The CLLGRF U.S./European Alliance participants certainly have a “can-do” attitude. The Alliance is off to an outstanding start with tremendous energy being placed in new drug development, exploring the impact of the CLL cellular environment, and using the immune system to improve outcomes and potentially lead to cure of the disease. The Alliance has generated significant momentum, and we have sufficient support committed to maintain the program for the next year. Continuation beyond that will be determined by our success in attracting gifts to support the research.

CLLGRF is committed to ensuring accountability of our research. Other research environments fund investigators for three to five years with only cursory oversight of productivity in subsequent years. In contrast, CLLGRF has maintained that a second year of funding is not guaranteed without sufficient progress being made.

We are unquestionably accountable to you, our donors. We aim to provide accurate and timely information on the progress of the research, and we will continue to update you through this newsletter and our website. I can enthusiastically state that this is the best time for CLL research. The future has never looked better, and CLLGRF is committed to accelerating progress to limit the suffering and loss of life caused by this disease.

Who will be responsible for CLL research?

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Cancer can be a genetically linked disease; chronic lymphocytic leukemia (CLL) is no exception. Researchers study the genetics of cancer by evaluating chromosomes. Chromosomes are made up of DNA which contains genes that carry genetic codes of information. This information directs the development and function of the body. Individuals normally have 23 pairs of chromosomes, all of which code for different traits and functions. By evaluating genes and chromosomes, researchers are able to figure out the root cause of cancer and other diseases. However, CLL cells seldom divide, making the traditional evaluation of chromosomes for genetic purposes difficult.

Until the last decade limited genetic research was available on CLL. Development of new technology such as fluororescent in situ hybridization (FISH) now allows researchers to evaluate the frequency of some commonly described genetic abnormalities that have been found in patients with CLL. These abnormalities most frequently involve chromosomes 11, 12, 13, and 17.

Historically, chromosome 17 abnormalities, and to a lesser degree chromosome 11 abnormalities, were associated with a poor response to treatment and short survival. Patients with a chromosome 12 abnormality had a similar prognosis to those with no abnormality. Abnormality to chromosome 13 alone was considered to be somewhat better than other abnormalities associated with CLL. Ongoing research is better defining chromosomal characteristics, and identifying new chromosomes that add to the understanding of CLL characteristics.

**CHROMOSOME 11**

Chromosome 11 contains genes that play a role in the DNA repair process and that regulate abnormal cell growth. In 10-15% of patients with CLL, important DNA repair genes present on the long arm of chromosome 11 are lost. This loss is generally associated with very large lymph nodes and extensive disease. Combination regimens, fludarabine and cyclophosphamide (FC) and FC+ rituximab (FCR), have significantly reduced the negative impact of this chromosomal abnormality.

**CHROMOSOME 12**

Some CLL patients have three number 12 chromosomes instead of two, an abnormality known as trisomy 12. This abnormality makes the cells look somewhat unusual for CLL. Patients with trisomy 12 do not generally exhibit the chromosomal changes commonly associated with CLL.

The overall outcome of patients with trisomy 12 is similar to those who have no abnormalities on FISH testing. Trisomy 12 is generally associated with an increased likelihood of eventual disease.

**Left: Cytogenetic analysis of CLL patient chromosomes with aberrations on chromosomes 8 and 14. Right: Floresent In Suto Hybridization (FISH) detection of chromosomes 8 and 14. The top portion of the photograph shows chromosomes after they have divided. The bottom of the photograph shows cells with non-dividing chromosomes.**
progression and need for treatment. These patients have a very high expression of CD20, which is the target for rituximab and other emerging antibodies such as ofatumumab and GA-101. These drugs may prove to be very beneficial to trisomy 12 patients.

Patients with trisomy 12 often have abnormalities involving chromosomes 6, 8, 14 and 19. Individuals with trisomy 12 alone tend to exhibit a better clinical response than those with the less common abnormalities. In the next two to three years it is likely that there will be treatments specifically targeting these abnormalities.

CHROMOSOME 13

A normal chromosome 13 suppresses the development of tumors. At the time of initial presentation, more than two thirds of CLL patients have loss of genetic material on chromosome 13. Unsuccessful efforts have been made to explain the loss of genetic material in CLL patients.

A collaboration among investigators in the Clinical Research Consortium (CRC), including Dr. Carlo Croce of Ohio State University, Dr. Kanti Rai at Long Island Jewish Medical Center, and Dr. George Calin (previously at OSU, now at M. D. Anderson Cancer Center) identified a region on chromosome 13 that is associated with the loss of genetic material. It was suggested that the missing genetic material might be linked to microRNA genes.

MicroRNA genes were first discovered in worms in 1993, and later found in other species. When discovered in humans, microRNA was considered to be “junk DNA” (DNA with no function) because these genes were so small and did not code for anything. Almost 10 years later, the investigators mentioned above began looking at microRNA in CLL. They found that two microRNAs, 15 and 16, were lost from chromosome 13. CLL is considered to be a genetically silent disease. Therefore, it was an unexpected breakthrough that CLL would lead to the conceptual understanding of microRNAs role in regulating cancer.

CHROMOSOME 17

The loss of information on the long arm of chromosome 17 (17q deletion) is often associated with a mutation in a very important regulatory gene, p53. A properly functioning p53 gene is needed for a beneficial response to chemotherapy and radiation. Previously, chromosome 17 abnormalities did not indicate a positive prognosis. Now, many early stage patients with a 17q deletion show no evidence of progression for long periods of time. The true impact of the loss of 17q continues to be defined.

The path to understanding the role of genetics in CLL will not be straight or short. However, exciting breakthroughs will come at a steady pace. Eventually researchers will understand what causes CLL to occur and to progress. In the meantime, the role of clinicians is to develop treatments that will cure the disease without necessarily understanding all the genetic ramifications of the disorder.
Are treatment decisions determined by my genetic factors?
Genetic factors can help predict rapid disease progression and the requirement of early treatment intervention; however, these decisions are always made in conjunction with a patient’s clinical features including symptoms, blood counts and evolution of disease. Recently it has been found that genetic factors may also help in determining the type of therapy to use for a greater likelihood of response.

Am I born with the genetic abnormalities for CLL or do they develop? What causes the development?
Although this is not completely known, most people likely acquire genetic abnormalities throughout their life. The causes for these changes can be varied. During the DNA replication process (which occurs many times a day in blood cells), genes sometimes make mistakes when copying the genetic material. Problems can also evolve in the structure of the genes. Some changes may occur randomly while others may be caused by external or environmental factors. Although environmental factors seem like an attractive explanation, no specific environmental factor has yet to be convincingly identified.

Is there a classification of subgroups in CLL based on genetics?
At the moment, clinical features are generally used for CLL subgroup classification. A standardized form of genetic classification is needed to better understand which patients should be treated earlier, who can be observed, and what treatments would most benefit patients. This form of classification is in developmental stages as newer technologies are improving the understanding of CLL genetics.

Can my prognostic markers change?
It is possible that a patient’s prognostic markers may change over time. Some people will develop new genetic abnormalities if they have progressive disease. However, the majority of CLL patients with a good prognosis will have slowly progressive or non-progressive disease and their prognostic markers are unlikely to change significantly.

What is cytogenetics?
Genetic material is packaged into cells in the form of chromosomes. Cytogenetic tests examine all 23 pairs of chromosomes to look for genetic and molecular abnormalities that may be associated with a malignancy. This test provides limited information relative to CLL because it has to be performed on cells preparing to divide, and CLL cells generally do not divide. Cytogenetics does provide useful information in a subset of CLL patients with complex abnormalities that are not detected by FISH tests.

Why are fluorescence in situ hybridization (FISH) tests useful in CLL?
FISH tests look at specific chromosomes commonly linked to a malignancy. Fluorescent light is used to determine if specific genetic abnormalities are present on these chromosomes. The features identified help in deciding the best course of treatment. Unlike cytogenetics, FISH tests can be applied to chromosomes during any phase of cell division. FISH is also more sensitive than cytogenetics, and therefore more likely to pick up an abnormality if present.

How is flow cytometry used?
Cells have tags on them called surface markers which are identifiable to other cells and molecules in the body. Flow cytometry analyzes these surface markers. CLL cells have a very characteristic set of markers on their surface, and flow cytometry helps distinguish CLL from similar lymphocyte disorders, making it an important test for diagnosis. Flow cytometry can also offer useful prognostic information (measurement of CD38 and ZAP-70) and is useful in determining if patients have any residual disease in their bone marrow after chemotherapy.

What is IgVH?
Immunoglobulin gene variable heavy (IgVH) chain is a gene in lymphocytes. IgVH rearrangement occurs as lymphocytes mature. Maturing lymphocytes undertake a high number of random mutations to allow the immune system to recognize a wide variety of antigens. The cell stores a library of these mutations so antigens can be easily recognized and destroyed. The IgVH mutation process is part of the normal function of the immune system to recognize foreign organisms.

Q & A:

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Why is IgVH mutation a good prognostic factor?

Patients with a mutated IgVH are capable of recognizing a larger number of antigens and are genetically stable, resulting in a lower likelihood of disease progression. CLL cells with unmutated IgVH are likely to be more immature and genetically unstable, thus associated with a higher chance of progressive disease.

Why does a mutation to chromosome 11 cause enlarged lymph nodes?

A number of genes on chromosome 11 are involved in the regulation of abnormal cells. When these genes become mutated or deleted in CLL cells, the abnormal cells (in this case lymphocytes) are allowed to grow uncontrollably. This uncontrolled growth forces the lymph nodes to enlarge.

What causes a patient to be refractory to chemotherapy?

Most chemotherapy drugs target essential functions of a cell that help them divide or grow. CLL cells seldom divide and can also develop tricks to bypass these functions. They may repair the damage caused by chemotherapy drugs, use alternative mechanisms to grow or they may develop proteins to pump the chemotherapy out of the cell. Some of the genetic changes that underlie the malignancy can also help the cell to fight the effects of chemotherapy.

Why does CLL generally not show up until age 50 or older?

Most malignant disorders increase with age. The immune system is constantly growing and dividing, and is generally kept in check by regulatory genes and self-regulation. Unfortunately, mistakes are made and occasionally these mistakes take place in genes that can predispose to cancer. Since it is believed that more than one event must occur to produce diseases like leukemia, it takes time for CLL mutations to accumulate. Also, it appears that the immune system may be less able to control the malignant cells as people get older.
The combination treatment of fludarabine, cyclophosphamide and rituximab for CLL was developed at M. D. Anderson Cancer Center in July 1999. A decade later, M. D. Anderson will document the first ten-year complete remission. The accompanying story, FCR- Putting it all Together, on page 6 describes how the regimen was developed.

The FCR regimen has achieved results not previously seen in CLL patients. Although met with initial skepticism from the research community, preliminary outcomes led to interest in initiating studies to demonstrate FCR's benefit for a greater number of patients. The results from these studies are beginning to emerge, confirming the claims of the superiority of FCR.

The German CLL Study Group led a randomized comparison of fludarabine and cyclophosphamide (FC) versus FCR in relapsed and untreated patients. At the American Society of Hematology (ASH) meeting in San Francisco in December 2008, the study results demonstrated a significantly higher complete response rate, overall response rate, and longer progression-free survival in the FCR group. The addition of rituximab was not associated with any significant increase in toxicity.

Dr. Tadeusz Robak from Poland presented a comparable study in patients with relapsed disease. His study exhibited a similar advantage of FCR compared to FC. These two randomized comparisons of FCR versus FC in Europe demonstrated that FCR is unmatched at the present time. There is still some discussion as to whether FCR is better than the combination of fludarabine and rituximab. Studies are ongoing in the U.S. to address this question.

The pentostatin, cyclophosphamide, rituximab (PCR) regimen, developed initially at Memorial Sloan-Kettering and later amplified at the Mayo Clinic, has attracted significant interest. This regimen was said to have a lower toxicity than FCR and still be as effective. A randomized clinical trial conducted in the United States demonstrated a higher complete and overall response rate and lower toxicity for FCR compared to PCR.

For the first time there is a growing consensus that FCR is the standard against which all other studies should be compared. Is FCR the best treatment for everyone? Probably not. There is little information in patients over the age of 70. Does FCR work in patients with deletion of 17p chromosome and mutation of the p53 gene? While FCR may not be as beneficial in this patient population, it may still be the best treatment option currently available.

Beyond FCR, other agents have recently been introduced into the overall strategy of CLL. Bendamustine was recently approved by the FDA; alemtuzumab is now being used for frontline treatment, and lenalidomide is undergoing extensive investigation. The new monoclonal antibody, ofatumumab, has been submitted for approval for treating CLL. Drs. Anders Österborg and William Wierda, both members of CLL Global’s U.S./European Alliance, provided leadership for the pivotal studies of ofatumumab. Additional research is needed to better understand these compounds.

Many of the new agents are being evaluated in conjunction with FCR. The cost effectiveness of the FCR regimen is also being evaluated. Healthcare organizations and insurance carriers in the U.S. and around the world are looking at the cost effectiveness of the addition of rituximab to chemotherapy. Roche in Europe undertook two studies looking at FCR compared to FC and other regimens. The studies demonstrate that FCR is one of the most cost effective regimens in oncology. This type of information convinces healthcare providers that FCR is not only beneficial, but lowers the cost of healthcare for their patients.

Is FCR a Gold Standard? Gold is probably too strong of a word at the present time. A Gold Standard will be achieved when the majority of CLL patients are cured of their disease, as has occurred in childhood leukemia since the mid-1960s. There is no reason why this occurrence cannot be replicated in adults with CLL.
Have you ever wondered how physicians and scientists develop combination regimens? It certainly is not as easy as mixing together ingredients in a recipe or cocktail. Years of research and insight are often needed. The FCR program was developed based on the acumen of several scientists who integrated results from precursor studies. The initial study, conducted many years ago, evaluated fludarabine in patients with all leukemias and lymphomas. The study showed that fludarabine was a powerful new agent in low grade lymphomas, CLL, and Waldenström’s macroglobulinemia.

Based on his laboratory expertise, Dr. Bill Plunkett proposed the combination of fludarabine and the alkylating agent cyclophosphamide (FC). Cyclophosphamide kills cells by damaging DNA. Dr. Plunkett’s studies showed that cells become resistant to cyclophosphamide by repairing the DNA damage. Knowing that fludarabine inhibits the DNA repair process, Dr. Plunkett, the chair of CLLGRF Scientific Advisory Board and Alliance member, put forward the FC combination. Early exploratory studies demonstrated its effectiveness, and three major clinical trials confirmed the usefulness of the FC regimen.

Subsequently, the monoclonal antibody, rituximab, was investigated as an alternative method in eliminating CLL cells. However, the response was dismal in CLL patients. Low doses were given during initial studies, and it was thought that patients might not be getting enough of the drug. Dr. Susan O’Brien, Scientific Advisory Board and Alliance member, conducted a single-agent study to evaluate whether increased doses of rituximab would produce a higher response rate. She demonstrated that increasing the dose improved the response rate from 15% to 75% and was still well tolerated by patients.

Dr. Michael Keating, CEO of CLLGRF, believed that combining rituximab with FC would be an optimal regimen for CLL patients, and he was right! By putting together the correct dose and schedule, the FCR regimen was born. Complete response rates rose from 30 – 40% with FC to 70% with FCR.

While insight and previous studies were important elements in the development of FCR, the willingness of patients to participate in the clinical trials was the real key. A decade has now passed since the FCR regimen was piloted; the long term follow-up statistics of FCR patients show a very significant improvement in complete response rate, time to disease recurrence, and overall survival for CLL patients.

FCR is considered the best treatment option for most CLL patients. A window of opportunity exists to build upon FCR by rebuilding and educating the immune system to prevent recurrence of disease. As new therapies are brought to the table, it is very likely that FCR will continue to play an important role in decreasing the amount of leukemia.

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**Microbeads- the jewel to stimulate immune system recovery?**

A Phase I multi-center trial is now open evaluating whether laboratory processed T-cells will help CLL patients’ immune system recover faster after chemotherapy. Both the University of Pennsylvania (UPenn) and M. D. Anderson Cancer Center (MDACC) are currently enrolling patients.

CLL patients often develop weakened immune systems, particularly after treatment. This puts CLL patients at greater risk of infectious complications. It is hypothesized that the infusion of CD3/CD28 expanded and activated T-cells, given after treatment, may lower the chance of infections.

For this study, a patient’s T-cells are collected prior to initial treatment and frozen until needed. The patient then receives treatment with either fludarabine or alemtuzumab-based chemotherapy. Patients that respond to chemotherapy will be infused with a modified version of their own cells once the chemotherapy administration is completed. In the laboratory the frozen T-cells are thawed and co-stimulated with CD3/CD28 microbeads and infused back into the patient.

CD3 and CD28 are proteins found on T-cells that do not function properly in CLL patients. The microbeads contain antibodies which attach to CD3 and CD28 on T-cells and activate and expand them. By activating the T-cells, the microbeads turn on the anti-cancer and anti-infection activity and allow T-cells to multiply at an accelerated rate. Properly functioning CD3 and CD28 allows the immune system to better fight infection and cancer.

The trial is being supported by the CLL Global Research Foundation’s (CLLGRF) U.S./European Alliance program. Drs. Chitra Hosing (MDACC) and Stephen Schuster (UPenn) are chairing the study. The CD3/CD28 microbeads were created and are being supplied by Dr. Carl June (UPenn). Drs. Elizabeth Shpall (MDACC), Bruce Levine (UPenn), and John Gribben (Bart’s, UK), are collaborating in the research.

Similar clinical trials have been conducted in patients with other hematologic malignancies. These studies show the treatment was well tolerated and results were favorable. The hope is that CD3/CD28 microbeads will result in rapid immune recovery, reduced rate of infectious complications, and delayed disease progression for CLL patients.

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**Find out more about the projects we are accelerating at** [www.cllglobal.org](http://www.cllglobal.org)