IX CHRONIC MYELOGENOUS LEUKEMIA RESEARCH PROGRAM

Chronic myelogenous leukemia (CML) is also known as chronic granulocytic leukemia or chronic myeloid leukemia. CML is an overgrowth of granulocytes, a type of white blood cells. The cause of this disease is unknown. The disease accounts for about 14% of adult leukemias in Western countries. It was predicted that in 2005, approximately 4,600 individuals would be diagnosed with CML, and an estimated 850 would die from the disease.[1] In most cases, CML is characterized by a chromosomal abnormality that is known as the Philadelphia chromosome. Treatment usually consists of various chemotherapeutic agents used to disrupt the production of leukemic cells. These treatments may be followed by stem cell transplant. More recently, targeted therapy for CML with STI571 (Gleevec®), an Abl-specific tyrosine kinase inhibitor, has shown significant success in patients with advanced disease. However, resistance to this therapy has been observed, indicating research is still needed.

Vision: To perfect the existing and develop new diagnostic and therapeutic approaches for chronic myelogenous leukemia.

Mission: To sponsor basic and clinically oriented research in the field of chronic myelogenous leukemia.

Congressional Appropriations for Peer Reviewed Research:

- $9.25M in FY02–03
- $4.25M in FY04
- $4.25M in FY05

Funding Summary:

- 28 awards from the FY02–03 appropriations
- 8 awards from the FY04 appropriation
- ~9 awards anticipated from the FY05 appropriation

PROGRAM BACKGROUND

The Department of Defense (DOD) Chronic Myelogenous Leukemia Research Program (CMLRP) was established in fiscal year 2002 (FY02) by Joint Appropriations Conference Committee Report No. 107-350, which provided $5 million (M) for CML research. The CMLRP has managed $17.75M through FY05 to fund peer reviewed research, and 36 awards have been made through FY04 to improve the diagnostic and therapeutic approaches to CML. The key initiatives of the CMLRP are supporting basic and clinically oriented research, encouraging innovative research (read the box story on page IX-3 about the novel fish model of human CML supported by the CMLRP), and sponsoring preclinical phases of development of potential CML-specific therapeutic agents. Appendix B, Table B-6, summarizes the congressional appropriations and the investment strategy executed by the CMLRP for FY04 through FY05.

THE FISCAL YEAR 2004 PROGRAM

The CMLRP was continued through an FY04 congressional appropriation of $4.25M. A new award mechanism, called the Therapeutic Development Award, was launched to move the field significantly closer to the development of new therapeutics for CML. Of the 23 proposals received, 4 were funded to support preclinical phases of development of potential CML-specific therapeutic agents with the intent to conduct clinical trials after completion of the proposed

[1] *National Cancer Institute Physician Data Query and American Cancer Society,* Cancer Facts and Figures, *2005*.

Signs and Symptoms

CML can be divided into three phases called chronic, accelerated, and acute depending on the maturity of the leukemia white blood cells. Symptoms of CML usually develop gradually over time. In the early stage of CML (chronic), there are usually few to no symptoms of leukemia present. However, as CML progresses, symptoms may be present but are often nonspecific and gradual in onset. The accelerated phase can be characterized by such signs as weakness, fatigue, fever, poor appetite, weight loss, increased sweating, and an enlarged spleen. The disease then progresses to the acute phase, also called the blast phase, where significant symptoms are usually experienced that may include weight loss, anemia, fever, bone pain, and recurring infections.

work. Table IX-1 provides a summary of the Therapeutic Development Awards in terms of number of proposals received, number of awards, and dollars invested. (Because of excess FY04 funding, four FY05 Exploration-Hypothesis Development Awards were included in the FY04 portfolio.) As illustrated in Figure IX-1, the FY04 CMLRP has supported a multidisciplinary portfolio of research.

Table IX-1. Funding Summary for the FY04 CMLRP

Category and Award Mechanism's	Number of Proposals Received	Number of Awards	Investment
Research			
Therapeutic Development	*23*	*4*	*$3.22M*
FY05 Exploration-Hypothesis Development	*n/a*	*4*	*$0.53M*
Total	*23*	*4*	*$3.75M*

THE VISION FOR THE FISCAL YEAR 2005 PROGRAM

Congress again appropriated $4.25M to the CMLRP in FY05. The emphasis for the FY05 program was placed on therapeutics and innovation. Two award mechanisms were offered, both of which were previously offered by the program, the Therapeutic Development Awards and the Exploration-Hypothesis Development Awards. A total of 54 proposals were received, as detailed in Table IX-2, and approximately 9 awards are anticipated.

Table IX-2. Award Mechanisms Offered and Proposals Received for the FY05 CMLRP

Category and Award Mechanisms	Number of Proposals Received
Research	
Exploration-Hypothesis Development	*45*
Therapeutic Development	*9*
Total	*54*

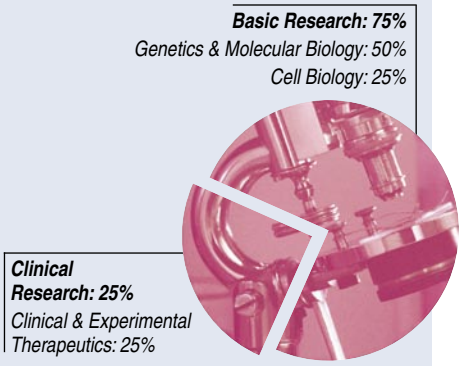


Figure IX-1. FY04 CMLRP Portfolio by Research Area

SCIENTIFIC OUTCOMES AND ADVANCES

While the CMLRP is a relatively young program, CMLRP-supported research provides hope for the future. The following projects represent examples of the advances that are being made in the therapeutic approaches for CML supported by this program.

LITTLE FISH, BIG POTENTIAL

The common home aquarium zebrafish, *Danio rerio*, has moved from "pet status" to an important whole organism model added to the repertoire of tools used for gene expression and protein activity studies. Whole organism studies are invaluable because the biological questions asked may be more encompassing and when combined with data obtained from in vitro studies the resulting knowledge can lead to powerful insights into the specific mechanisms and general consequences of disease. Developmental and biological conservation between zebrafish and mammals is evident down to the level of gene expression and function. Because of their biological and genetic complexity, zebrafish have been used as model organisms for studies of initiation and progression of many human diseases including cancers such as leukemia and melanoma. There exists a strong conservation of sequence, expression, and function of key hematopoietic genes and proteins between zebrafish and mammals, which suggests that the zebrafish may be a superior model for investigating CML disease initiation and progression, and potential therapeutic intervention. The CMLRP has funded two independent Exploration-Hypothesis Development Awards focused on creating transgenic zebrafish models of human CML through the myeloid-specific expression of the BCR/ABL protein, which is implicated in the development of disease. The BCR/ABL protein is expressed as a result of a genetic translocation that creates the Philadelphia chromosome found in the majority of CML patients. The approach to model development may be similar for the two groups with such research on zebrafish funded; however, their research interests are distinct. The first study by A. Thomas Look, M.D., of the Dana Farber Cancer Institute, will use the transgenic BCR/ABL-expressing fish to evaluate changes in myeloid cell proliferation, growth, and differentiation that occur in the presence of the BCR/ABL protein expression. The second award, from Kevin J. P. Griffin, Ph.D., of the University of California, Los Angeles, will focus on identifying genetic mutations that act in combination with BCR/ABL and lead to disease development and disease progression. It is anticipated that both of these studies will significantly increase the understanding of CML development and progression because of the many similarities that exist between the hematopoietic systems of zebrafish and mammals. Thus, they are truly "little fish" with very "big potential."

Pre-Clinical Evaluation of PD166326, a Potential New CML Therapeutic Agent

Robert L. Ilaria Jr., M.D., University of Texas Southwestern Medical Center, Dallas, Texas

Most CML arises as a result of a reciprocal chromosomal translocation event causing the expression of a fusion protein, BCR/ABL, a tyrosine kinase with potent leukemogenic activity. Imatinib mesylate (Gleevec®, STI571) has successfully treated CML patients in the early stages of disease; however, treatment of patients in the later stages of CML has not been as successful, and there is evidence of emerging imatinib mesylate-resistant CML. The development of alternative treatment options such as the compound PD166326 might benefit CML patients.

Fiscal Year 2005 Integration Panel Members

Richard A. Van Etten, M.D., Ph.D. (Chair)
Tufts-New England Medical Center

Moshe Talpaz, M.D. (Chair Emeritus)
The University of Texas M.D. Anderson Cancer Center

Alan J. Kinniburgh, Ph.D.
The Leukemia and Lymphoma Society

Rose McCullough, Ph.D.
Health Policy Professional

Jerald P. Radich, M.D.
Fred Hutchinson Cancer Research Center

"I have participated in two peer reviews as a Consumer reviewer and I am tremendously excited to now serve on the Integration Panel—I cannot imagine a more rewarding experience. The CDMRP/CMLRP program is run by some of the most dedicated and professional people I have ever worked with. The process brings out the best thinking from amazingly talented researchers and clinicians in the field of CML to allocate funding to the most promising grants..."

Kelvin J. Dickenson, FY03–04 CMLRP Consumer Peer Review Panel Member

Dr. Robert L. Ilaria Jr. received an FY02 CMLRP Investigator-Initiated Award for research designed to study PD166326, a promising potential CML therapeutic agent, in a pre-clinical mouse model of human CML.

Using this mouse model, Dr. Ilaria and his colleagues produced in vivo data that were the first to move studies of PD166326 from the in vitro setting to the in vivo setting, a critical first step that will bring this agent closer to testing in human clinical trials. Dr. Ilaria and his colleagues demonstrated that PD166326 (1) suppressed leukemic cell growth, (2) suppressed tyrosine phosphorylation, (3) was well tolerated, (4) quickly reached therapeutic levels in the blood stream, and (5) was useful in treating an imatinib-resistant CML mouse model. PD166326 treatment of a mouse model with a CML-like myeloproliferative disorder reduced leukemic cells better than treatment with imatinib mesylate. Additionally, splenomegaly, a characteristic of this CML-like myeloproliferative disorder, was reduced when compared to imatinib mesylate- or placebo-treated mice. PD166326 also suppressed tyrosine phosphorylation events. PD166326 and imatinib mesylate suppressed overall tyrosine phosphorylation when compared to each placebo-treated mouse; global suppression of constitutive phosphotyrosine was greater with PD166326. Specifically, the phosphorylation status of Crk-L, a target of BCR/ABL kinase activity, and Lyn, a Src family member implicated in the development of imatinib mesylate-resistant CML, was suppressed in PD166326-treated mice.

To address the issue of emerging imatinib mesylate-resistant CML, Dr. Ilaria and colleagues studied the effects of PD166326 using a mouse model of imatinib-resistant CML in which the bone marrow was reconstituted with cells expressing mutants of BCR/ABL implicated in imatinib-resistant CML. In addition to this model system, PD166326-treated mice survived twice as long as placebo-treated mice. These data taken together suggest that PD166326 may be an effective addition to the arsenal of CML treatment options in the future.

Please refer to the following publication for additional information about this research:

Wolff NC, Veach DR, Tong WP, et al. 2005. PD166326, a novel tyrosine kinase inhibitor, has greater antileukemic activity than imatinib mesylate in a murine model of chronic myeloid leukemia. *Blood* 105:3995–4003.

Advancing the Understanding of CML Progression

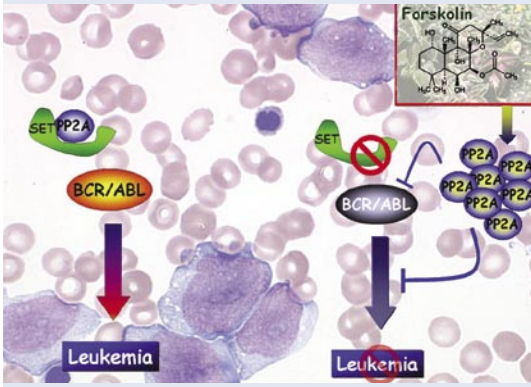
Danilo Perrotti, M.D., Ph.D., The Ohio State University, Columbus, Ohio

Underlying mechanisms of CML progression from the chronic phase to the blast crisis stage are not well understood and probably involve changes in a range of cellular and molecular processes. For example, changes in the regulation of mRNA metabolism may correlate with CML progression. Expression levels of hnRNP K, hnRNP E2, hnRNP A1, and La, four RNA-binding proteins involved in the regulation of mRNA processing and translation, are increased in BCR/ABL-expressing myeloid cell lines and in primary cells from CML patients in the blast crisis stage. Danilo Perrotti, M.D., Ph.D., of The Ohio State University, designed a research program focused on evaluating the effects of the BCR/ABL protein, the oncogenic tyrosine kinase responsible for leukemic transformation of the hematopoietic pluripotent stem cell and found increased in CML blast crisis patients, on mRNA metabolism. In FY02, Dr. Perrotti received a DOD CMLRP Investigator-Initiated Award for research that evaluates the role of RNA binding proteins in CML

Using CML-derived BCR/ABL-expressing cells, Dr. Perrotti proposed to use Ribonomics to identify hnRNP-associated mRNAs that may contribute to the blast crisis CML cell phenotype. The Ribonomics screen demonstrated that La was associated with the mRNA of the protein phosphatase 2A (PP2A) regulatory subunit B alpha (PPP2R3A) and that another RNA binding protein hnRNP A1 was associated with the inhibitor of PP2A (I2PP2A; SET), suggesting that expression and function of PP2A might be regulated during transition of CML into blast crisis. Dr. Perrotti and his colleagues demonstrated that expression of active PP2A, through either molecular or pharmacologic means, leads to the dephosphorylation, and therefore inactivation, of regulators that are important for the proliferation and survival of CML progenitor cells. Moreover, pharmacologic activators of PP2A significantly prolonged survival of mice injected with leukemic cells bearing the imatinib-resistant T315I BCR/ABL mutant. These data suggest that new CML treatments may be possible through the development of therapeutics aimed at upregulating PP2A activity in BCR/ABL-positive CML.

Please refer to the following publication for additional information about this research:

Neviani P, Santhanam R, Trotta R, et al. 2005. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity ot the BCR/ABL-regulated SET protein. *Cancer Cell* 8(5):355–368.



"Being a member of the CMLRP Peer Review Panel was an extraordinarily rewarding experience. The panel members were a broad mix of researchers interested in CML: basic scientists, clinical scientists, and consumer reviewers. This breadth brought new perspectives to the review process and allowed exceptionally good ideas to be supported, not just the status quo. The panel was not just "insiders," but thinkers with very different perspectives—all with the same goal. We were able to accomplish a great deal of work with efficiency and fairness. New and established researchers were given equal priority in this egalitarian system. I was proud to be part of this panel and am looking forward to reading the results of the research supported by this worthwhile effort."

Gwen L. Nichols, M.D., FY03–05 CMLRP Scientific Peer Review Panel Member

"The advances from the Human Genome Project, information technology, and basic science research have converged on the study of human cancer. The work of medical scientists in this field has never been more exciting, creative, and full of potential. The importance of this work reaches everyone. The Army's CDMRP program in general, and the CMLRP in specific, offers research another essential avenue of funding for innovative research. The peer review by top-notch scientists insures the quality, and ultimately the productivity, of the science. The program is a dramatic example of how government programs can make a positive benefit to society at large."

Jerald P. Radich, M.D., FY04–FY05 CMLRP Integration Panel Member

BOTTOM LINE

Since FY02, the DOD CMLRP has been responsible for managing $17.75M in congressional appropriations, resulting in 36 awards through FY04. Projects funded by this newly established program are anticipated to improve the understanding of the basic science of CML, advance the diagnostic and therapeutic approaches to CML, and enhance the quality of life for individuals and their families living with the disease. Research highlights, award data, and abstracts of funded CMLRP proposals can be viewed on the Congressionally Directed Medical Research Programs website (http://cdmrp.army.mil).