

## FAMILIAL CLL:

Do microRNAs hold key to genetic predisposition?



**F**amily members of CLL patients are more likely to develop CLL or other types of cancer than expected by chance. Evidence suggests that approximately 10% of chronic lymphocytic leukemia (CLL) patients have an inherited genetic predisposition for the disease. Familial CLL is often characterized by three affected members in two generations, earlier onset and more severe disease in next generation, and increased frequency of secondary tumors.

Several collaborative groups are actively conducting research on the genetic predisposition of CLL in order to identify gene(s) responsible for the development of CLL in familial cases. These groups are collecting blood and tissue to perform a variety of studies to identify candidate genes. If genes are identified, they will be evaluated for mutations or loss of controlling function. The hope is that eventually specific genes and mutations will be identified to allow for screening of family members to evaluate for possible risk such as in breast cancer.

Dr. George Calin and his colleagues at the Ohio State University have been working to identify the genes behind genetic predisposition to CLL. Recently identified, microRNAs are very small genes, approximately 20 to 22 nucleotides. Rather than coding for proteins, microRNAs interact and destroy the message of several protein coding genes.

Dr. Carlo Croce and Dr. Calin previously reported that two microRNAs, mir-15 and mir-16-1, are located at a deletion in chromosome 13 which is known to be associated with the familial risk of CLL. (The deletion is found in greater than 50% of both sporadic and familial B-Cells.) A mutation in mir-16-1 gene is influencing the level of this microRNA's production in the cells and decreasing the efficiency of downregulation of BCL2 antiapoptotic protein.

Calin and colleagues recently screened 97 patients and identified three microRNA "signatures" that were able to differentiate good from bad prognosis CLL. The pattern of microRNAs was different in patients with familial CLL compared with patients that have no known family history of cancer.

Calin continues to work on identifying the unique microRNA signatures associated with prognosis and disease progression in CLL. Mutations in microRNAs are frequent and may have functional importance and an association with higher risk to familial history of cancers in CLL patients.

The next step for the study is to use computer-assisted research and various experimental approaches to study how microRNAs act on different targets and what cellular mechanisms are altered. This will identify a list of possible molecules that may have important therapeutic implications. ::

## The promise of PROGNOSTIC FACTORS & FAMILIAL LINKS



**I**n many ways, 2006 was a consolidation year for clinical research in CLL.

We began to see the results of previously initiated studies. Studies of chemotherapy combined with monoclonal antibodies are demonstrating a distinct improvement in

achieving remission, the duration of remission, and a significant extension of survival compared to regimens or protocols of the past. Mini-transplants or reduced intensity conditioning transplants are effective even in patients with far advanced disease and help those patients who would not benefit significantly from traditional approaches to treatment.

The results of new regimens were presented at the American Society of Hematology meeting in Orlando in December. Mature information on the pentostatin, cyclophosphamide, and rituximab (PCR) regimen from Mayo Clinic showed that this is a well-tolerated regimen even in older patients. Two new agents are demonstrating significant promise. Investigators at both Roswell Park and M. D. Anderson Cancer Center demonstrated that lenalidomide is active even in CLL patients with advanced disease, a large amount of prior therapy, and adverse genetic features. Flavopiridol is also demonstrating marked activity. While this drug is complex to administer, it shows promise as a new strategy for dealing with fairly advanced patients.

The single dose gene therapy protocol is advancing and the study has completed enrollment. A phase II study of multiple doses should open in the next few months.

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# CAN T CELLS BE TAUGHT TO RECOGNIZE AND kill leukemic cells?

**P**rofessor John Gribben and his colleagues at St. Bartholomew's Cancer Center and the Royal London Hospital are teaching a different constituency. Their pupils are not students but rather T cells. Gribben is working to educate T cells from patients to recognize and kill CLL cells. Eventually these educated T-cells may replace current transplant technologies. Existing allogeneic transplantation technologies have proven effective. However, there is a significant risk that a patient will develop graft vs. host disease because of the use of foreign cells during the transplantation.

In an allogeneic transplant, patients receive a variety of cells from siblings or unrelated, matched donors. Following stem cell transplantation, the donated immune T cells are able to recognize CLL proteins as foreign and subsequently an immune reaction is stimulated to kill the CLL cells. This effect is known as graft vs. leukemia.

Gribben hopes molecular techniques will identify the proteins that are recognized by the T cells. He then plans to expand the donor immune cells in the laboratory and show that these cells are capable of killing CLL cells.

Gribben and his colleagues demonstrated that donor derived T cells in patients that have undergone allogeneic transplant are capable of "seeing" a protein expressed only by the CLL cells. Subsequently, Gribben has expanded these cells in the laboratory and demonstrated that these cells can recognize and kill the patients' CLL cells. These experiments demonstrate that it is possible that donor cells are capable of recognizing "tumor-specific" proteins.

Gribben plans to use a mouse model of CLL to determine if these expanded cells can be given back safely. These pre-clinical studies could then lead to a clinical trial. In the proposed clinical trial, blood samples would be obtained from matching donors. The CLL specific immune cells would be expanded outside the body and then infused back to CLL patients that have undergone stem cell transplants and still have evidence of disease. The approach could lead to an increase in the cure rate for CLL after stem cell transplantation, as well as a decrease in the risk of the procedure. The immune response will be generated using the patients own cells rather than foreign cells.

In addition to Professor Gribben's work, CLL Global Research Foundation is supporting gene therapy studies of Dr. William Wierda. Dr. Wierda's ongoing studies transfer a small amount of genetic information into the leukemia cells using a virus which cannot multiply but passes on genetic information causing a variety of leukemia cells to be recognized by the immune system. The next step for improving the gene therapy technology is to combine the gene therapy with the educated T-cell approach. Improving immunotherapy is a major focus of the CLL Global Research Foundation. In future issues of CLL Research Momentum, we will share our efforts to encourage collaborative research in this arena. ::

# mapping genes:

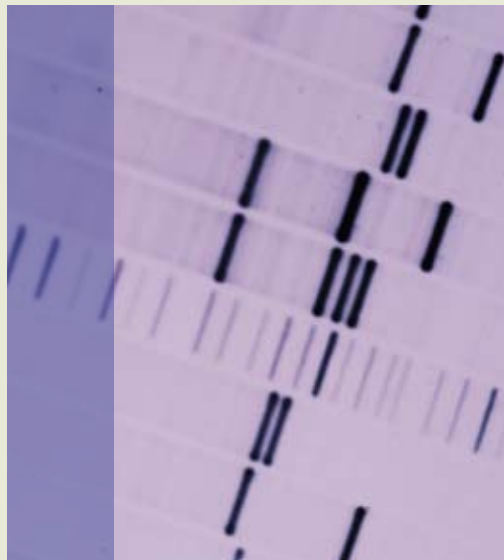
## THE PROMISE OF SMALL GENETIC DIFFERENCES

Scientists continue to search for clues to predict the course of CLL. Dr. Claire Dearden and Dr. Gareth Morgan at the Institute of Cancer Research in the United Kingdom are hoping to identify small genetic changes that may influence disease progression and prognosis. They are using novel high resolution gene mapping technology to identify the genetic alterations.

Dearden and Morgan are using comparative genomic hybridization (CGH), a more sensitive molecular technique than fluorescence-in-situ hybridization (FISH) or conventional chromosome analysis. CGH can better define genetic changes and detect smaller chromosomal changes.

CGH is used to examine the deletion of the P53 gene on chromosome 17. This deletion and genetic alterations on chromosome 11 are known to be poor prognostic factors. Several other recurring changes are being identified. These mutations appear as the disease evolves. Identifying these vulnerable genetic areas will help to detect evolving resistant clones of cells.

Drs. Dearden and Morgan are using clinical samples from a recently closed clinical trial in the U.K. of 580 patients to perform laboratory research to identify the genetic events occurring in 'high risk' CLL. As this technology is linked to a real clinical trial, the data will have major clinical relevance. An improved understanding of CLL prognostic markers should lead to better designed clinical trials as well as the ability to select treatment strategies based on risk-stratification. ::



# epigenetic profiling:

## UNDERSTANDING THE IMPORTANCE OF EPIGENETIC CHANGES

The genetics of CLL (and cancer in general) have become increasingly complicated. We are making research advances; however, we are learning not only that the genetics of the disease are very complex but also that epigenetic alterations (gene modifications) play a role in CLL.

Dr. Guillermo Garcia-Manero has led an investigation on methylation that includes a large scale epigenomic profiling of CLL. He is pursuing the identification of new targets of DNA methylation (addition of methyl groups to specific regions of the genome) that may allow the discovery of new tumor suppressor genes in CLL.

To do so, he has used a new technology to scan hundreds of genes. In his first experiment, he used DNA from patients with chromosome 17 alterations. The intention is to identify poor prognosis genes. So far, his group has identified 300 genes for which methylation had not been previously described in CLL.

This is important because drugs which have not traditionally been used in CLL will now enter into clinical trial to try and control the expression of these key genes. These drugs include hypomethylating agents which decrease methylation and HDAC inhibitors which increase acetylation. These drugs may allow the re-expression of genes (such as tumor suppressor genes) that may be turned off when cells become malignant. The CLL Global Research Foundation is also supporting the investigation of HDAC inhibitors by Dr. Deepa Sampath (M. D. Anderson Cancer Center). Sampath is studying the mechanism of action of HDAC inhibitors in leukemia cells. ::

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During the past five years, studies have demonstrated that prognostic factors such as CD38 mutation status, ZAP-70 and beta-2-microglobulin are important in assigning prospects of long term control to CLL patients. As studies mature, the data shows an impact on the time to needing treatment, response to treatment, and remission duration after treatment. These prognostic factors will undoubtedly be used to develop our strategies in the future.

The study of familial CLL will likely drive the development of future treatment strategies. A recent grant by the National Cancer Institute has led to a significant study on the familial nature of CLL. While the genes responsible for familial CLL have not been identified, national studies will be able to identify high risk families so appropriate counseling can be given to patients concerned about their siblings, children, etc. Collaboration with Europeans in this activity is ongoing, and with the genetic tools available to us at the present time, I anticipate that in the next five years the genes involved with familial CLL will be identified. Replacement gene therapy should then be possible. ::

**Dr. Michael J. Keating**

Dr. Michael Keating, Professor of Medicine at M. D. Anderson Cancer Center, serves as president and CEO of the CLL Global Research Foundation. He is an internationally renowned CLL clinical scientist dedicated to patient care and to development of potentially curative CLL therapies.



## TARGETING ZAP-70 TO DISRUPT

# CLL cell survival



**P**atients with aggressive CLL aberrantly express ZAP-70 an enzyme which makes CLL cells grow. Leukemia cells that express ZAP-70 undergo stronger cellular activation and grow faster than ZAP-70 negative CLL cells. This suggests that abnormal expression of ZAP-70 provides a growth factor stimulus to CLL cells that may drive disease progression.

Dr. Januario Castro and colleagues at the University of California, San Diego, recently found that activated ZAP-70 becomes a companion enzyme for another chaperone protein called heat shock protein 90 (HSP90). ZAP-70 expression level is known to identify patients needing treatment earlier in their course.

Present indications are that ZAP-70 is required for cell survival. This activation occurs through the B cell receptor (BCR) present on CLL cells in poor prognosis CLL patients. HSP90 is necessary for ZAP-70 expression and activity exclusively in

CLL B cells but not in normal B or T cells, suggesting that HSP90 inhibitors could be valuable therapeutically in high risk CLL without suppressing the immune system.

Linking this to patients, a clinical trial is underway of 17-AAG, a targeted HSP90 inhibitor. HSP90 inhibitors including 17-AAG have been under clinical study in cancer for almost two decades.

Recent data by Castro and colleagues shows that HSP90 inhibitors promote specific degradation of ZAP-70 in CLL and the CLL cells die by apoptosis. CLL cells that express activated HSP90 complex are more sensitive to HSP90 inhibitors such as 17-AAG. Castro and colleagues tested 110 samples from patients at time of CLL diagnosis and found that activation of the HSP90 protein is an independent prognostic factor. Even in patients with good prognostic factors (such as low expression of ZAP-70), the presence of HSP90 indicates as a more aggressive clinical course. ::

## CD38 AND THE FATE OF CLL CELLS: innocent bystander or culprit?

**C**D38 is a protein which is present on the surface of CLL cells. High levels of expression of this protein are associated with a more aggressive form of disease. Dr. Silvia Deaglio and Dr. Fabio Malavasi at the University of Torino have demonstrated that CD38 activation is very important in allowing ZAP-70 to function. ZAP-70 is a protein that regulates the growth of CLL cells. This is the first time that linkage between ZAP-70 activation in CD38 engagement has been demonstrated. CD38 activation of ZAP-70 results in CLL cells which are more likely to grow or proliferate. This makes the disease more rapidly moving.

However, it also raises the possibility that the CLL cells will be more susceptible to drugs which are active in cells moving rapidly through the cell multiplication phase. It is clear that CD38 can be activated by proteins present not only on the cells themselves but on the surrounding supportive stromal elements. Antibodies against CD38 are being developed and will be studied in clinical trials.

In addition to Dr. Deaglio's work on CD38 expression and kinetics or growth of CLL cells, CLLGRF is also supporting the kinetics research being conducted by Dr. Nicholas Chiorazzi at the Feinstein Institute for Medical Research. Both Deaglio and Chiorazzi's findings should facilitate the identification of patients more or less likely to respond to inhibition of ZAP-70. ::

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