NEW INVESTIGATOR AWARDS

Ioannis Aifantis, Ph.D.
The University of Chicago, Chicago, IL
$75,000.00 – ***Cooperation of Notch and pre-TCR Signaling in the Induction of T Cell Leukemia***
The pre-T Cell Receptor (pre-TCR) is a receptor expressed during the early developmental stages of T cells in the thymus. It's signaling is essential for the maturation of T cells as it induces proliferation and differentiation of immature T cell progenitors. One of the main functions of the pre-TCR is the induction of cell survival as cells unable to express the receptor die by programmed cell death. However, the mechanisms of the pre-TCR mediated survival are poorly understood. In this proposal we initially attempt to identify the molecular mechanisms responsible for the induction of survival of pre-T cells. To achieve that, we will characterize the nature of pre-TCR derived anti-apoptotic signals and in particular, the expression pattern and the function of the pre-TCR induced pro-survival protein BCL2A1. We are also connecting constitutive pre-TCR signaling inducing abnormal survival of pre-T cells to the induction of T cell acute lymphoblastic leukemia (T-ALL). This experimental aim is based on recent observations on the genetic characterization of human and murine T-ALL models suggesting that the pre-TCR plays a key role in the generation of T cell malignancy. To study the role of the pre-TCR in leukemogenesis we will a) address the role of pre-TCR target-genes regulators of both survival and proliferation and b) study the role of the unique pre-TCR structure, expression pattern and signaling properties in the induction of T cell leukemia. The proposed experiments will identify the specific molecular mechanisms regulating early T cell survival and pinpoint pre-TCR-induced signaling pathways responsible for the malignant transformation of pre-T cells.

Michele K. Anderson, Ph.D.
Sunnybrook and Women's College Heath Sciences Centre
$66,500.00 – Roles ***of HEB Transcription Factors in T Cell Development and Leukemia***
Leukemias often arise from the inappropriate activity of transcription factors, which are normally responsible for controlling the expression of genes involved in both function and cell growth. We have isolated a new transcription factor, HEBAlt, that is expressed in developing T cells at the time that they are most prone to become cancerous. Our preliminary experiments indicate that HEBAlt can interfere with the function of the anti-oncogene E2A. We propose to make HEBAlt knockout mice to test the normal function of HEBAlt in T cell development, and to make another strain of transgenic mice which express HEBAlt but not HEBCan. HEBCan is a transcription factor that works with E2A in developing T cells in limiting cell growth. These mouse models will enable us to test the suspected ability of HEBAlt to promote leukemia in T cell precursors, and may provide access to new molecular markers or therapeutic targets in the treatment of T-cell leukemia.

Stacy Blain, Ph.D.
Suny Downstate Medical Center Health Science Center at Brooklyn
$75,000.00 – ***The Role of p27Kip1-Cdk4 Inhibition and Activation in Leukemia and Lymphoma***
Cells divide in order to make duplicate copies of themselves during the growth of the organism, following injury to regenerate the lost tissue, or during normal processes such as an antibody response when we are ill. But, cells also rest and are maintained in a quiescent or silent state until needed. For example, lymphocytes circulate in our blood in a resting phase. When we become ill, the lymphocyte must leave the quiescent state and duplicate itself to produce enough antibodies to attack the infection. Obviously, the decision to divide or rest is tightly controlled, as the consequence would be too much antibody during periods of health, or too few antibodies during infection. A group of proteins called the cyclin-cdks coordinate this cell division process. These proteins sense signals from both inside and outside the cell and use this information to decide whether it would be safe and/or beneficial for the cell to divide, or better for it to rest. These proteins have the ability to stop cell division if they sense a problem, such as environmental stress or a mutation in the cell's DNA. Leukemias or lymphomas arise when this tightly regulated control system is perturbed. Cells no longer

interpret signals to stop dividing, and the lymphoid cells continue to grow. The cyclin-cdk proteins are part of the "Rb pathway", which is destroyed in almost all human cancers. Thus, the study of this pathway will shed light on proteins, which are mutated in cancer, as well as offer potential diagnostic, prognostic, and therapeutic targets. In a normal cell, the activity of the cyclin-cdks is tightly regulated by several mechanisms, including interaction with p27Kip1. p27 itself appears to be lost in many types of cancer, including lymphomas and leukemias, and this loss correlates with aggressive cancers and poor prognosis. Thus, it is clear that p27 and the cyclin-cdks are involved in cancer progression, and understanding how they are activated and regulated is imperative to understanding their role in the development of leukemia and lymphoma. It appears that p27 sometimes blocks the activity of one of the cdks in the "Rb pathway", cdk4. Alternatively, p27 sometimes activates cdk4, and this ability to turn the complex "on" or "off" is used by the cell in its decision to divide or rest. In a lymphoma or leukemia cell, the p27-K4 complex is permanently locked into an "on" mode, and the cells divide without stopping. Our research project will determine why p27 is a cdk4/6 inhibitor under certain conditions, but not others. Elucidation of the signals that turn this complex on or off should shed light on the development of lymphoma and leukemia, and may offer potential therapeutic targets for intervention.

Gino Cingolani, Ph.D.
Suny Upstate Medical University, Syracuse
$74,620.00 – ***Molecular Characterization of Epstein-Barr Virus BBRF1 Gene Product: A Putative Portal Protein***
Epstein-Barr virus is a widespread herpes virus, which infects about 90% of the adult human population causing a variety of diseases, from infectious mononucleosis to deadly leukemia. We have identified *in silico* a viral gene that encodes a protein, pBBRF1, which we believe to be essential for virus infectivity. In analogy to simple viruses infecting bacteria, pBBRF1 is expected to be located at single vertex of the EBV capsid and drive movement of the viral genetic material in and out of the capsid. Our goal is to clone the gene encoding pBBRF1 and to over-express it in bacterial cells. Such approach will allow to purify a large amount of recombinant pBBRF1, which will be used to characterize the biochemical and biophysical properties of the molecule as well as to determine the three-dimensional structure of the protein by X-ray crystallography. These studies will open the doors to future pharmacological applications aimed at synthesizing inhibitors that block EBV infection by targeting an essential component of the viral machine.

Andrew Herr, Ph.D.
University of Cincinnati
$74,347.76 – ***Laying the Groundwork for Design of FcaRI-Activating IgG Anti-Tumor Antibodies***
The human immune system uses proteins called antibodies to specifically seek out germs and attract immune cells to destroy the germs. In the last several years, antibodies have been used to fight tumor cells in certain types of leukemia and breast cancer. These treatments show a lot of promise; however, the most commonly used antibody type, IgG1, has certain limitations. One important limitation is that IgG1 antibodies are able to recruit only half of the immune cells in the blood. Another type of antibody, IgA, is able to activate those immune cells that do not respond to IgG1. The Principal Investigator has used the technique of protein crystallography to determine how an IgA antibody interacts with its receptor (called Fc‹RI) on immune cells. This finding reveals precisely the amino acid building blocks in IgA that are involved in attaching to the receptor and activating the immune cell. Using this information, we intend to transplant the amino acid residues that IgA uses to interact with Fc‹RI onto an IgG1 antibody. This will create a hybrid antibody that should be able to activate nearly all immune cells in the blood, leading to more effective killing of tumor cells. This proposal covers the first stage of the project, in which we will alter individual amino acids in IgA and measure how tightly the mutated IgA can interact with Fc‹RI. We need to know which amino acids are the most important before we can transplant the IgA amino acids onto an IgG1 antibody.

Wladyslaw Krajewski, Ph.D.
Institute of Developmental Biology, Russian Federation, Moscow
$49,500.00 – Interaction *of MLL and Related SET-Domain Proteins With Active Chromatin Structures*
A typical human cell contains more than 30,000 genes, however only a few percent of them work in each particular cell. It is essential not only to keep the right genes on but also to keep the right genes off. The Polycomb and trithorax groups of proteins are the cell components that ensure correct states of gene activity. In general, Polycomb proteins stably repress genes in cells where these genes must remain inactive, while trithorax proteins maintain active gene states. Mistakes of this system have severe consequences. For example, misregulation by MLL, the human trithorax group protein, results in aggressive lymphoid and myeloid acute leukemias in both children and adults. The molecular principles of these events are essential not only for therapy of leukemias, but also for understanding the basics of tumorigenesis in general. However, this knowledge is limited. The Polycomb and trithorax group proteins are present in all cells but they selectively repress or activate each particular gene only in cells descended from cells in which such repression/activation was initiated in the early development. We propose a set of experiments designed to approach molecular mechanisms of how the Polycomb and trithorax proteins can recognize such predetermined gene activity states, to access if these proteins can selectively recognize and bind specific DNA structures, typical for active genes, whether the occurring *in vivo* unusually structured DNAs can participate in such regulation.

Chengming Zhu, Ph.D.
Anderson Cancer Center, Houston, TX
$75,000.00 – *Role of ATM in Lymphocyte Development and Tumor Suppression*
In a normal cell, genetic information carrying molecules, DNA, is condensed and packed into a structure called chromosome, and the entire set of chromosomes in a cell is genome. To pass all the genetic information to next generation, each cell must keep the stability of its genome: to faithfully replicate the entire sets of DNA. However, cells suffer DNA damage constantly, from exposure to damaging agents or from normal physiological programs. If damaged DNA is not properly repaired, an altered genome maybe passed to next generation, the phenomena we describe as genomic instability, it is a hallmark of all cancers, including leukemia and lymphoma. During the development of lymphocytes, specialized cells for fighting against infections, a DNA “cutting and paste” process must occur according to their developmental program. If this process is incomplete or not properly controlled, genomic instability occurs and subsequently results in lymphoma. This proposed research uses genetically made deficient mice as animal models, to define molecules that are important for controlling this process, to study how genomic instability leads to lymphoma, and how lymphocytes maintain their genomic integrity and prevent tumor.

POSTDOCTORAL FELLOWSHIP RESEARCH GRANTS

Silvia Buonamici, D.SC., Ph.D.
UIC School of Medicine, Chicago, IL
$60,000.00 – ***The Role of EVI1 in the Pathogenesis of Myelodysplastic Syndromes (MDS)***
The myelodysplastic syndromes (MDS) are a group of blood cancers that originate in blood-forming cells in the bone marrow. In patients with MDS, the marrow produces insufficient red blood cells, white blood cells and often platelets. For most MDS patients there is no cure and the only available therapy is supportive transfusion with blood products. In about 10% of MDS patients there is an abnormal expression of a gene called EVI1, the role of which is unknown. EVI1-positive MDS patients have a very poor prognosis. To better evaluate the role of EVI1, we generated a mouse model that recapitulates the development and progression of human EVI1-positive MDS. EVI1-positive mice have insufficient red blood, white blood cells, and abnormal platelet counts as observed in MDS patients. Analyses of the data from the mouse model show that the inappropriate expression of EVI1 inhibits the activity of certain genes that are important for normal blood formation. The goals of this project are to understand the mechanism by which EVI1 inhibits the activity of these genes and to develop a new treatment strategy that can reverse the effects of EVI1 in MDS patients.

Mwe Mwe Chao, M.D.
University of Michigan, Ann Arbor, MI
$60,000.00 – ***Mechanisms of MLL-CALM-Mediated Leukemic Transformation***
Acute myeloid leukemia (AML) accounts for 15-25% of childhood leukemias. Despite increased understanding of the causes of AML, fewer than half of children with this disease are cured. Chromosomal translocations involving the **M**ixed **L**ineage **L**eukemia (*MLL*) gene are observed in a significant number of AML cases and its presence confers a particularly poor prognosis. Over 50 genes fuse with *MLL* and previous studies suggest that *MLL* fusion partners are crucial for the development of leukemia. We recently identified the **C**lathrin **A**ssembly **L**ymphoid **M**yeloid Leukemia (*CALM*) gene as a novel partner for *MLL* in an infant with fatal AML. The CALM protein participates in internalizing cell surface signaling molecules, a process that turns off growth signals. The goal of this proposal is to characterize the mechanisms by which MLL-CALM causes AML. We will determine whether expression of MLL-CALM in mouse blood cells results in the development of leukemia. Next, we will determine whether CALM alters the genes normally regulated by MLL. Finally, we will explore the possibility that disruption of normal CALM function results in a failure to inactivate growth signals. Through these studies we will better understand mechanisms associated with poor prognosis leukemias, which may translate to improved therapy.

Hong Chen, Ph.D.
University of Florida College of Medicine
$60,000.00 – ***Reversal of Asparaginase Resistance by RNA Interferance***
Acute lymphoblastic leukemia (ALL) accounts for approximately 30% of newly diagnosed childhood cancer. Although the initial remission rate has now reached 80-90%, 20-30% of the children suffer relapse and often drug resistance occurs. One of the many drugs used in ALL treatment is the enzyme asparaginase (ASNase). Although the basis for drug resistance in ALL children is not well understood, it has been documented that ASNase resistance is associated with increased production of another enzyme called asparagine synthetase (ASNS). The proposed research is designed to further elucidate the mechanisms involved in ASNase-resistance using a model system involving a leukemia cell line in laboratory cultures. The pathways responsible for acting on the ASNS gene after ASNase therapy will be investigated. A thorough understanding of the molecular mechanisms regulating ASNS expression by ASNase treatment will lead to improved drug treatment for ALL patients. This research project is also designed to apply the knowledge gained from the basic research to use a gene therapy approach to block the increased expression of ASNS and thereby, reverse ASNase resistance in leukemia cells. By doing so, we maintain the efficiency of ASNase-related therapy. The therapeutically useful molecules tested in these gene therapy studies will provide new strategy treating childhood ALL patients.

Kim Rice, Ph.D.
Mount Sinai School of Medicine
$60,000.00 – ***Identification of Genetic Targets of the Promyelocytic Leukemia Zinc Finger***
The abnormal growth of the immature white blood cells characteristic of leukemia is due in many cases to the creation in the leukemic cell of an abnormal gene regulator that causes a disruption in the normal pattern of cell differentiation. One such regulator, studied in the mentor's lab, is called PLZF. PLZF is affected in an unusual form of acute promyelocytic leukemia, although developing evidence suggests it may be malfunctioning in other cases of leukemia as well. We believe that the PLZF gene suppresses cell growth by regulation of a network of other genes. I intend to identify these genes using Affymetrix gene chips which measure the levels of thousands of genes at the same time. In addition, I will directly isolate fragments of DNA bound by the PLZF protein as well as by a mutant form of the protein called RAR-PLZF found in promyelocytic leukemia. The identification of PLZF target genes may lead to significant insights into the mechanism by which aberrant transcriptional regulation, a common theme in many leukemias, may perturb cellular differentiation leading to the development of leukemia. Understanding how the gene networks of the white cell are shuffled in leukemia may allow for the identification of new proteins to target with drugs with the eventual aim of increasing the cure rate of leukemia.