Karin Grunebaum Cancer Research Foundation

Founded in 1958

October, 2006 • Volume 3

Dear Friends of the Karin Grunebaum Cancer Research Foundation:

What a fabulous year this has been for the Foundation!

In the past twelve months, in addition to our numerous on-going cancer research projects at Harvard Medical School and Boston University School of Medicine, we established a new joint 5-year post-graduate cancer research Fellowship program with the Massachusetts General Hospital (MGH). **The Foundation is delighted to be associated with such a superb research, educational and medical treatment facility.**

The joint program at MGH is a surgical cancer research Fellowship which will be based in the world-renowned Pancreatic Cancer Research Laboratory of Dr. Andrew L. Warshaw, Surgeon-in-Chief and Head of the Department of Surgery at MGH. The first joint Fellowship research project at MGH already started work in August 2006.

As many of you know, the Karin Grunebaum Foundation's Board of Trustees is somewhat unique amongst charitable foundations because it is composed both of direct descendents of Karin Grunebaum, as well as some of the most respected medical researchers and educators in the world. To this august group of medical professionals, we are very pleased to add the names of Dr. Warshaw and Dr. Karen H. Antman, Dean of the Boston University School of Medicine and former Deputy Director at the National Cancer Institute. The Foundation is greatly honored that these two outstanding cancer researchers have agreed to join the Board of Trustees.

I am very pleased and proud that the Foundation has made such enormous gains over the past year. New Fellowships, new trustees and new research recognition did not happen by accident, but rather through the on-going, dedicated efforts of the Foundation's Fellows and Trustees, alike.

Our Foundation was further honored when one of our current Fellows, Tim Daskivich, was asked to present his research findings at the annual meeting of the American Society of Clinical Oncologists. At the same time, the other incoming and outgoing Fellows presented summaries of their research projects to the Trustees and to each other at the June Trustees' meeting at Boston University's School of Medicine. The Trustees and Fellows were also privileged to hear excellent summary research presentations by Dr. Sarah Thayer of the MGH Pancreatic Cancer Research Laboratory and by Dr. Douglas Faller, Boston University's Karin Grunebaum Professor in Cancer Research.

As part of the Foundation's dedication to increasing both professional and social interaction between the Fellows, after the Trustees' meeting, current and former Fellows joined the Trustees for a most enjoyable reception at the Harvard Club in Boston.

I am very pleased and proud that the Foundation has made such enormous gains over the past year. New Fellowships, new trustees and new research recognition did not happen by accident, but rather through the on-going, dedicated efforts of the Foundation's Fellows and Trustees, alike. I would like to express my thanks and appreciation to all of you for all of your hard work in making these goals come true.

My immediate future goal is to now increase the Foundation's capital through a series of fund-raising activities, so that we can continue to expand our research-funding projects. We are actively looking for corporate and other charitable donors, and will be planning fund-raising events in the future to accomplish our next goal.

Steven Wallach, Chairperson
Karin Grunebaum Cancer Research Foundation

What's "Fueling" your Leukemia?

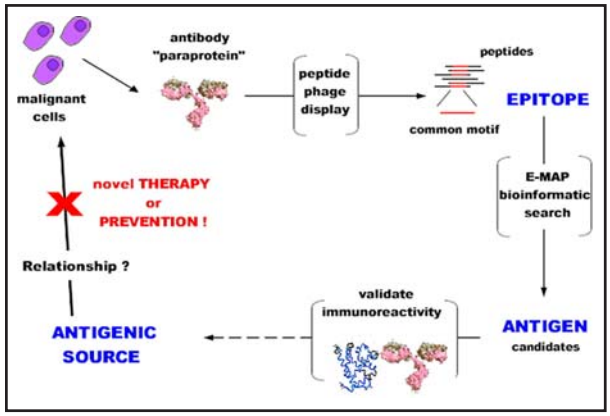
The Role of Antigenic Stimulation in Lymphomagenesis

Gerassimos Bastas , MD/PhD candidate
Boston University School of Medicine
Advisor: Steven Bogen, MD/PhD
Associate professor of Pathology and Laboratory Medicine
Boston University School of Medicine

B-lymphocytes are those cells of the immune system that produce antibodies against foreign substances and invading microorganisms. When B-cells become malignantly transformed, i.e. cancerous, the result is a leukemia or lymphoma. In general, a B-cell's specific antibody is known to play a major role in the life and behavior of that cell: Presence of the target substance the antibody recognizes (the "**antigen**") stimulates a B-cell to activate and divide. Increasing evidence suggests a major role for antigenic stimulation in lymphomagenesis. In other words, when a malignant B-cell maintains responsiveness against the foreign substance or microorganism against which it was elicited, the presence of that antigen may serve as a trophic stimulus, "fueling" the cancer.

The paradigm case is that of gastric MALT lymphoma (stomach cancer), whereby the infiltrating lymphocytes were discovered to be reactive to Helicobacter pylori (the ulcer causing bacterium). Chemotherapy had been largely ineffective in these patients. However, treatment with antibiotics cured the underlying infection, removing the antigenic stimulation maintaining the lymphoma, resulting in durable remissions in 70% of cases.

It would be extremely helpful if we could discover what the antibodies of other clonal B-lymphoproliferative diseases had been initially elicited against. That information might, for instance, implicate a cancer-causing virus in the development of the leukemia, or even suggest potentially new treatments. However, with no suggestive clinical evidence (such as e.g. the stomach – H. pylori connection) there is currently no way to discover or implicate antigen candidates.



We have been working on **Multiple Myeloma** (MM), which is a B cell hematological malignancy. It is a highly aggressive disease which despite treatment, with chemotherapy and stem cell transplant, maintains a generally poor prognosis. The cause of MM is not known and even the precise epidemiologic pattern remains obscure. According to the American Cancer Society the median prognosis is three years. There are approximately 45,000 people in the United States with MM, with 14,600 new cases diagnosed each year. Multiple myeloma is the second most prevalent blood cancer (10%) and is responsible for 2% of all cancer deaths in Western countries.

In MM, the malignant B-cells proliferate and produce their antibody in copious amounts, to the point where the patient presents as if seemingly having only one antibody in their system (the so called "**paraprotein**"). The paraprotein can be easily purified from the patients' serum. In what is truly a "reverse immunology" approach we look at the antibody's binding site and attempt to perform a "casting process" on a molecular scale to figure out what the target epitope looks like (the "**epitope**" is the specific region of the foreign substance that the antibody attaches to). We do this by using phage-displayed peptide combinatorial libraries, which are best thought of as vast libraries of random peptides. Only certain of these peptides "fit" into the antibody's binding site. We analyze several of these specific binders, scrutinizing them for commonalities. In the end we can reconstruct a virtual profile of what the true cognate epitope must look like. Armed with this knowledge we then use an advanced bioinformatics algorithm to perform searches that ask "where is this motif found in nature?" We call this process E-MAP (Epitope-Mediated Antigen Prediction). Discovering plausible antigen candidates from short, experimentally-determined, oligopeptide sequences is a tall order indeed, but we have made it work. Following the identification of plausible antigen candidates we have validated that the patient's antibody does in fact specifically bind the proposed antigen.

We have managed to show that different patients' paraproteins seem to have been elicited against the same epitopes, suggesting that these antibodies are raised in response to a limited pool of antigens. This finding is significant because it indicates that identification of the antigenic source could suggest a generalizable therapeutic strategy. We have also managed to discover the cognate antigens of at least three patients' paraproteins and they are from Human Cytomegalovirus (HCMV), a ubiquitous herpes virus. We have managed to demonstrate that several MM patient's paraproteins react with determinants from HCMV. The road is now open to elucidate the potential role of HCMV in the development of MM and other leukemias: does the virus cause MM by directly transforming B-cells? Do the viral antigens serendipitously provide a trophic stimulus to the leukemic cells? Would antiviral medication be protective or therapeutic?

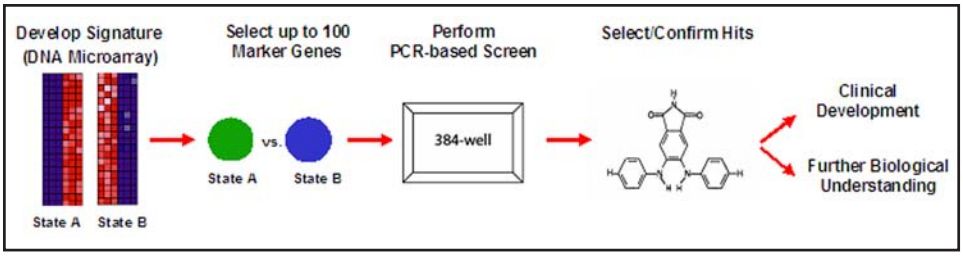
We believe that identification of the cognate antigens of different patients' paraproteins will further implicate antigenic stimulation in pathogenesis, revealing common risk factors and suggesting generalizable therapeutic interventions. In what is a truly unprecedented approach, we have managed to analyze antibodies' binding sites and back-track the information in discovering the true cognate antigens. **By looking at the immunologic "fingerprint" of the disease we are now better poised to understand something new about its natural history and pathophysiologic progression.** The hope has been and always remains that this insight will help us create better therapeutic strategies for this aggressive disease and many others.

Finding the Target: Identifying the Mechanism of Gefitinib Induced Acute Myeloid Leukemia Differentiation

Rose Kakoza, MD
Harvard Medical School

Acute myeloid leukemia (AML) is the most common form of leukemia among adults in this country. Prognosis remains poor, with an overall five-year survival for adults of only 20%. New treatment approaches are clearly needed. A block in differentiation is a critical feature in the pathogenesis of AML. Overcoming this block has been shown to have therapeutic impact in some forms of AML. Gefitinib is a newly identified myeloid differentiation agent. Understanding gefitinib's mechanism of action is expected to lend new biological insight into the AML maturation block, as well as further the development of second generation drugs for AML.

Recently, a new method of small molecule library screening, Gene Expression-based High-throughput Screening (GE-HTS), was developed by my project investigator, Kimberly Stegmaier, M.D., and colleagues (Figure 1). Our lab used GE-HTS to discover AML differentiating agents. This work resulted in the discovery of gefitinib



(Iressa) as an AML differentiation agent. Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor previously FDA-approved for the treatment of lung cancer.

Gefitinib induces AML differentiation via a new, unknown, non-EGFR mechanism of action. Understanding its mechanism of action is ultimately very important from a scientific and clinical perspective. Knowledge of the target would enable confirmation of biological effect in patients on clinical trials and would guide future trials as more potent modulators of the target become available. Our project will focus on the identification of the target of gefitinib in induction of AML differentiation. There will be three parts to this investigation: 1) identifying a gene expression signature for gefitinib induced AML differentiation, 2) screening a lentiviral-based short-hairpin RNA (shRNA) library designed against the human kinome for RNAi constructs that phenocopy the expression signature of gefitinib induced AML differentiation, and 3) confirming identified shRNA hits.

Thus far, a gene expression signature for gefitinib induced AML differentiation has been identified. For the next phase of this project, we are preparing to screen for potential targets. Because we believe that the target of gefitinib is a serine, threonine, or lipid kinase, we will use an shRNA lentivirus-based library developed by the RNAi consortium in Boston consisting of approximately 3,500 hairpins designed against the human kinome (tyrosine, serine-threonine, and lipid). The purpose of this assay is to identify those kinases that, when inhibited by the shRNA, induce the same gene expression signature as gefitinib. Finally, we will confirm these shRNA hits through a series of analyses.

At the completion of this work, we expect to identify the target of gefitinib and, possibly, additional kinases important in AML pathogenesis and myeloid differentiation. These findings should contribute to a better understanding of AML pathogenesis and lead to the identification of new and more potent agents that promote AML differentiation. These agents will serve as therapeutic leads and ultimately may contribute to more effective treatment for this life-threatening disease.

I would like to thank the Karin Grunebaum Cancer Research Foundation for the opportunity to do this important and needed research.

A Novel Mouse Model for the Study of Chronic Graft-versus-Host Disease

Karen S. Lee, MD
Boston University School of Medicine

Allogeneic stem cell transplantation (alloSCT) is a potentially curative therapy for malignancies of blood cells, inherited disorders of stem cells, and acquired nonmalignant diseases, and has revolutionized cancer therapy. Distinct from autologous SCT in which blood forming stem cells from the same patient are used, alloSCT obtains stem cells from a donor whose tissue type is as close as possible but not identical to the patient's tissue type. Generally, tissue types are determined by the presence of specific cell-surface molecules known as histocompatibility antigens. These antigens are further classified into major and minor histocompatiibility complexes (MHC), and confer a person's tissue type. In an effort to match patients with an appropriate donor, clinicians detect the major histocompatibility differences, however they are unable to detect minor histocompatibility differences. Therefore, although alloSCT restores the bone marrow to enable the use of higher doses of chemo- and radiation therapy that function to destroy cancer-forming cells., alloSCT is limited by persistent minor differences that result in a disorder known as graft-versus-host disease (GVHD).

GVHD describes the phenomenon of donor cells reacting to host organs and/or tissues that results in organ damage. Regardless of the potential of alloSCT in the treatment of cancer, the occurrence of GVHD is a major cause of late mortality. GVHD is divided into two categories, acute and chronic. Occurring within days following a transplant, acute GVHD reflects the immediate reactiv-

(continued on next page)

ity of donor cells to host tissues that are not matched across major MHC barriers. In contrast, chronic GVHD (cGVHD) follows a slower, progressive course, and occurs even when the MHC barriers are matched (reflecting current clinical practice.) Occurring in up to 80% of patients, cGVHD is thought to be on the rise due to the increased use of alloSCT treatment for cancer. Therefore, a better understanding of the induction and pathogenic mechanisms of cGVHD is needed.

cGVHD is caused when alloreactive T cells respond and expand to unknown MHC differences, also known as allo-antigens. Therefore in the absence of T cells, cGVHD is prevented. While the occurrence of cGVHD can be prevented through the blockade of T cells, decreasing the number of T cells renders the immune system helpless to foreign pathogens, and more susceptible to infection; thus not a practical solution to prevent cGVHD. In addition, phenotypically cGVHD has a wide spectrum of clinical and pathologic features. For example, one form of cGVHD is characterized by scleroderma-like changes, such as skin thickening and intestinal disorders; another form of cGVHD mimics a lupus–like disease, resulting in kidney disease. However, what determines whether cGVHD develops into one form of disease over another remains unclear. In order to address this issue, numerous murine cGVHD models have been studied. Similar to human disease, mice models that mimic the donor-recipient MHC-pairings cause very different phenotypic outcomes of cGHVD. Researchers hypothesize that these models vary according to some unknown target allo-antigen and how it is distributed in various tissues and/or organs, consequently manifesting distinct forms of disease. This suggests that selection and location of expression of allo-antigens can determine the quality of cGVHD. Therefore, differences in allo-antigen distribution (target organs and cell types), or the qualitative nature of T cell responses in response to the allo-antigen, could in turn contribute to changes in cGVHD phenotype. Based on this evidence, we are proposing to create a novel mouse model that will enable the manipulation of the site and level of expression of allo-antigens in order to discern what key factors influence the changes in cGVHD phenotype. We hypothesize that persistent allo-antigen presentation in specific tissue locales influences the varied subsequent clinical features of cGVHD pathogenesis.

Since we do not know what the allo-antigens are that incite cGVHD, we propose to use a pseudo-alloantigen that can be regulated to mimic cGVHD, ultimately to delineate what factors contribute to disease pathology. In order to regulate expression of a pseudo-alloantigen, we adapted an *in vivo* tetracycline (tet)-regulatable gene expression system (Fig.1). This is a system in which expression of the pseudo-alloantigen is dependent on binding of another molecule known as the tetracycline-sensitive transactivator (rtTA). As the term implies, this molecule is sensitive to the presence of tetracycline, a drug that can be provided exogenously, as in the drinking water of mice. Hence, the rtTA molecule can only "turn on" the pseudo-alloantigen when tetracycline is present (mouse ingests tetracycline), versus "turned-off" when tetracycline is not provided. Due to the easy administration of tetracycline, this enables us to manipulate how often rtTA can 'turn-on' or 'turn-off' the pseudo-alloantigen, an important facet that we believe influences disease progression. In addition, the location of the pseudo-alloantigen can be altered through the use of tissue- or cell-specific promoters that change where the pseudo-alloantigen is expressed; thus once we have one mouse that expresses the tet-regulated element (TRE) with the pseudo-alloantigen, we can mate it to multiple types of rtTA mice that will drive the expression of in different locations (only in the presence of tetracycline). Therefore, this mouse model has both regional selectivity in addition to the ability to change the level of expression through the addition or removal of exogneous tetracycline.

In the past year, we have begun to generate the initial TRE-regulated mouse model (Fig. 2). Currently, we are beginning the initial tests to determine whether we can in fact regulate our pseudo-alloantigen in a cell-specific manner. Once we have confirmed that we have a functional and regulatable mouse model, we hope to further delineate factors that will influence the cGVHD phenotype. Ultimately, this will enable us to contribute to the understanding of cGVHD in the hopes of improving the outcomes of alloSCT for the treatment of cancer.

Figure 1: Tet-regulatable system: The pseudo-alloantigen, denoted as TGO, cannot be expressed unless the rtTA is present, and tetracycline (tet) is provided exogenously. This system can be manipulated to change level of expression, through the addition or removal of tet, or by altering the location of the rtTA; i.e. only allowing TGO to be expressed in specific organs and/or tissues.

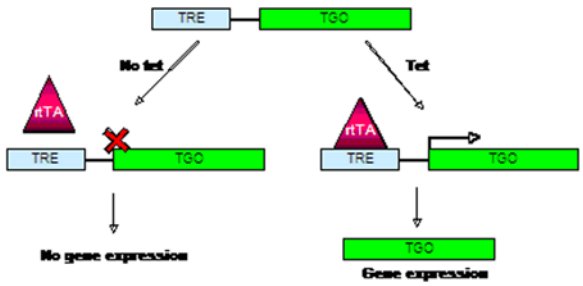
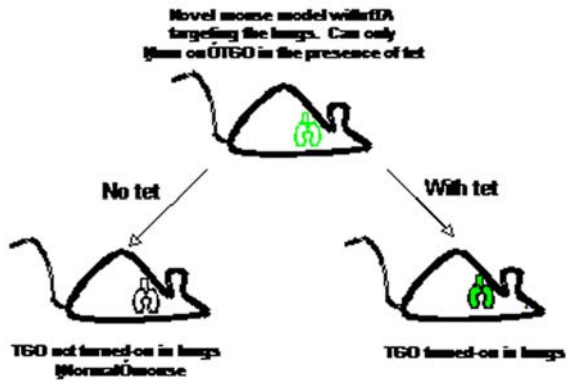


Figure 2: Example of a potential mouse model given lung expression of the rtTA:



Meet The Foundation's New Trustees

Karen H. Antman, M.D.
Provost and Dean of Medicine
Boston University School of Medicine

Karen H. Antman, M.D is Provost of the Medical Campus and became Dean of the Boston University School of Medicine on May 1, 2005. She was previously the Deputy Director of Translational and Clinical Sciences at the National Cancer Institute.

Dr. Antman received her M.D. from Columbia University, College of Physicians & Surgeons. She joined the Harvard Medical School faculty in 1979 and served as the Clinical Director of the Dana-Farber Cancer Institute Solid Tumor Autologous Marrow Program and of the sarcoma and mesothelioma clinical research and treatment programs until July of 1993, when she returned to Columbia University. There she was the Wu Professor of Medicine and Chief, Division of Medical Oncology, Columbia University, from 1993 to 2003, and the Director of Columbia's Herbert Irving Comprehensive Cancer Center.

Her research accomplishments include the development of now standard regimens for the treatment of sarcomas and mesotheliomas. She has also developed regimens for breast cancer and supportive care of patients receiving chemotherapy including pharmacology, growth factors and mobilization of peripheral blood derived stem cells for blood and marrow transplant.

She has published reviews and editorials on medical policy and the impact of research funding and managed care on American clinical research. She has considerable past and present grant support and is an author on more than 160 peer reviewed articles, and more than 100 chapters and reviews. She has edited 2 text books (Asbestos Related Malignancies and High Dose Chemotherapy).

She has lectured to lay audiences and has written articles in Vogue and in Readers Digest on cancer prevention and screening.

She has served as President of the American Society of Clinical Oncology in 1994-19955, the American Society of Blood and Marrow Transplant in 1996-1997, and the American Association for Cancer Research in 2003-2004. She has served on many Federal and foundation boards and committees.

Her husband is a cardiologist. Their two children are both in medical school.

Andrew L. Warshaw, M. D.
Surgeon-in-Chief and Chairman
Department of Surgery
Massachusetts General Hospital

Dr. Andrew Warshaw is a graduate of Harvard College and of Harvard Medical School. His residency training was at the Massachusetts General Hospital. He also spent two years as a Clinical Associate in the Section on Gastroenterology of the National Institutes of Health, as well as a year as Research Fellow in Medicine at Harvard Medical School. Since 1972, he has been on the staff at the Massachusetts General Hospital and the faculty of Harvard Medical School. In 1987, he became Professor of Surgery at Harvard and in 1997, the W. Gerald Austen Professor of Surgery, Surgeon-in-Chief and Chairman of the Department of Surgery at the Massachusetts General Hospital.

Dr. Warshaw is a member of most of the important surgical societies, including the Society of University Surgeons, the American Surgical Association, Society of Clinical Surgery and the Halsted Society. He has been President of the Society for Surgery of the Alimentary Tract, the International Association of Pancreatology, the Massachusetts Chapter of the American College of Surgeons, the New England Surgical Society, the Halsted Society, the Boston Surgical Society, and the Society of Surgical Chairs. He was a Director of the American Board of Surgery and its Chairman in 1993. He has served as a Governor of the American College of Surgeons and chair of its Socioeconomic Issues Committee. He was First Vice-President of the College and remains a member of its Health Policy Steering Committee and Chair of the Board of Directors of the American College of Surgeons Professional Association/Political Action Committee.

Dr. Warshaw has had a distinguished career as a teacher and editor. He is Editor-in-chief of the journal SURGERY and has served on the editorial boards of 15 other major journals. He has been a visiting professor at more than 50 major universities in the U.S. and around the world.

Dr. Warshaw's major interests focus on benign and malignant diseases of the pancreas. He has done much to define the pathogenesis and treatment of inflammatory and malignant lesions in the pancreas. His bibliography lists 325 original reports as well as 230 book chapters, reviews, and monographs and 10 books.

FROM THE TRUSTEES

The trustees of the Foundation want to remind everyone that the support given to the students is possible only through private donations and the generous contributions from those who have benefited from the foundation and those who are dedicated to the hope for a cancer-free world.

We ask that you send your tax- free donations to: KGCRF 85 Sherman Street #8, Cambridge, MA 02140. Tax ID # given upon request.

Karin Grunebaum Cancer Research Foundation
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