



Leukemia Research Foundation

2010-2011 Scientific Research Grant Recipients

NEW INVESTIGATOR AWARDS

Yehudit Birger, PhD - The Chaim Sheba Medical Center

\$99,500.00 - *Decipher AMKL progression events for targeted therapy: Collaborative factors and downstream targets of the megakaryocytic oncogene ERG*

Leukemia is characterized by arrest in the normal differentiation coupled with increased cell survival and proliferation which results in the pathologic accumulation of immature cells. Often leukemia is caused by inappropriate expression of proteins called transcription factors that regulate the normal differentiation program of blood cells. Down Syndrome (DS) is characterized by an extra copy of chromosome 21. Children with DS are in high risk for developing Acute Megakaryocytic Leukemia (AMKL) that is associated with mutation in GATA1 gene leading to expression of a shorter isoform GATA1s. Here we propose to study the collaboration of the chromosome 21 transcription factor ERG that is also an oncogene, with GATA1s and identify the molecular events leading to the initiation and progression of DS AMKL. We will also identify ERG targets which will be further studied for functional analysis and targeted in therapeutic experiments aimed to improve ERG-related myeloid leukemia therapy. Our studies are not only likely to decipher the mechanism by which ERG causes leukemia, but could also provide a platform for preclinical studies of novel therapeutics of the poor prognosis ERG associated leukemia.

Glen Raffel, MD, PhD - University of Massachusetts

\$100,000.00 – *Yolk sac progenitors as a developmentally-restricted target population for congenital leukemia*

Megakaryocytes are cells in the bone marrow which make platelets, a blood component responsible for stopping bleeding. Leukemia is the uncontrolled growth and faulty development of blood-forming cells and is often fatal even with treatment. A mutation (OTT1-MAL) has been identified found exclusively in infant leukemias of the megakaryocyte cell type. We have generated mice with an identical mutation and are able to reproduce megakaryocytic leukemia. The cells from the diseased mice may have developed from yolk sac progenitors, which are a transient bloodproducing cell type from the earliest stages of the fetus. We are examining whether the mutation in mouse yolk sac progenitors show functional changes similar to the leukemia cells and whether these isolated cells are capable of producing leukemia alone or in combination with other mutation. If yolk sac progenitors show evidence of being the origin of OTT1-MAL associated infant megakaryocytic leukemia, it would offer a novel explanation for the unique age-dependence and megakaryocytic predilection of this disease. Yolk sac progenitors differ significantly from more mature blood-forming cells which could lead to the development of new targeted therapies.

Justin Kline, PhD - The University of Chicago

\$100,000.00 - *Immune evasion by acute myeloid leukemia through induction of T cell tolerance*

Acute myeloid leukemia (AML) is the most common acute leukemia occurring in older adults. Modern chemotherapy will lead to cure in less than half of those diagnosed with AML, and progress in improving patient survival has been slow over the past 20 years. Thus, new therapeutic approaches are needed. Immunotherapy with cancer-specific vaccines or adoptive cell therapy may be of benefit as an adjunct to chemotherapy. In fact, it is known that leukemia cells can express unique antigens which could serve as targets for immune-based therapies. Unfortunately, our two decade experience with immunotherapy for solid cancers like melanoma has taught cancer immunologists the important lesson that the tumor and its environment exploit a number of pathways which prevent immune cells from rejecting a growing cancer. Blood cancers, like their solid tumor counterparts, probably also promote immune evasion, though the mechanisms through which this occurs have been underexplored. In order to begin to address this question, our laboratory has developed a mouse model of acute myeloid leukemia. Preliminary experiments have suggested that when AML cells are inoculated into mice intravenously, a minimal anti-tumor immune response is generated. On the other hand, when the same cells are injected under the skin, a potent tumor-specific immune response ensues which can be detected shortly following tumor implantation. In fact, when AML cells are inoculated intravenously, they actually prevent a subsequent anti-tumor immune response against a challenge with AML cells subcutaneously in the same mice. Collectively, these experiments have suggested that AML cells growing in the blood are able to induce a state of T cell tolerance. We suspect that this tolerance results from process called T cell anergy which occurs when T cells are not fully activated by their targets, and results in their inability to proliferate and kill. The set of experiments outlined in this research proposal will help us to clarify whether anergy of tumor-specific T cells occurs in mice harboring AML intravenously. If so, we have developed approaches aimed at preventing tumor cells from inducing T cell anergy, some of which, if successful, are amenable to future clinical translation for patients with AML.



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Uttiya Basu, PhD - Columbia University

\$100,000.00 - *Regulation of the Mono-Ubiquitination of the Fanconi Anemia D2 Protein*

The production of antibodies in our body is a complicated and highly regulated process. Inside our body, the antibody recognition of antigen is facilitated by important processes like class switch recombination (CSR) and somatic hypermutation (SHM). The protein Activation Induced cytidine Deaminase (AID) has a central role in both these processes. Failure of SHM and CSR in patients with hyper IgM syndrome (HIGM) leads to recurrent infection at germinal centers and hypertrophy of lymph nodes. Mutation in AID gene leads to HIGM2. In addition, overexpression of AID in mice leads to mature T cell lymphomas. Furthermore, a large number of B cell lymphomas are associated with mutations and chromosomal translocations of the Ig locus. These translocations occur in germinal centers, where CSR and SHM take place. However, little knowledge is available regarding the mechanism of these aberrant translocations. AID is a potent mutator and initiates programmed DNA breaks at the switch regions leading to CSR and cause SHM. This work is directed towards elucidating the mechanism of function of AID during SHM and CSR.

Koren Mann, PhD - Lady Davis Institute for Medical Research

\$100,000.00 - *The role of tungsten exposure in the development of preB acute lymphoblastic leukemia*

The toxic effects of the metal tungsten are not well defined. However, high levels of tungsten have been found at the sites of several childhood leukemia clusters. Most of these leukemias are derived from a blood cell called the B lymphocyte or B cell. We propose experiments designed to determine whether tungsten can change the development of B cells or can cause the generation of leukemia in mice.

David Langenau, PhD - Massachusetts General Hospital

\$100,000.00 - *Identifying Collaborating Genetic Events in Relapse T-cell Acute Lymphoblastic Leukemia*

Patients with T-cell acute lymphoblastic leukemia (T-ALL) have a very poor prognosis when their tumors relapse after treatment. Self-renewal, or the process by which tumor cells make more of themselves, is thought to be responsible for driving tumor relapse. If self-renewal could be blocked, these leukemias would be unable to grow. However, the mechanisms governing self-renewal in T-ALL are unknown. Relapsed T-ALLs often harbors new genetic mutations compared to the original leukemias, and it is likely that these mutations increase both tumor self-renewal and aggression, or how quickly the tumor grows. While many genes have been associated with relapsed and refractory T-ALL, their function tumor re-growth remains unclear. In this proposal, we will use a zebrafish model of T-ALL to screen over 50 genes that are known to be recurrently amplified or over-expressed in relapsed and refractory human T-ALL. Zebrafish leukemias, which will express each of the genes of interest, will be analyzed for increased time to T-ALL development, and *in vivo* effects of the gene on self-renewal and tumor growth will be assessed. Additionally, any gene that has an effect in zebrafish T-ALL will be used in *in vitro* assays with human T-ALL cell lines to verify their function in human disease. The ultimate goal of this work is to identify novel genes associated with T-ALL self-renewal or aggression in the hopes of developing new therapeutics for relapsed T-ALL.

Shuo Dong, MD, PhD - Baylor College of Medicine

\$100,000.00 - *The modulation of STAT3 pathway in the suppression of MLL fusion protein mediated transformation*

Acute leukemia (AL) is one type of cancer affecting blood cells, come from the cancer stem cells and characterized by specific chromosomal translocations that occur within the malignant cells. Breakage and rejoining of the chromosomes results in fusion of the gene encoding MLL (mixed lineage leukemia) to a number of partner genes. This leads to abnormal genes that produce hybrid proteins made up of pieces from MLL plus one of different proteins that have the abbreviations ENL, AF9, AF10, AF6 et al. MLL leukemia has unique clinical and biological features and the patients with MLL rearrangement frequently present with poor prognosis. In this proposal, we will use biological and chemical studies to develop small molecular drugs that can target the cancer stem cell but not normal stem cell in leukemia. These new medicines, which will be developed in this proposal, will treat these leukemia patients that might not harm their normal organs.



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Sathees Raghavan, PhD – Indian Institute of Science

\$100,000.00 - Mechanism of *BCL6* fragility and incidence of *BCL6* translocation in Diffuse Large B-cell Lymphoma in India

Cancer is a disease of uncontrolled proliferation of cells. Deregulation of cellular proliferation can be caused by mutations in oncogenes and tumor suppressor genes. Mutations affecting these genes can be point mutation, deletion, inversion or translocations. A chromosomal translocation involves exchange of arms or segments of the chromosome, between two different non-homologous chromosomes. Majority of the hematological malignancies like leukemia and lymphoma are characterized by the presence of such translocations. One unique and interesting set of translocations, characteristic of the most common lymphoma worldwide, Diffuse Large Bcell Lymphoma (DLBL), is the one involving the *BCL6* gene on chromosome 3. In such *BCL6* translocations, the partner chromosome is not fixed and as many 26 different chromosomal loci maybe involved. The precise mechanism underlying the fragility of the *BCL6* gene is not known. Moreover, this particular translocation can serve as a good model system for understanding the fine mechanistic details of generation of chromosomal translocations as a whole. The presence of altered DNA structures in this region will be examined by various *in vitro* as well as *in vivo* methods, including chemical probing, NMR, cellular transfection assays etc. In addition, we aim to discover the proteins or physiological phenomena like replication and transcription, responsible for inducing the double-strand breaks. We also propose to determine the incidence and frequency of the occurrence of the *BCL6* translocation in healthy individuals due to previous reports of occurrence of other translocations in the peripheral blood of normal volunteers. We also plan to characterise the breakpoint junctions from both healthy individuals, and patients so as to get better understanding on the development of such translocations during the progression of DLBL.

Marta Muzio, PhD - San Raffaele Research Institute, Milano, Italy

\$69,000.00 - Toll-like receptors in chronic lymphocytic leukemia

The general aim of this project is to test the hypothesis that stimulation through Toll-like receptors favours leukemogenesis. This hypothesis will be verified in mouse models of Chronic Lymphocytic Leukemia (CLL). TLR are best known for their role in host defence from infection; they recognize a set of different molecular patterns of microbial origin, and immediately trigger an innate immune response. In addition, several recent reports demonstrated that TLR stimulation is involved in neoplastic transformation and progression of solid tumors. Emerging evidence suggest that TLR may play a role also in hematologic malignancies and especially in chronic lymphoproliferative disorders. First, microbial components directly sustain the proliferation of multiple myeloma cells *in vitro* through TLR. Second, infections are known to play a role in B cell lymphomagenesis, especially in the case of MZL (Helicobacter pylori and HCV infections). Third, there is growing evidence that malignant CLL cells may recognize self-antigens and microbes through their surface Immunoglobulins, and we recently showed that bacterial lipopeptides triggering TLR2 activate and protect CLL leukemic cells from spontaneous apoptosis. Based on these observations, it is becoming apparent that both infection and autoimmunity are involved in the development and/or progression of chronic B-cell malignancies, that distinct TLR may play a direct role in this overall process and that CLL provides an excellent model to test this hypothesis. We hypothesize that chronic exposure of the malignant cell to distinct TLR ligands and/or persistent cell activation and signalling may have a role in the development, progression and/or leukemic cell accumulation of CLL. Specific aims of this project are:

- 1) To analyze leukemogenesis before and after inflammation in mouse models of CLL.
- 2) To analyze the occurrence of B cell neoplasia before and after induction of autoimmunity in mouse models of CLL.

To approach these questions we carefully designed the following three experimental tasks:

- 1) To analyze development, progression and/or accumulation of CLL cells before and after TLR4 ligation *in vivo*.
- 2) To analyze leukemogenesis before and after intestinal inflammation in CLL mouse models.
- 3) To analyze CLL pathobiology before and after induction of autoimmunity *in vivo*. Dissecting the molecular interactions that occur in the microenvironment and are responsible for the growth and accumulation of CLL is crucial to understand its pathogenesis and may pave the way to a more proper understanding of the mechanisms



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that account for the natural history of indolent B cell lymphomas. It will be essential to identify novel molecules or pathways to be explored as targets for therapeutic purposes.