acute myeloid leukemia (AML) is the most common leukemia in the adults and the second in childhood. Yet, despite recent advances and diffuse use of therapeutic bone marrow transplantation, AML is still associated with a dismal outcome for over 50% of affected patients. Innovations in gene engineering have made it possible to reprogram immune cells to attack specific targets on cancer cells, allowing the first adoptive cellular immunotherapies, known as CAR T cells, to be approved by the FDA for the treatment B lymphoblastic leukemia. A similar approach is currently under development for AML, but in contrast to B-ALL, there is no leukemia-specific target which would be amenable to targeting by immune cells without incurring in severe adverse effects. This is due to the great similarity between leukemia cells and normal bone marrow stem cells, which would be killed along with the targeted tumor cells, leading to anemia, low white blood cells, low platelets and susceptibility to infections and hemorrhage. To create a new treatment strategy for high risk AML patients, we aim to modify normal bone marrow stem cells used for allogeneic transplantation in order to make them resistant to targeted therapies such as CAR-T cells. This would allow the administration of highly effective immune-therapies targeting a protein essential for tumor survival without the risk of severe toxicity on the healthy tissue counterpart.

Md Kamrul Hasan, PhD
University of California, San Diego – La Jolla, California
$100,000.00 – ***Investigating the Role of DOCK2 in Cells Expansion, Drug Resistance and Pathogenesis of Chronic Lymphocytic Leukemia***

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia in adults' accounts for over 20,000 new cases every year in the United States and 37% of all newly detected leukemia cases. CLL is characterized by the accumulation of B cells (a type of white blood cell that express B-cell receptor (BCR)) in the bone marrow and lymphoid tissues (e.g. spleen). DOCK2 is a cytoplasmic protein of hematopoietic cells (blood cells), and can directly activate Rac1/2. Wnt5a is a growth factor found at high levels in the plasma of patients with CLL, and a ligand (have high affinity) of ROR1, which is present on the surface of CLL cells. Recently, we described that Wnt5a can induce ROR1/DOCK2 association, activate Rac1/2 or ERK1/2 and promote leukemia-cell proliferation (increase cell number/growth), and could be inhibited by cirmtuzumab (anti-ROR1 antibody), which is under phase-II clinical trial to treat CLL patients (Hasan et al., Blood, 2018; Leukemia, 2020). Moreover, an FDA approved front line therapies to treat CLL patients

such as ibrutinib (BTK inhibitor) that could inhibit BCR signaling, was unable to block Wnt5a enhanced activation of DOCK2 or ERK1/2, suggesting potential drug resistance in CLL therapy. Here, we will examine the molecular events of activation of DOCK2 or ERK1/2 in CLL. This study will provide better understanding of DOCK2 protein in CLL cells expansion and pathogenesis, and suggest new therapeutic strategies to treat CLL.



Sangmoo Jeong, PhD
Johns Hopkins University – Baltimore, Maryland
$100,000.00 – ***Identify Metabolic Vulnerabilities in Leukemic Stem Cells***

One of the main challenges in curing acute myeloid leukemia (AML) is the recurrence of disease, which occurs in about 50% of patients. A 5-year survival rate after the recurrence is less than 20%. It is now widely accepted that a small, distinctive population of malignant cells, called leukemic stem cells (LSCs), is responsible for disease relapse and drug resistance. Therefore, there has been a significant interest in identifying and developing therapeutic methods targeting LSCs. Recently, multiple groups have started investigating the metabolic vulnerabilities of LSCs, as they have been shown to exhibit unique metabolic phenotypes. Still, our research progress has been limited, mainly due to a lack of experimental tools that model the tumor metabolic environment. In this Proposal, my lab aims to address this problem by developing a powerful bioreactor system and identifying the unique metabolic characteristics of LSCs. Specifically, we will design a multi-well plate format bioreactor that does not require any complicated and expensive components while providing in vitro experimental conditions that mimic the leukemic metabolic environment. With this innovative model system, we will investigate the mitochondrial metabolism of leukemic cells and identify which pathways are essential for LSCs. Our engineering platform and biological findings would help us find a more effective treatment for this devastating disease.

Huacheng Luo, PhD
Pennsylvania State University College of Medicine – Hershey, Pennsylvania
$100,000.00 – ***The N6-Methyladenosine (m6A) of HOTTIP lncRNA Coordinates with CTCF Mediated TAD Boundary in AML Leukemogenesis***

Acute myeloid leukemia (AML) is an aggressive and fatal hematologic malignancy characterized by malignant transformation of myeloid cells. The development of AML is associated with accumulation of acquired genetic and epigenetic events in the differentiation of hematopoietic stem/ progenitor cells (HSPCs). HOX loci- associated long noncoding RNA (lncRNA), HOTTIP, is specifically up-regulated in MLLr+ AML patients that account for up to 50% of infant and 10% of adult acute leukemia with very poor prognosis. The m6A writer complex METTL3/METTL14 is required for development and maintenance of AML and self-renewal of leukemia stem/initiation cells (LSCs/LICs). Consistent with our findings that HOTTIP is overexpressed in MLLr+-driven AML, both METTL3 and METTL14 are overexpressed in AMLs, especially in MLL+ pediatric AML. Here, we found that AML blasts express higher mRNA levels of METTL3 and METTL14 than normal cells, and expression levels of METTL3 or METTL14 are correlated with poorer prognosis in AML patients. Moreover, the expression of METTL3 or METTL14 has a positive correlation with the expression of HOTTIP in AML patients from TCGA datasets. In this proposal, we will explore the role of m6A modification of HOTTIP lncRNA in hematopoiesis and leukemogenesis, and we anticipate that our studies will identify novel therapeutic targets for the treatment of AML disease.

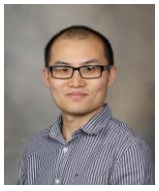
Mithun Shah, MD, PhD
Mayo Clinic – Rochester, Minnesota
$100,000.00 – ***Predicting and Preventing Therapy-related Myeloid Neoplasm in Multiple Myeloma Patients Undergoing Stem Cell Transplantation***

Therapy-related myeloid neoplasm (t-MN) is a leukemia that develops following DNA-damaging therapies. t-MN is one of the most aggressive malignancies with no effective therapies, making early prediction and prevention critical. Multiple myeloma (MM) patients who develop t-MN after undergoing stem cell transplantation (SCT) provide a unique insight into t-MN pathogenesis. MM patients are at 12-fold higher risk of t-MN compared to general population, suggesting genetic predisposition. This risk increases to 100-fold in patients undergoing SCT, suggesting that selection pressure exerted by chemotherapy also contributes to t-MN. We performed targeted sequencing and methylation analysis using paired samples obtained pre-SCT and at t-MN diagnosis and showed that those

that develop t-MN (cases) have a unique genetic and methylation profile compared to matched-controls years prior to developing t-MN. Whether pharmacological intervention targeting these differences can prevent and treat t-MN is not known. In aim 1, we will investigate if hematopoietic stem cell (HSC)-intrinsic factors such as TP53-defiient state and spindle assembly checkpoint (SAC) may be present years prior to leukemic transformation, and whether SAC inhibitors can be used for prevention or therapy. In aim 2, we will study how melphalan, directly or by increasing 8-oxoguanine incorporation, predisposes to chromosomal breaks. We will study if MTH1 inhibitor, by reducing 8-oxoG incorporation prevents t-MN.

Alexander Valvezan, PhD
Rutgers, The State University of New Jersey – Piscataway, New Jersey
$100,000.00 – ***Repurposing IMPDH inhibitors for Selective Targeting of PTEN-deficient T-ALL Cells***

This project will test a new strategy for selectively killing a molecular subtype of T-cell acute lymphoblastic leukemia (T-ALL) using drugs that are already being used in humans as safe and well-tolerated immunosuppressants. This strategy exploits a molecular vulnerability that I recently discovered in tumors with abnormal activation of a critical group of proteins called mTOR complex 1 (mTORC1). mTORC1 is activated to promote cell growth and proliferation in the majority of human cancers. I discovered that tumor cells with active mTORC1 could be selectively killed, without affecting normal cells, in pre-clinical models of a genetic tumor syndrome called Tuberous Sclerosis Complex (TSC), in which tumor growth is driven by high mTORC1 activity. Due to key molecular similarities between cells in TSC tumors and T-ALL, I hypothesize that the same strategy could be effective in killing T-ALL cells in which mTORC1 is activated by specific genetic mutations which are common in leukemia and lymphoma. These studies will rigorously test this hypothesis in T-ALL cells, laying the foundation for in vivo testing of these drugs alone and in combination with current standard-of-care therapies to provide the pre-clinical rationale for clinical trials in patients with T-ALL. The use of clinically approved therapeutics means that efficacy in these studies could lead to rapid repurposing of these drugs for leukemia patients.



Zhiquan Wang, PhD
Mayo Clinic – Rochester, Minnesota
$100,000.00 – ***Exploring an NFATc1-Dependent Epigenetic Program as a Determinant of BTK inhibitor Treatment in B-Chronic Lymphocytic Leukemia and other B-cell Malignancies, including Non-Hodgkin Lymphomas***

CLL is the most common leukemia in the U.S. with ~21,000 new cases diagnosed each year. Although CLL is considered an indolent cancer with a median survival of ~10 years, it has a heterogeneous clinical course. About 5% of the CLL cases have a very aggressive disease at diagnosis with a 5-year survival of 14-19%; whereas 15-20% of the CLLs have a 5-year survival of 68-72%, and the remaining CLL cases have over 90% 5-year survival. MCL is an uncommon but aggressive subtype of B cell malignancy that comprises 3% to 6% of non-Hodgkin lymphomas, with an annual incidence of 0.5 per 100,000 population in Western countries. Despite the improved clinical outcomes Bruton's tyrosine kinase BTK inhibitors (BTKi, e.g., Ibrutinib, Acalabrutinib, Zanubrutinib), disease progression is an emerging therapeutic challenge in treated patients. Therefore, there is an urgent need to develop more effective therapies to maintain and enhance BTKi treatment among patients whose tumors develop resistance to BTKi. We will focus on CLL as a model to elucidate the mechanism underlying malignant B-cell survival and BTKi therapeutic efficacy. These mechanistic studies will also include MCL. Most importantly, we will test if our discovery can be exploited to improve BTKi efficacy and/or overcome drug resistance during BTKi treatment in B-cell malignancies. Together, successfully completing the proposed studies will lay the foundation for subsequent manipulations that will enhance B-cell malignancy therapies.