



Leukemia Research Foundation

2006-2007 Scientific Research Grant Recipients

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NEW INVESTIGATOR AWARDS

Paul Andreassen, PhD

Children's Hospital Medical Center of Cincinnati

\$100,000.00 - Functional Analysis of the Interaction of BRCA2/FANCD1 with Monoubiquitinated FANCD2

Fanconi anemia (FA) is a rare, inherited disease that is associated with a strong predisposition to leukemia. Importantly, both FA and cancer are characterized by an elevated rate of rearrangement of the genetic material (DNA). Thus, the study of Fanconi anemia serves as a critical model for the mechanisms involved in this process. The proteins made from the many FA genes are organized into a biochemical pathway that leads to the chemical modification of another FA protein, FANCD2, by a process termed monoubiquitination. Among the FA proteins, BRCA2/FANCD1 has a defined role in the repair of DNA damage. We have shown that monoubiquitinated FANCD2 interacts with BRCA2/FANCD1 and localizes it following DNA damage. We aim to understand whether this interaction is critical to the collective function of all of the FA genes in preventing the genetic alterations found in leukemia. Results from this study should lead to a better understanding of the cellular defects which underlie leukemia. Further, this could lead to a potential screen for cases of sporadic leukemia which may be susceptible to treatment with bifunctional alkylating agents, a group that is utilized extensively in chemotherapy.

Jan Burger, MD, PhD

MD Anderson Cancer Center, University of Texas

\$100,000.00 - *Anatomy of the microenvironment of CLL: focus on the chemokine CXCL13*

Chronic lymphocytic leukemia (CLL) represents the most common type of adult leukemia in western societies and is characterized by the relentless accumulation of neoplastic B lymphocytes (CLL cells) in the blood, lymphatic tissues, and the bone marrow. Despite major advances in treatment, CLL currently is considered incurable with standard treatments and new therapeutic approaches therefore are urgently needed. Unlike normal B lymphocytes, CLL cells are long lived and gradually accumulate in the patient not because they grow faster, but because they survive longer. However, when CLL cells are removed from their microenvironment in patients and placed into cell culture, they rapidly undergo cell death unless they are cultured in contact with "feeder" cells. Therefore, it is hypothesized that contact with "feeder" cells is essential for growth and survival of CLL cells. A variety of studies suggested a particularly important role for feeder cells termed "follicular dendritic cells" (FDC), "marrow stromal cells" (MSC), and "nurselike cells" (NLC) in regulating CLL cell growth and/or survival. FDC are considered the major source for the chemokine CXCL13, which attracts normal B cells for migration to lymph nodes. In the lymph nodes, CXCL13 helps to tether B cells to the FDC "feeder" cells. Due to its high activity to attract cells, in particularly B cells, this factor is termed B lymphocyte chemoattractant or chemokine, and it acts through cognate receptors termed chemokine receptors. We recently found that CLL cells have high levels of CXCR5 chemokine receptors for CXCL13. We also found that CXCL13 attracts CLL cells through CXCR5. We now propose to examine the role of CXCR5 in CLL cell interactions with different feeder cells. CLL cell co-cultures with such accessory cells will allow us to study and manipulate interactions between these cell types related to the CXCL13-CXCR5 axis. We will also determine CXCL13 serum levels and CXCR5 surface levels on CLL cells from different CLL patients to confirm our preliminary data that demonstrate higher CXCL13 and CXCR5 levels in CLL patients, compared to healthy controls. Intracellular signaling pathways related to growth and cell survival will be evaluated after stimulation of CLL cells with CXCL13. Collectively, the experiments described in this proposal will give us a better insight into regulation of growth and survival of CLL B cell by the microenvironment. As such, these studies may lead to a new therapeutic approach for CLL patients by targeting molecules involved in CLL cell crosstalk with the microenvironment.



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Loren Erickson, PhD

University of Virginia

\$100,000.00 - *Immune tolerance by regulatory T cells in multiple myeloma*

One of the main cell types of the immune system is the B cell. The primary function of B cells is to generate proteins called antibodies that recognize and bind to invading pathogens, resulting in the demise of the microorganism. B cells that are most efficient at producing antibodies are called plasma cells that reside within the bone marrow. Some plasma cells can become malignant leading to the cancer multiple myeloma (MM). Similar to plasma cells, MM cells live and are sustained in the bone marrow where they will ultimately destroy the tissue. How MM cells are able to go undetected by the immune system is not understood. This research project is focused on a unique population of cells known as "regulatory T cells" that we believe shield MM cells from being killed. Thus, devising therapeutic strategies that target these regulatory T cells should render MM cells susceptible to death.

Rhett A. Kovall, PhD

University of Cincinnati

\$100,000.00 - *Structure-function of Notch-CSL transcription complexes: a structural basis for developing anti-leukemia drugs*

The causes of leukemia and lymphoma are often linked to signaling pathways in cells, which through mutation signal abnormally. The development of targeted drugs, such as Gleevec, which specifically inhibits abnormal signaling in chronic myeloid leukemia, has met with great success. Aberrant signaling from the Notch pathway underlies the leukemogenesis of T-cell acute lymphoblastic leukemia, the most common type of leukemia in children, and is associated with the pathogenesis of multiple myeloma and acute myelogenous leukemia. Therefore, medicinal manipulation of Notch signaling holds great potential for novel antileukemia chemotherapeutics. The transcription factor CSL is the primary nuclear effector of the Notch pathway and therefore represents an attractive target for therapeutic intervention. The objective of this proposal is to characterize CSL-Notch transcription complexes using structural and thermodynamic approaches. Resolving the structural and thermodynamic details of CSL-Notch transcription complexes will lead to a more fundamental understanding of Notch signaling and will facilitate the rational design of small molecule drugs that target CSL for new anti-leukemia therapies.

Brian Lannutti, PhD

University of Washington

\$70,000.00 - *Megakaryocyte differentiation and polyploidization; deciphering the roles of Lyn and Fyn kinases.*

The orderly production of blood cells during the life of an organism is referred to as hematopoiesis. Bone marrow is carefully regulated to provide a constant supply of red blood cells, white blood cells, and platelets. These processes are controlled by growth factors or cytokines, which interact with receptors on the cell surface and transmit specific signaling to the nucleus stimulating growth and maturation. Occasionally, mutations in signaling molecules occur which release a cell from normal control mechanisms and lead to unrestricted growth and leukemia. Megakaryocytes (MK) arise in the bone marrow and produce platelets, the cells that mediate primary hemostasis. Precise regulation of platelet production is critical over the life of an organism and can be abnormal during disease states. Myelodysplastic syndrome (MDS) is a clonal disorder affecting bone marrow stem cells that causes ineffective hematopoiesis and may evolve into acute myeloid leukemia (AML). For many individuals with MDS, thrombocytopenia is a major clinical problem, resulting in bleeding, bruising, and the need for frequent platelet transfusions. The growth factor Thrombopoietin (TPO) and its receptor, Mpl, are part of a signaling pathway that is essential both for optimal growth of hematopoietic progenitors and MKs. The potent proliferative stimulus delivered by TPO may play a role in the etiology or progression of myeloid leukemia. We have shown that Mpl stimulation results in the activation of both Fyn and Lyn kinases in primary MKs, and inhibition of these signaling molecules results in enhanced proliferation and differentiation. Furthermore, we have demonstrated that inhibition of Lyn and Fyn kinases induces rapid MK differentiation in leukemic cell lines as well as in primary bone marrow cells and bone marrow from patients with MDS. A more complete understanding of the roles of these kinases and the signaling mechanisms of Mpl may lead to novel approaches in the treatment of ineffective thrombopoiesis associated with MDS and other hematologic diseases.



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Sami N. Malek, MD

University of Michigan

\$99,996.00 - *Genomic profiling and clinical outcome in CLL*

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world with about 10,000-15,000 new patients diagnosed each year and about 5,000 deaths attributed to the disease. CLL remains incurable and demonstrates a varied clinical course, with some patients progressing and requiring therapy shortly after diagnosis and others living with the disease for decades. This proposed research study aims at identifying and improving markers in the CLL genome that predict the clinical course of the disease at presentation. This data should guide patient counseling and design of better therapies in the future.

Mariam Merad, MD, PhD

Mount Sinai School of Medicine (New York)

\$100,000.00 - *Novel immunotherapy strategies for the treatment of graft versus host disease*

Bone marrow transplantation is the treatment of choice of blood cancer leading to the cure of 50-60% of patients with acute and chronic leukemia and aggressive lymphoma and myeloma disorders. This treatment consists on injecting bone marrow cells (also called graft) from a family-related or unrelated donor to patients after administration of chemo/radiotherapy. Bone marrow transplantation therapy has two important goals: 1/ To allow the delivery of large dose of chemotherapy and radiation to patients to kill most of the circulating cancer cells (and normal blood cells) and replace them with donor blood cells. 2/ To deliver an army of donor cells called donor T lymphocytes that are able to recognize and attack the leukemic cells that have not been killed by chemo and radiotherapy: this effect is critical and is called the graft anti-leukemic effect. Unfortunately, occurrence of graft versus host disease, a serious and life threatening complication limits the extended use of this important therapy. Graft versus host disease is the counterpart of the graft anti-leukemic effect and occurs when donor T lymphocytes, in addition to recognizing leukemic cells recognize and attack normal cells. Donor T lymphocytes commonly attack the skin and the gut. Skin lesions include skin rashes that may progress to total-body desquamation with severe and sometimes fatal infections. Gut graft versus host disease presents as diarrhea, abdominal pain with significant loss of fluids and blood which, can be life threatening. Graft versus host disease affects 30-60% of patients after bone marrow transplantation and most of the strategies to prevent this dramatic complication have centered on the depletion of donor T lymphocytes. However, the major drawback of these strategies has been the loss of the donor T lymphocyte-mediated antileukemic effect and cancer relapse in patients. In this application, we propose to test a novel strategy to prevent graft versus host disease that occurs after bone marrow transplantation, without affecting the graft anti-leukemic effect. Indeed, we have recently established that a population of skin cells called dendritic cells present in the recipient mice (the equivalent of patients) are responsible for activating donor T lymphocytes to induce skin graft versus host disease and that elimination of this cell population prior to injection of donor bone marrow cells prevents skin graft versus host disease. In this application we propose to extend these studies and explore: (1) whether in humans as in mice, persistence of patient skin dendritic cells correlates with skin graft versus host disease; (2) whether persistence of gut dendritic cells (in mice and in patients) also correlates with gut GVHD; (3) whether a new drug called Flt3 TKI (fms-like tyrosine kinase inhibitor) recently shown (by us and other) to be able to eliminate dendritic cells in vivo can eliminate dendritic cells in recipient mice after bone marrow transplantation and modulate skin and gut GVHD outcome in mice. Information gained from these studies should lead to new strategies in clinical transplantation and improve GVHD, a major medical problem in the treatment of patients with Leukemia, Lymphoma and Myeloma disorders.



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Eishi Noguchi, PhD

Drexel University

\$100,000.00 - *Roles of the Replication Fork Protection Complex in Genomic Integrity*

Cells must replicate the millions or billions of DNA base pairs with absolute fidelity every time cells divide to produce daughter cells. This is a remarkable accomplishment because cells are under the stress of many agents that cause DNA damage or stop DNA replication. Ultraviolet (UV), radiation, and reactive oxygen species generated during our normal metabolisms are among the many factors that cause problems during DNA replication. These agents cause mutations and rearrangements in chromosome DNAs, resulting in development of a variety of genetic diseases, including cancer. To avoid these situations, cells are equipped with a quality control system, termed the DNA replication checkpoint. This checkpoint responds problems during DNA synthesis and ensures that daughter cells inherit accurate copies of parental DNAs. This checkpoint is controlled by a group of proteins, called "checkpoint proteins". These include ATM, p53, Chk2, all of whose mutations cause cancer, including leukemia, lymphoma, and myeloma. The proposed studies will address the critical questions regarding the activation of the checkpoint. More specifically, we will work on the Swi1-Swi3 protein complex that is required to activate Chk2 and maintenance of DNA replication. The proposed experiments will elucidate how Swi1-Swi3 ensures accurate DNA replication. Therefore, our studies will provide important insights into checkpoint mechanisms, thereby facilitating our detailed understanding of how defects in checkpoints are involved in the occurrence of mutations and development of cancer.

Chih-Cheng Tsai, PhD

University of Medicine and Dentistry of New Jersey

\$100,000.00 - *Molecular mechanisms of PLZF-mediated acute promyeloid leukemia*

The promyelocytic leukemia zinc finger protein (PLZF) is a DNA-binding transcriptional repressor that encodes a conserved BTB/POZ domain at its N-terminus and Kruppel-like zinc fingers at its C-terminus. PLZF was identified by virtue of its fusion with retinoic acid receptor- α (RAR α) in t(11; 17) acute promyelocytic leukemia (APL) patients. Aberrant transcriptional repression, a result of PLZF-RAR α 's constitutive interactions with the transcriptional co-repressor SMRT (Silencing Mediator of Retinoid and Thyroid hormone receptors) and with HDACs (histone deacetylases), has been implicated in the pathogenesis of t(11; 17)APL. In this proposal, we summarize our recent characterization of two SMRT-interacting proteins, ataxin-1 (Atx1) and Brother of ataxin-1 (Boat), and report our discovery that these two related proteins also interact with PLZF. Because the interactions between PLZF-Atx1 or Boat are mediated through PLZF's zinc fingers whereas the PLZF-SMRT interaction is mediated through the BTB/POZ domain of PLZF, we here propose that PLZF-SMRT-Atx1-Boat form a stable protein complex that is involved in the differentiation of normal myeloid cells. Chromosomal translocations, as in the case of t(11; 17)APL, lead to aberrant protein associations which may contribute to arresting hematopoietic cells at the promyeloid stage and, as a result, to the development of leukemia. In specific aim 1, in order to establish the in vivo connections of Atx1 and Boat to PLZF, we will investigate whether Atx1 and Boat are coexpressed with PLZF in hematopoietic cells in bone marrow and in several existing promyeloid cell lines. Additionally, we will also use HL-60 cells to investigate whether Atx1 and Boat participate in their differentiation into granulocytic cells. In specific aim 2, in order to delineate how Atx1 and Boat participate in PLZF's regulatory pathways, we will probe whether expression of any direct target genes of PLZF is regulated by Atx1 and Boat. If so, we will further investigate whether Atx1 and Boat, by means of their associations with PLZF, bind the promoter regions of direct target genes of PLZF at the chromatin level. In specific aim 3, to assess how Atx1 and Boat modulate the properties of PLZF, we will investigate whether the ability of PLZF (and its fusion counterparts) to bind DNA, to associate with SMRT, and to form complexes with HDACs is affected by its association with Atx1 or Boat. The outcome of this study will shed new light on the properties of PLZF and pave ways for developing novel therapeutic approaches to treat t(11;17)APL.