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CLL RESEARCH Momentum

MOVING CLL RESEARCH FORWARD – A PROGRESS UPDATE

FALL 2007

INTERRUPTING THE FOOD CHAIN

for CLL cells

Jan Burger's project deprives CLL cells of nutrition. He is exploring the nutrients that CLL cells require for survival. For a number of years we have known that the environment in which CLL cells live (in the bone marrow, lymph nodes and spleen) is important in keeping them alive.

In test tubes outside the body, CLL cells die very rapidly- often in two to three days. In the body the cells survive for 100 days. Dr. Burger, while working with Dr. Thomas Kipps at the University of California San Diego, demonstrated that a type of cells called nurselike cells could sustain CLL cells for a long time. The challenge has been to explore how the nurselike cells function and, from a treatment point of view, how this chain of nutrition can be interrupted.

In his project supported by CLLGRF, Dr. Burger has found an attraction factor called CXCL13 which is secreted by the nurselike cells and attracts CLL cells via corresponding CXCR5 receptors abundantly present on CLL cells. This mechanism allows for CLL cell attachment to the nurselike cells, where CLL cells are nourished with CXCL13 and other nutrients. This is an important mechanism for sustaining the life of the CLL cells. Interruption of the dialog between the nurselike cells and the CLL cells will lead to the CLL cells dying in the body or becoming much more vulnerable to other chemotherapy drugs and antibodies.

We have also known for a number of years that the other family of lymphocytes (T-cells) is important in maintaining the life of CLL cells. Dr. Burger, now at M. D. Anderson Cancer Center, has discovered with collaborators in Germany that the interaction between the CLL cells and nurselike cells activates the T-cell mechanisms. The activation of T-cells (key immune cells) is a fertile area for exploration of a totally new way to approach the treatment of CLL and should be a major improvement over the traditional chemotherapy drugs which damage DNA. Nurselike cells are more selective and have great promise for the future.

One of the limits of testing new drugs against CLL is that the cells die quickly when they are taken out of the patient's body (as mentioned above). By using nurselike cells, we prolong the survival of the CLL cells in test systems and create a more similar environment to what happens in the patient, which will facilitate the testing of new drugs. Dr. Burger's research on the micronenvironment should facilitate the discovery process of effective new agents in CLL. : :

inside this issue:

CURE CLL NOW

Many patients with CLL do not progress or need treatment. However, most patients who have *progressive* CLL die of their disease. In the past, it was said that most CLL patients die of some other disease. However, as management of diseases such as heart disease, stroke, high blood pressure and infectious diseases improves, more patients who require treatment for CLL will eventually die of some complication of their CLL. As our population ages, more and more patients with CLL will be diagnosed and need to be treated.

The recent deaths from CLL of public figures such as Ed Bradley and Tom Snyder crystallizes for many patients and their families that CLL is a life threatening condition. It creates fear that their life span will be significantly shortened. These fears are realistic.

However, this reality must be coupled with optimism. It is clear that new treatments have extended the life of patients with CLL who need therapy and, for most of their lives, the majority of patients are in excellent health. There are a multitude of new technologies to analyze CLL cells. The availability of new chemotherapy drugs, antibodies and immune modifying approaches has never been better. New transplantation technology has taken away much of the fear of transplantation. In its place, we now have realistic optimism about the cure of this disease in patients able to receive a transplant.

The stage is set. The tools are available. Patients are willing and anxious to participate in research that changes the whole concept of CLL. CLL Global Research Foundation (CLLGRF) continues in its mission to fund projects directly related to CLL patients.

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IS THERE A "universal" protein?

The amazing success of Imatinib (Gleevec) in chronic myelogenous leukemia is a result of its ability to neutralize a specific protein which activates the CLL cells. This protein is called a tyrosine kinase and results from the new hybrid gene formed in CML. The search for the presence of such a protein in CLL in the vast majority of cases has been disappointing.

Dr. Zeev Estrov (M. D. Anderson Cancer Center) has found that a protein called Stat-3 is activated in a very specific way in almost all CLL cases. The Stat-3 protein is important in inducing CLL cells to stay alive and multiply. This occurs when a phosphate is attached to a specific amino acid on Stat-3, resulting in its activation. Interfering with this activation pathway should lead to promising new treatments in CLL.

Dr. Estrov has initiated a clinical trial of 5-azacytidine for CLL. This drug has anti-leukemic properties and inhibits the activation of Stat-3. 5-azacytidine has been explored in the management of leukemia for 30 plus years, is well tolerated with minimal side effects and has shown to be active in myelodyplastic syndrome and acute leukemia. Data regarding the usefulness of this approach should be forthcoming in the next 6 to 12 months. This drug decreases methylation of genes which is a way of silencing several genes. Dr. Estrov's work interfaces nicely with Dr. Garcia-Manero's work on gene methylation. Dr. Garcia Manero, also a CLLGRF grant recipient at M. D. Anderson, has found consistent characteristic gene methylation patterns in CLL. These patterns represent an opportunity for further research. The intention is to identify poor prognosis genes and subsequently develop agents that control the expression of the key genes. : :

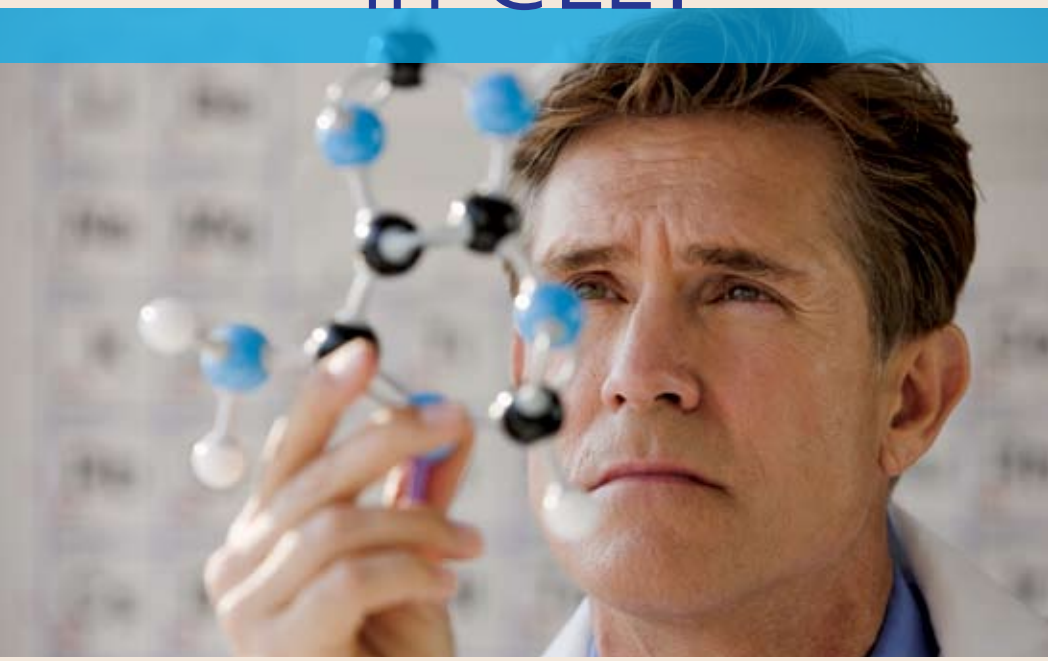
A COMPREHENSIVE genetic analysis

Richard Houlston, supported in part by CLLGRF, has applied a very powerful new technology called SNPs (single nucleotide polymorphisms) to characterize a large amount of genetic material in patients with CLL. Whereas 50 years ago it was thought that there may be a single dominant genetic characteristic driving malignant cells of all types, it is now known that multiple genetic abnormalities exist. Initial chromosome analysis helped us to identify key changes and fertile areas for exploration of genetic abnormalities.

We now know that genes can be switched on or off by a variety of mechanisms. There is a fine tuning process which occurs in regulating the expression of genes. The structure of a gene can be modified dramatically by very small changes in single nucleotides (building blocks of DNA). Thus, SNPs are a fertile way to investigate whether these changes occur in crucial parts of the gene.

Dr. Houlston (Institute of Cancer Research, United Kingdom) has recently conducted an extensive survey which is published in Blood (August 2007) to pinpoint areas which are important in identifying risk of having familial cases of CLL. However, it is also likely that the information gathered from such exploration will help to define patients who are likely or unlikely to respond to particular treatment. This powerful technology is being applied to a very well documented clinical trial (CLL4) in the United Kingdom. This protocol is also being used by another CLLGRF grantee at the Institute of Cancer Research, Dr. Claire Dearden, to investigate the role of various abnormalities in FISH cytogenetics and their function in establishing prognosis. Once again the integration of projects funded by the CLLGRF is helping to unlock weaknesses in the CLL cells. These weaknesses can be exploited to give us very important prognostic information that we can apply to the care of patients with this disease. : :

TCL1: A KEY MOLECULE in CLL?



TCL1 is a gene whose protein product is key in the development of CLL. For many years CLL research has been frustrated by the lack of a suitable mouse model to investigate new approaches to treatment and new understanding of the biology of CLL. The laboratory of Dr. Carlo Croce (Ohio State University) developed a mouse model where insertion of the TCL1 gene into mice would reproducibly cause a disease very similar to human CLL in the mice.

Dr. George Calin, a CLLGRF researcher now at M. D. Anderson Cancer Center, worked with Croce to establish that a unique set of genes called micro-regulatory genes (miRs) were deleted in many cases of CLL. This finding has led to an explosion in the search for the role of these mir genes in all cancers. Over 250 miR genes have been identified. The exploration of these genes has led to questions regarding what makes TCL1 vital in CLL and how it interacts with other findings. A CLLGRF project of Yuri Pekarsky (Ohio State) has found that two miR genes, miR-181 and miR-29, have a profound influence on TCL1 expression.

Another CLLGRF investigator, Dan Jones (M. D. Anderson), has found that TCL1 is over expressed in more aggressive forms of CLL (those with unmutated immunoglobulin genes and high expression of the ZAP70 tyrosine kinase), and that TCL1 functions to regulate growth signaling in both CLL and T-cell leukemias. TCL1's effects are mediated through activating interactions with the protein AKT which has a profound influence on regulating the growth and death of CLL cells.

It is apparent that understanding of this biologic pathway, initially in mice and more recently in humans, provides many opportunities for interfering with the well-being of CLL cells. The miR genes are very small. They can be manufactured and the potential exists for replacing the missing miR genes, such as miR-29 and miR-181, in CLL cells making them behave more like normal lymphocytes. The second feature is that if particular miR genes are causing the cells to behave badly by the over expression, chemicals which interfere with the activity of these miR genes could turn off the CLL cells, causing them to die.

Understanding that the TCL1 protein and the AKT protein are interacting closely suggests that chemicals that interfere with these pathways are likely to have a major effect not only in CLL but also other cancers. The possibility of developing these very targeted molecules to exquisitely block pathways is exciting. The reality will take a lot of work but the carrot is dangling in front of researchers who follow the TCL1/miR research area to lead to potentially curative strategies in the management of CLL. : :

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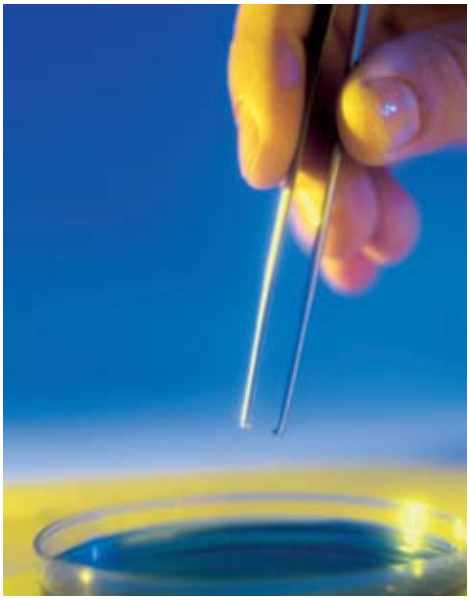
As president and CEO of the CLLGRF, I am angry. I am angry that this disease kills so many special people. I am angry that the familial nature of the disease puts fear in the minds of CLL patients and their families as to their future. I am angry that the pace of clinical research is too slow. I am angry that the regulatory requirement of research has become a massive burden which limits the willingness of brilliant people to continue in the research environment.

At the same time, I am optimistic that present-day CLL research, especially that funded by the CLLGRF, will dramatically change the present state of CLL so that patients can routinely be cured in the next five to ten years. All of you receiving this copy of *Momentum* would like to see this happen.

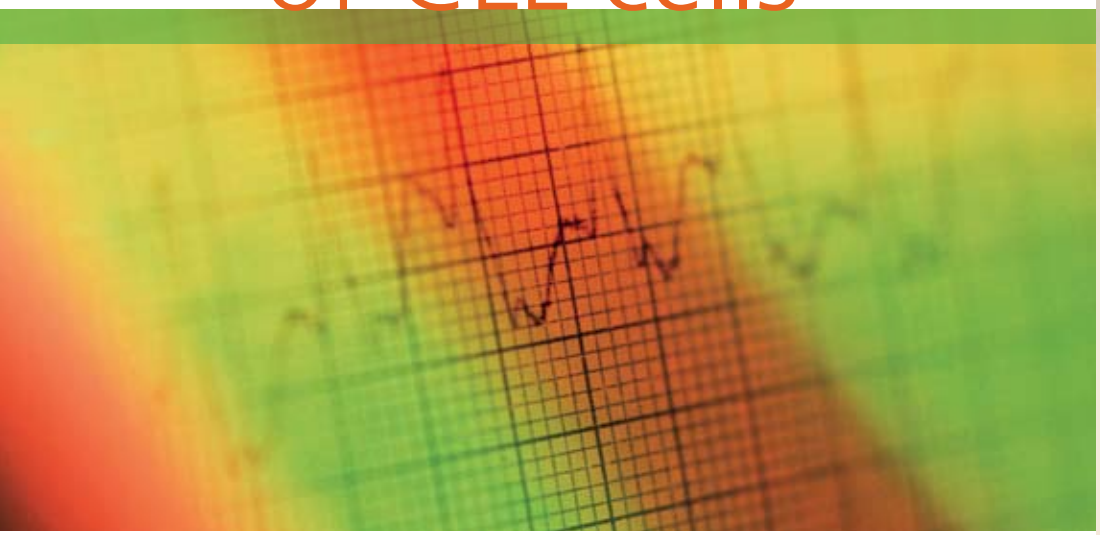
The CLLGRF offers you the opportunity to have a say in support of CLL research. We are grateful for the financial support that we have received from many of you. I encourage all of you to join our partnership by using whatever means are at your disposal whether it may be financial, community outreach, or volunteering to assist in the attack on CLL now. : :

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.



MEASURING THE BIRTH RATE of CLL cells



In recent times, it has become well understood that CLL cells live for a long time. The mantra is that CLL cells do not know how to die. However, we do know that there is a definite death rate of CLL cells, and without the help of T-lymphocytes, the disease would disappear. CLL cells will vary from patient to patient in the rate of turnover. The development of a new technology which uses heavy water (holds deuterium instead of hydrogen) to determine the rate of new cell development is truly fascinating.

Heavy water is incorporated into the DNA of dividing cells. It is not a radioactive molecule. As the patients drink heavy water, the deuterium is taken up into the DNA and patients with CLL can then be classified into those who have a very low birth rate of new CLL cells and those with a higher birth rate. This suggests that drugs which are effective in killing off dividing cells may be effective in CLL, whereas formerly it was thought because of a low growth rate of CLL cells that these agents would not be effective.

The growth rate analysis links to another area of Dr. Nicholas Chiorazzi's research, CD38 expression on the leukemic cells. Dr. Chiorazzi's group at the Feinstein Institute for Medical Research was the first to demonstrate that CD38, a protein on the surface of CLL cells and other cells, is high in patients with more aggressive disease. The heavy water studies helped delineate that the CD38-expressing cells, which are present in the patient, are the active cells which are dividing and manufacturing new progeny. Based on Chiorazzi's work, we can imagine that there is a group of CLL cells which are working hard dividing and making daughter cells. Eventually, these daughter cells accumulate in the body for long periods of time.

While a number of treatments may kill off the daughter cells, it is important that we address how best to destroy proliferating or growing CD38 positive cells. Selecting agents that are more effective against the CD38 positive cells is an important element of future research strategies. Monoclonal antibodies against CD38 have been developed and will be coming into clinical practice within the next year. These agents show great promise in their ability to interact with presently available drugs and other antibodies to increase the number of CLL cells killed. ::

FIND OUT MORE ABOUT THE PROJECTS WE ARE ACCELERATING AT **WWW.CLLGLOBAL.ORG**

OPTIMIZING TREATMENT

with Alemtuzumab (Campath)

Two drugs have been approved in the last twenty years for management of CLL. The first was fludarabine, a chemotherapy drug, and the second was alemtuzumab (Campath), a monoclonal antibody. Antibodies are proteins which are normally manufactured by the B-cells in the body to fend off invading organisms and foreign proteins. The development of monoclonal antibodies in mice and rats has enabled the production of antibodies which will attach to CLL cells and use normal human proteins called complement and T-lymphocytes (major immune cells) to kill off the CLL cells. The antibodies initially developed in mice and rats have been humanized so that 98% of the antibody protein is now human. Fully human antibodies are also being developed and used in clinical practice at this time.

The role of various mechanisms of cell killing by the antibodies is poorly understood even in CLL where access to the cells is usually not a problem. Dr. Stephan Stilgenbauer (University of Ulm) has developed a method of identifying how the CLL cells are being killed. He is studying the relationship between the killing mechanism and various genetic abnormalities identified in CLL patients. The approach to evaluate the method of killing has been broad-ranged, exploring 17 proteins, all of which are involved in sustaining the life or activating the death of CLL cells.

Understanding how particular types of CLL cells are killed enables us to look at different ways of optimizing this therapy. Alemtuzumab is an extremely potent drug. Combination studies are already underway of alemtuzumab with other antibodies (rituximab and ofatumumab). The downside of alemtuzumab is that it causes an immune deficiency for a significant period of time, increasing the risk of getting infections. However, methods to restore immunity are already being developed. ::