n children with sickle cell disease, one of the most devastating long-term sequelae is a stroke. Prior to 1998, approximately 11 percent of the children with sickle cell disease would have strokes. Unfortunately for the majority of children, the only option of secondary stroke prevention was the burdensome responsibility of monthly blood transfusion therapy followed by the eventual requirement of iron chelation therapy. Without regular blood transfusion therapy, approximately 50 percent of the children would have a stroke recurrence within two years of the initial stroke, and a significant proportion would go on to have a third stroke or additional strokes. In 1998, the landmark STOP trial demonstrated that among children with sickle cell disease and elevated transcranial Doppler (TCD) measurements, regular blood transfusion therapy (experimental arm) resulted in a 92 percent relative risk reduction in strokes when compared with observation (standard arm). The impact of implementing TCD screening coupled with blood transfusion therapy has resulted in up to a log-fold decrease in the rate of strokes in children with sickle cell disease. However, the impact of decreasing the rate of strokes is not without its consequences, and the most significant of these is the commitment to lifelong blood transfusion therapy and the potential for the majority of children treated with elevated TCD velocities.

With this background, Dr. Russell Ware and colleagues completed a randomized, open-label, noninferiority trial referred to as the TWiTCH (TCD With Transfusions ChangIng to Hydroxyurea) trial. The team tested the hypothesis that children with elevated TCD measurements (> 200 cm/sec; nonimaging technique) who had been placed on blood transfusion therapy for primary stroke prevention could be safely switched to hydroxyurea therapy. The two arms of the trial were regular blood transfusion therapy with iron chelation (standard arm) versus hydroxyurea therapy and phlebotomy (experimental arm). The primary endpoint was the 24-month TCD velocity with a non-inferiority margin set at 15 cm/sec. A minimum of 12 months of blood transfusion therapy for elevated TCD measurements were required for inclusion and individuals with severe magnetic resonance angiogram-defined vasculopathy were excluded from the trial. The Data and Safety Monitoring Board stopped the trial early because of the convincing evidence that the experimental arm was noninferior to the standard arm. Significantly, no strokes occurred in either arm, as would be expected because the stroke event rate in children with elevated TCD measurements treated with blood transfusion therapy is very low (0.06 to 0.9 events per 100 patient years), and the trial had a short follow-up with only 121 total participants. For children who met the inclusion and exclusion criteria, the main conclusion of the trial is that for children with sickle cell disease and high TCD velocities, maximum tolerated dose of hydroxyurea therapy is noninferior to blood transfusion.

This is a landmark trial that will have a lasting impact on primary prevention of strokes in thousands of children in high-income countries. Due to the early cessation of the trial, the remaining question is the permanence of hydroxyurea therapy for primary stroke prevention in children with elevated TCD measurements. Hopefully the investigators can continue to formally follow TWiTCH participants for a longer span of time to determine the long-term benefits and risks, if any, of being treated for an indefinite period on hydroxyurea therapy.

Since 1998, the ability to now complete the fifth National Institutes of Health–sponsored randomized clinical trial for primary or secondary stroke prevention in children with sickle cell disease should lay to rest any concerns among parents and healthcare providers about the safety of not providing blood transfusions. Unfortunately, approximately 50 percent of the children would have a stroke recurrence within two years of the initial stroke, and a significant proportion would go on to have a third stroke or additional strokes. In 1998, the landmark STOP trial demonstrated that among children with sickle cell disease and elevated transcranial Doppler (TCD) measurements, regular blood transfusion therapy (experimental arm) resulted in a 92 percent relative risk reduction in strokes when compared with observation (standard arm). The impact of implementing TCD screening coupled with blood transfusion therapy has resulted in up to a log-fold decrease in the rate of strokes in children with sickle cell disease. However, the impact of decreasing the rate of strokes is not without its consequences, and the most significant of these is the commitment to lifelong blood transfusion therapy and the potential for the majority of children treated with elevated TCD velocities.

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Since 1998, the ability to now complete the fifth National Institutes of Health–sponsored randomized clinical trial for primary or secondary stroke prevention in children with sickle cell disease should lay to rest the perception that the African American community is unwilling to participate in patient-oriented research. The community will participate when there is a compelling research question to be answered, coupled with a trusted provider network.
How ASH Spreads the News

In an increasingly connected, information-driven society, the impressions we make each day and the conversations in which we take part — our “footprints” in the world at large — are of utmost importance. With this in mind, ASH expends a great deal of effort to maintain awareness of the larger conversation around hematologic breakthroughs and developments, to consistently share information that directly affects the community of hematologists, and also to lead these conversations when it is most appropriate.

It is worth mentioning that early in my tenure with ASH, the Society purposely kept a low profile, operating under the assumption that the science should speak for itself. But in the last 12 years, ASH leadership has recognized the importance of raising the profile of hematology in order to help support a number of strategic goals — specifically, ensuring the future of hematology and promoting the best care of patients. To that end, ASH has built a comprehensive strategy to foster healthy interactions with the trade and lay press, which includes sustaining and tapping into our media relationships before, during, and after the annual meeting.

If you attended the most recent meeting, you couldn’t miss the proverbial “gauntlet” of reporters in the halls of the Orange County Convention Center. The 2015 ASH annual meeting drew an impressive 271 reporters (96 U.S. and 175 international), from top-tier news sources including Bloomberg, Reuters, and The Wall Street Journal, which ran the widely circulated story “New Weapons in the Fight Against Multiple Myeloma.” And for the second year in a row, CNBC broadcast live from the meeting. Through the presence of these and other outlets, a greater public appreciation for the important work we do is created, and a deeper understanding of major scientific breakthroughs — from newly approved drugs, to CAR T cells, to gene therapy for sickle cell disease — moves far and wide throughout society.

Similar to the annual meeting, the influence of Blood is always top of mind in terms of maintaining and increasing our visibility in the public eye. When a Blood article gains momentum in the popular press, important conversations are sparked. For example, back in 2013, ASH published a Blood Forum article in which more than 120 experts in CML wrote that the high cost of drugs is “unsustainable.” News of this timely, controversial, and consumer-friendly piece appeared in 323 stories in outlets such as The New York Times, CBS Evening News, CNN, Consumer Reports, CNN Money, Forbes, and Harvard Business Review, and stimulated a critical national dialog about the sometimes crippling prices of life-saving drugs.

There has been much discussion lately about the exciting times that we are witnessing in hematology, and the ground that is being broken in drug development, genome editing, and numerous other areas. With this whirlwind of innovation comes a flood of information that can overwhelm not only us as practitioners, but also the patients and families that we are united in serving. ASH is dedicated to disseminating the most credible, clear, informative news in the field, and to harnessing all our media relationships to do the essential work of educating the broader public and elevating the perception of hematologists and all that we do.

Charles Abrams, MD

LETTERS TO THE EDITOR SOLICITATION

The Hematologist welcomes letters of up to 200 words. Please include a postal address, email address, and phone number. Publication will be based on editorial decisions regarding interest to readers and space availability. We may edit letters for reasons of space or clarity. The Hematologist reserves the right to publish your letter, unless it is labeled “not for publication.”

Letters should be sent to:
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CORRECTION:
In the January/February 2016 article, “57th ASH Annual Meeting Wrap-Up,” on page 4, there is an incorrect statement. The phrase should read “ daratumumab, a monoclonal antibody to CD38,” not “CD138.” This has been corrected online, and we apologize for this error.
New Location: Highlights of ASH Latin America

Improve your patient management and care strategies by attending Highlights of ASH in Latin America in Porto Alegre, Brazil. Hear internationally recognized experts analyze the latest updates in hematology research from the 57th ASH Annual Meeting. This is your chance to evaluate your diagnostic techniques and therapeutic approaches and discuss with leading hematology experts and colleagues how new research and clinical updates can be translated into new patient care strategies. Simultaneous translations into Spanish and Portuguese will be available at this meeting.

Program Co-Chairs
Dr. Eduardo Rego, University of São Paulo
Dr. Carlos Chiatone, The Santa Casa Medical School, São Paulo

The meeting will take place April 29-30, 2016, at the FIERGS Exhibition & Convention Centre. Learn more at www.hematology.org/Highlights/Latin-America.aspx

ASH Meeting on Lymphoma Biology

The 2016 ASH Meeting on Lymphoma Biology will take place June 18-21 at The Broadmoor in Colorado Springs, CO. The meeting brings together experts from around the world to discuss the latest breakthroughs in basic and translational lymphoma research. Learn about current challenges in the field and exchange ideas on how to move the field forward. Laboratory-based and pharmaceutical scientists, translational and junior investigators, and other health professionals will be able to establish collaborations with leading experts and colleagues and earn CME credits.

Visit www.hematology.org/Lymphoma-Biology for more information and to register.

ASH Workshop on Genome Editing

ASH’s Workshop on Genome Editing will be held July 14-15, 2016, in Washington, DC. This is a new opportunity to discuss current genome targeting methodologies; outline vital steps necessary to improve the specificity, efficacy, and versatility of these methods; and highlight the transformative potential of genome editing in basic and clinical research for hematology and beyond. The program will include a review of ongoing clinical trials; present examples of application of genome editing technology; and provide additional information on regulatory frameworks, use of this technology as a research tool, and successful translation into the clinic.

These data provide the most convincing evidence that ionizing radiations cause leukemias, and the dose-response relationships developed from these studies are the basis of most radiation protection guidelines. Hematologists such as Dr. Masao Tomonaga have spent their lives studying hematologic consequences of the atomic bombings.

Much of what we know regarding the etiology of leukemias and related disorders also comes from studies done by the atomic hematologists. An increase in leukemia was first noted by Japanese hematologist Dr. Takusu Yamawaki in the late 1940s. This led to the establishment of the Joint Japan-US Atomic Bomb Casualty Commission (now the Radiation Effects Research Foundation) in 1950 and the start of the Life-Span Study of 121,000 exposed persons and controls conducted by Japanese and American hematologists and epidemiologists. These data provide the most convincing evidence that ionizing radiations cause leukemias, and the dose-response relationships developed from these studies are the basis of most radiation protection guidelines. Hematologists such as Dr. Masao Tomonaga have spent their lives studying hematologic consequences of the atomic bombings.

Back to Fliedner. In 1963 he returned to Europe to become director of EURATOM Institute for Radiation Hematology Research affiliated with the Faculty of Medicine at the University of Freiburg. He and his team studied the behavior of hematopoietic stem cells in rats using a model of continuous 3H-thymidine infusion. They were able to distinguish resting and dividing stem cells and early progeny and postulated that the resting stem cells were attached to the bone marrow endothelium. This prescient observation is the basis of much of the current research into the so-called stem cell niche (see Diffusion article in this issue by Dr. Jonathan Hoggatt), a field most people think was recently invented. Their model was also applied to other settings such as high-dose radiation exposures and leukemia. These experiments in rodents and dogs required a continuous 24- to 48-hour 3H-thymidine infusion, and the team often spent nights and weekends in the laboratory.

Fliedner’s interest in hematopoietic stem cells and radiation naturally led to studies of leukemia. He considered both sides of the issue: radiation as a cause of leukemia and using radiation to treat persons with leukemia. His team developed a technique of extracorporeal radiation as a therapy in persons with chronic lymphocytic leukemia (CLL), whereby blood was shunted into a chamber and exposed to ionizing radiations. This brings us to bone marrow transplants and the atomic bomb. Several U.S. physicians, including hematologists, were part of the Manhattan Project (see photo on page 5), which developed the atomic bombs detonated over Japan in 1945. These participants included Louis Hempelmann, who treated victims of a famous criticality accident at Los Alamos.* Immediately after World War II, the U.S. government, especially the Navy, which had nuclear submarine and aircraft carrier programs, was anxious to find ways to reverse the destruction of the bone marrow caused by exposure to high doses of ionizing radiations. The fear was that a nuclear attack by the Russians or a submarine accident would have devastating effects on military forces and on civilians. Hematologists were assembled at several sites including the National Institutes of Health and the University of Chicago. These efforts led to many important discoveries, including transplanting bone marrow cells into irradiated animals by Dr. Egon Lorenz and colleagues, and later into humans, as pioneered by researchers including Drs. E. Donnell Thomas, Joseph Ferreehe, and Georges Mathé, who with his colleagues in Paris treated live victims of a nuclear reactor accident in Vinca, Yugoslavia, in 1958. These radiation-related studies also led to the discovery of hematopoietic growth factors such as erythropoietin by Drs. Alan Erslev, Eugene Goldwasser, and others, and of myeloid growth factors by Drs. H. Sachs and Dow Pluznik in Israel, Drs. Benjamin Bradley and Donald Metcalf and colleagues in Melbourne, and Dr. Malcolm Moore and colleagues in New York. Similar radiation protection programs were started in Europe, including in the Netherlands at the NTO, the Netherlands Organisation for Applied Scientific Research, by Dirk van Bekkum.

Dr. Eugene P. Cronkite

L,000). However, Fliedner was an expert, and as recounted by Cronkite in his 1989 oral history for ASH, he was often found in the lab at 4:00 a.m. laboring over an autoradiograph. He also studied bone marrow transplants in dogs given high doses of nitrogen mustard, in collaboration with Dr. E. Donnell Thomas, then at Cooperstown, New York. Using this model, Fliedner was able to show that a large proportion of hematopoietic stem cells were in the resting stage of the cell cycle.

Theodor Fliedner

(Cont. from page 1)

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Primary chronic cold agglutinin disease (CAD) is an autoimmune hemolytic anemia mediated by cold agglutinins (CA), without any obvious underlying disease such as aggressive lymphoma, other overt malignancies, or specific infections. CA are able to agglutinate red blood cells (RBC) at an optimum temperature of 3°C to 4°C, but are also active at higher temperatures, depending on the thermal amplitude. Most CA in CAD are IgM antibodies and have specificity for the surface carbohydrate antigen termed I, whereas IgG, IgA, or IgM phenotype are found in less than 10 percent of cases.

Clinical features and diagnostic workup

Following CA-binding to the I antigen at the RBC surface, the antigen-antibody complex C5b6789 complex has also been shown to occur to some extent, at least in some patients. By flow cytometry, the cellular x:o ratio in bone marrow aspirate is usually greater than 3.5.

Our understanding of the immunohistologic and cellular basis of the clonal immunoglobulin production has greatly improved during the last decade. Two large series have confirmed signs of a clonal lymphoproliferative bone marrow disorder in most patients. The individual hematologic and histologic diagnoses, however, showed a striking heterogeneity within each series. In one study, lymphoplasmacytic lymphoma (LPL) was the most frequent finding, whereas marginal zone lymphoma (MCL) and unclassified clonal B-cell lymphoproliferation were also reported frequently.

The explanation for this perceived heterogeneity has probably been revealed by a recent, comprehensive study of 54 patients with CAD. The bone marrow findings in these patients were consistent with a surprisingly homogeneous disorder, termed by the authors “primary CA-associated lymphoproliferative disease”, which was distinct from LPL, MZL, and other previously recognized entities. The authors “primary CA-associated lymphoproliferative disease”, which was distinct from LPL, MZL, and other previously recognized entities. The MYD88 L265P mutation, found in most cases of LPL, could not be detected in 15 samples from CAD patients analyzed for this mutation.

Mechanisms of complement-mediated hemolysis

Following CA-binding to the I antigen at the RBC surface, the antigen-antibody complex binds complement protein complex C1 and thereby triggers the classical complement pathway. The Figure shows the details of this process, resulting in predominantly extravascular, C3b-mediated hemolysis. Intravascular hemolysis mediated by the C5b6789 complex has also been shown to occur to some extent, at least in some patients and situations.

Clinical features and diagnostic workup

CAD is defined by chronic hemolysis, positive direct antiglobulin test (DAT), monospecific DAT positive for C3d, and CA titer ≥ 64 (much higher in most cases). DAT is usually negative for IgG but may be weakly positive.

Approximately 90 percent of patients have cold-induced acrocyanosis and/or Raynaud phenomena. By definition, all patients have hemolysis, but occasional patients are not anemic because the hemolysis is fully compensated. Median hemoglobin (Hb) level has been estimated at 8.9 g/dL. In cold climates, a majority of individuals experience seasonal variations with worsening of anemia and circulatory symptoms during winter or at low ambient temperatures. At least 70 percent of patients have experienced exacerbations precipitated by febrile infection, major surgery, or major trauma. Fifteen percent or more have hemoglobinuria. In a retrospective study, approximately 50 percent of patients were transfusion-dependent at some time during the course of the disease.

A focused history and clinical examination is, therefore, an essential part of the diagnostic workup. Quite often, “the history tells you the diagnosis.” Laboratory tests should include full blood counts, blood smear analysis, assessment of hemolysis (absolute reticulocyte count, lactate dehydrogenase, bilirubin, and haptoglobin), polyspecific and monospecific DAT, and CA titer. For differential diagnostic considerations, cryoglobulin assessment should be included. Thermal amplitude determination may be of interest but is time-consuming and often not necessary in routine clinical practice. Results of serum electrophoresis with immunofixation, immunoglobulin class quantification, complement protein (C3 and C4) levels and bone marrow examination (biopsy and flow cytometry) are not part of the disease definition, but should always be obtained.

Of critical importance, serum for CA titration, electrophoresis, and immunoglobulin assessments must be obtained from blood specimens that have been kept at 37°C to 38°C from the time of sampling until serum has been removed from the clot.

Management

The mainstay of nonpharmacologic management is warm clothing and avoidance of cold. Most patients, however, have discovered this measure before they see the hematologist. Transfusions can be given safely provided that specific precautions are observed; these are comprehensively described elsewhere. Splenectomy is inefficient because most of the extravascular hemolysis takes place in the liver. Because almost all IgM is located intravascularly, plasmapheresis is considered an efficient “first-aid” in acute situations or before surgery requiring hypothermia. However, such remissions are short-lived.

Not all patients require drug therapy. In our opinion, however, pharmacologic treatment is indicated more frequently than traditionally advised in the literature and should be offered to patients with symptom-producing anemia or disabling circulatory symptoms. Recommendations to avoid drug therapy have often been based on poor efficacy (until the last 10 to 15 years), combined with an underestimation of the patients’ clinical problems.

In two retrospective studies from Norway and the US, respectively, 70 to 80 percent of the patients had received pharmacologic therapy. Corticosteroids are inefficient, and unacceptably high maintenance doses are usually required to maintain remission in the few responders. Corticosteroids should, therefore, not be used to treat CAD.

Two prospective trials of rituximab monotherapy (375 mg/m² weekly for 4 weeks) showed response rates of about 50 percent according to strict criteria. Complete responses were rare. The median response duration was approximately one year. A prospective, ‘real life’ study confirmed these findings. Based on these results and its very favorable safety profile, rituximab monotherapy is now often recommended as first-line treatment.

The safety and efficacy of combination therapy with fludarabine and rituximab were studied in 29 patients in a prospective trial. The participants received rituximab 375 mg/m² on days 1, 29, 57, and 85; and fludarabine 40 mg/m² orally on days 1 to 5, 29 to 34, 57 to 61, and 85 to 89. The same response criteria were used as in the rituximab monotherapy trial. Twenty-two patients (76%) responded, with six (21%) achieving complete response and 16 (55%) achieving partial response. Median time to response was four months. An impressive estimated median response duration of more than 6 months was achieved. Short-term hematologic toxicity was significant, however, with 12 patients (41%) experiencing grade 3 or 4 toxicity. Furthermore, although not directly observed in this study, the possibility of long-term toxicity may be a concern, particularly in younger patients.

In conclusion, patients with CAD requiring therapy should be included in prospective trials if available. Outside clinical trials, fludarabine-rituximab combination therapy should be considered in those who definitely need efficient treatment, especially if they have failed rituximab monotherapy, are not too young, are reasonably fit, and have not previously received cytotoxic chemotherapy. In patients failing to meet these criteria, single-agent rituximab should remain first-line treatment.

The future

Favorable response to bendamustine-rituximab combination therapy has been reported in one patient, and a prospective study is ongoing. The targeted B-cell receptor pathway inhibitors ibritumomab and idelalisib have not been evaluated in CAD.

Given that hemolysis in CAD is entirely complement-dependent, studies of complement modulators are highly interesting. Favorable effect of the C5 inhibitor eculizumab has been described in a prospective trial. Since the hemolysis is predominantly C3b-mediated in most patients, complement blockade at a more proximal, classical pathway level might, in theory, be more successful. Preclinical studies with the anti-C1s monoclonal antibody TNT003 and its humanized counterpart TNT009 have shown favorable results. If sufficient clinical documentation for complement-directed therapies can be provided, such treatment will probably still not replace clonally directed therapies, which are more causal and do not need to be continued indefinitely. Complement-directed therapies seem very promising, however, in acute exacerbations, in patients undergoing surgery requiring hypothermia, and in those with severe CAD not responding to clonally directed immunochemotherapy.


Off-label use of pharmacological substances has been discussed. Dr. Berentsen has received research support from Mundipharma, lecture honoraria from Alexion, and consultancy honoraria from True North Therapeutics.

Theodor Friedner

(Gu phase), blood counts fell precipitously. Interestingly, there were also off-target (abscopal) effects such as reduced spleen and lymph node sizes. For perspective, Drs. Kanti Rai and Raster Storb treated persons with CLL with this technique when they were fellows. This approach was abandoned when anti-leukemia drugs were developed but is still biologically fascinating.

In 1967 the new Ulm University was inaugurated, with Friedner as the youngest of eight founders. He became the director of the Department of Clinical Physiology and later the Dean of the Theoretical Faculty of Medicine. In 1983 he was appointed President of the University of Ulm. His research group, staffed by associates from Europe and elsewhere, focused on characterizing hematopoietic stem cells, especially after total body radiation in dogs. The team showed that large numbers of hematopoietic stem and progenitor cells could be collected by continuous-flow centrifugation, frozen, and stored for a future transplant. Although their focus was on radiation accidents, these techniques are currently used in auto-transplants for cancer, especially lymphomas and myeloma. Friedner was often the volunteer for these experiments — he loved long, tedious, technically demanding experiments.

In later life, Friedner was best known internationally for his work on evaluating victims of radiation accidents. He chaired a European Consortium of Experts, which developed the 1981 publication Manual on the Acute Radiation Syndrome, which is widely used today. Quite remarkably, he developed a database of more than 800 detailed reports of radiation accidents. He also led a German research team studying the effects of space travel on bone marrow function in humans — studies continued by NASA and the Japan Space Agency.

Ted was active in scientific research throughout his life. Three years ago, he asked one of the authors (Dr. Gale) for details regarding a radiation accident in Israel. He wanted to review the pathology slides himself. In 2008, at age 79, he was writing letters to the editor of Blood, and in 2012, at age 83, he was first author of an article in the journal Dose-Response, “Hemopoietic Response to Low Dose-Rates of Ionizing Radiation Shows Stem Cell Tolerance and Adaptation.”

Seven atom bomb scientists look over a roengenometer at the site of the test atom bomb explosion on Sept. 13, 1945. Pictured (L to R): Dr. Kenneth T. Bainbridge; Dr. Joseph G. Hoffman; Dr. J. Robert Oppenheimer; Dr. Louis H. Hempelmann; Dr. Victor Weisskoff; Dr. Robert F. Bacher; and Dr. Richard W. Dooson. (AP Photo)
Angiogenesis, the formation of new blood vessels, is an essential physiological process for wound healing, reproduction, and development. In cancer, angiogenesis is crucial to sustain solid tumor growth and correlate with metastases. Studies of bone marrow biopsies from children with leukemia indicate that leukemic cells induce angiogenesis in the bone marrow. The central role of angiogenesis in cancer promoted the hypothesis that cancer may be treated with anti-angiogenic drugs. Moreover, other studies proposed that endothelial cells of tumor blood vessels within specific tumors express unique receptors, or zip codes, such that targeted delivery of anti-cancer drugs might be feasible and, simultaneously, could minimize collateral damage of healthy cells.

To map the molecular heterogeneity within the human vasculature, a combinatorial peptide bacteriophage library was screened by in vitro phage display of normal and tumor blood vessels in cancer patients undergoing terminal experimentation. The phage are engineered to display a short peptide sequence, a fusion protein on the gIII coat protein, and phage libraries typically have a peptide diversity of 10^10. Importantly, intravenously injected phage extractaevase from leukemicmouse cells to bind to receptor proteins present on the surface of tumor cells, as well as the extracellular matrix and perivascular cells. In addition to in vitro phage display, an in vitro peptide display method, Biopanning and Rapid Analysis of Selective Interactive Ligands (BRASIL), was developed and used to profile the expression of cell surface receptors of cultured human cancer cells in the NC360 panel (a National Cancer Institute panel of 60 human cancer cell lines from different histologic origins and grades). Recovered phage display peptide sequences that act as ligands to bind to accessible receptors expressed on the luminal surface of endothelial cells or on the surface of tumor cells. The experimental design enriches for phage that bind to accessible receptors and are internalized upon receptor binding.

In vitro phage display studies facilitated construction of a human vascular map from phage that specifically bind to receptors expressed on the vascular beds of numerous organs or tumor cells. As expected, various receptors that are uniquely expressed on the vascular beds in specific tumors were identified, as were receptors that are present on the extracellular matrix, perivascular cells, and tumor cells. Surprisingly, several common receptors expressed in different tumor cell types were also identified. For instance, the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST) recognized one such peptide, GRRAGGS, to be identical to a short peptide sequence within interleukin 11 (IL-11). Subsequently in vitro and in vivo analyses identified the IL-11 receptor alpha (IL-11Ra) as the cognate binding partner. In fact, high levels of IL-11Ra expression in tissue sections from prostate cancer patients correlated with disease progression. Clinical trial of BMTP-11 in castrate-resistant prostate cancer patients with osteoblastic bone metastases (NCT00672157) confirmed selective BMTP-11 localization and apoptosis induction in tumors in the bone marrow. Ex vivo studies showed that BMTP-11 preferentially induces cell death in MOLT-4 leukemia cells compared with normal white blood cells. Interestingly, the myristoylated BMPT-11 analog increased cell death by ninefold in cultured OCI-A3ML3, K562, and MOLT-4 cells compared to the efficacy of the parental BMTP-11 in MOLT-4 cells in the same time frame.

Other cancer-specific signatures have also been discovered and characterized. For example, screening human leukemia and lymphoma cell lines and patient-derived AML and acute lymphoblastic leukemia (ALL) bone marrow using a subtractive cell-targeting technology identified a cell-internalizing peptide motif, Phe-Phe/Tyr-X-Leu-Arg-Ser (FFXXLRS), where X is any amino acid residue. Further analyses revealed that FFXXLRS binds to neuropilin-1 (NRP-1) receptor. Treatment of cultured MOLT-4, CCRF-CEM, OCI-A3ML3, HL-60, K562, SR-786, U937, or RPMI-8226 cells with the NRP-1-targeting peptide, CGP56K, fused to KLAKKLAKL, decreased cell viability in the 5- to 30-µm range. Analyses of bone marrow samples from AML and ALL patients confirmed NRP-1 expression compared to normal bone marrow. These data, together with the promising results of BMTP-11 in targeting prostate cancer metastases and the improved efficacy of the myristoylated BMTP-11 analog against leukemic cells indicate that tumor-specific molecular zip codes exist and can be effectively exploited to design drugs to systemically treat cancers (Figure).

Figure: Schematic of drug development pipeline. Phage display in humans or with cultured tumor cells identified common and/or unique receptors that can be exploited for targeted drug delivery. In the case of BMTP-11 or myristoylated BMTP-11, the targeting peptide ligand, CRRAGGSGC, was synthetically fused to the apoptotic peptide, KLAKKLAKL, and validated in vivo and ex vivo, respectively. BMTP-11 was advanced to preclinical studies, and following FDA approval, BMTP-11 was tested as a new investigational drug in a first-in-man clinical trial.

Drs. Arap and Pasqualini have ownership interest (including IP licensing, royalty payments, and prior equity interest) in Arrowhead Research Corp., which is subject to certain limitations and restrictions under University policy.
ASH Calls on Congress to Help Address the Burden of Sickle Cell Disease

ASH is in the midst of a multifaceted initiative to identify the highest priority actions needed to improve outcomes for individuals with sickle cell disease (SCD) in the United States and globally and to map a plan to advance these actions in the short and long term. In 2016, the Society will announce different parts of this initiative through its “Call to Action on SCD.” A major component of this campaign is ASH’s congressional strategy to raise awareness for SCD on Capitol Hill and promote the need for comprehensive SCD legislation to enhance current federal SCD programs.

On February 9, ASH kicked off the Society’s SCD-focused advocacy activities by co-hosting a congressional staff roundtable meeting on SCD with the Sickle Cell Disease Association of America. The program featured ASH President Dr. Charles Abrams and ASH Vice President Dr. Alexis Thompson, as well as a sickle cell patient advocate, Mr. Kyle Smith, who presented startling facts about SCD and the burden of the disease. He also highlighted new practice-changing guidelines and research and facilitated a discussion of legislative strategies to help improve the lives of individuals with SCD.

The interactive and educational program was aimed at helping generate congressional staff interest in SCD and garner support for comprehensive SCD legislation. To continue the momentum, ASH will continue to work with Members of Congress to introduce SCD legislation, and the Society will be hosting a second briefing for congressional members and staff in conjunction with the Committee on Government Affairs’ spring Hill Day on March 23, 2016.

For the latest on ASH’s SCD initiatives and the status of the Society’s related advocacy efforts, visit www.hematology.org/scd.

ASH Continues Efforts to Pass Oral Parity Legislation in U.S. States

Ooral and patient-administered forms of chemotherapy have become more prevalent and represent the standard of care for many types of cancers. Despite their convenience, efficacy, and low rate of side effects, they are covered differently than intravenous drugs, leaving many patients responsible for unsustainable high monthly co-payments. As noted in the last “Headlines from Washington,” ASH is continuing efforts to pass legislation to ensure that cancer patients have equal access to oral chemotherapy. Appropriately, the President signed H.R. 2255 into law on February 10, 2016.

ASH has supported legislative efforts at both the federal and state levels and continues to work with stakeholders and advocacy groups on legislative efforts in numerous states in 2016. Since our last update, there has been significant action on legislation in several states, as noted below:

- **Alabama:** 2016 will be the first attempt to pass oral parity legislation in Alabama. ASH has been working as part of a coalition (the Alabama Cancer Treatment Fairness Coalition) since early fall 2015 to prepare for the 2016 legislative session. The coalition has lined up House and Senate sponsors, and as of the publication of this issue of The Hematologist, plans were to introduce the legislation soon after the start of the session (which begins in early February and runs through the spring).

- **Tennessee:** ASH is working as part of the Tennessee Fair Access to Cancer Treatment Coalition. In late January, the Cancer Treatment Fairness Act (SB209/HR2289) was introduced in both the state Senate and state House.

- **Michigan:** As with efforts in other states, ASH continues to work as part of a larger coalition of patient and provider organizations to seek passage of oral parity legislation in Michigan. The 2015 to 2016 legislative session will mark the third attempt to pass oral chemotherapy legislation in Michigan. The bill (SB 625) was officially read-in on December 1, 2015, and was assigned to the Senate Insurance Committee, where a hearing was held on the bill in late January 2016.

- **Alabama:** ASH’s Officers and a patient advocate participate in the February 9, 2016, Congressional Staff Roundtable on Sickle Cell Disease. Pictured (L to R): Dr. Kenneth C. Anderson, Dr. Susan B. Shurin, Dr. Alexis Thompson, Mr. Kyle Smith, Dr. Charles S. Abrams, and Dr. Stephanie J. Lee.
A Clearer View of HSCs


A new study by Dr. Melih Acar and colleagues has now added to these technological advances in imaging HSCs in tissue — application of tissue optical clearing and a new endogenous reporter mouse. Expanding upon whole-mount imaging techniques, the authors used a tissue-clearing technique previously employed in brain tissues or mouse embryos, to create “clear” bones, allowing for deeper penetration of confocal imaging. Using gene expression profiling from their earlier studies that identified the SLAM markers, the authors also found that v-catenin was highly expressed in HSCs compared with unfractionated bone marrow cells. A green fluorescent protein (GFP) was then inserted into the first exon of v-catenin, creating a reporter mouse in which approximately 50 percent of the SLAM cells were GFP+, and there was no detection of GFP in more mature hematopoietic cells. Coupling GFP expression with antibody staining for c-kit led to similar enrichment for long-term repopulating HSCs as SLAM markers. Using this two-marker system and optically cleared sections, the authors used similar spatial analysis to the prior report and demonstrated that these endogenously GFP-labeled c-kit+ cells were contained more commonly within the central marrow rather than in bone surfaces. Approximately 85 percent of the cells were within 10 μm of a sinusoidal vessel. However, if a similar analysis was performed by comparing the distance of randomly placed spots in the bone marrow cavity to sinusoids, there was no statistical difference between the random spots and the HSCs, due to the abundance of sinusoids in the bone marrow space. Surprisingly, there was no localization difference observed between cycling versus noncyling HSCs. The authors also coupled their imaging analysis with CXCL12 reporter mice and Leptin-receptor reporter mice and demonstrated that the vast majority of HSCs are within 5 μm of these reporter cells within the marrow niche. While this localization of HSCs was significantly different from randomly placed spots, 85 to 90 percent of random spots also were within 5 μm of these reporter cells, demonstrating how ubiquitous these cells and “niche” locations are within the marrow space.

Recently, a number of studies have described numerous cell types that comprise the HSC niche, largely driven by the availability of a specific Cre-recombinase or related reporter mouse strains. Consequently, a series of publications have described the “cell identity” of the HSC niche based on the mouse model including Lepr-Cre, Prx1-Cre, Nestin-Cre, NG2-Cre, CXCL12-GFP, Mc21-Cre, Osr-Cre, and others. While the field has advanced as a result of these new mouse models, many of these Cre systems were not conditionally activated, meaning that the phenotype observed may not faithfully represent what happens in the adult stem cell niche. Secondly, none of these Cre or reporter systems are restricted to an exclusive cell type, and there are many known and unknown overlaps. Finally, different variants of the same reporter system often result in different phenotypes, adding to uncertainty in niche cell identity.

The next decade of HSC niche research is likely to involve the use of still-emerging technologies, and our understanding of the niche (specifically what has occurred this past decade) will become simultaneously clearer and more convoluted. It will be important to apply these technologies both in steady-state situations as well as in situations of stress and disease, particularly HSC transplantation. Notably, optical clearing and deep tissue imaging also has been used by the authors to begin to explore HSC localization in the spleen (Figure) during extramedullary hematopoiesis.1 As more groups adopt these new techniques and apply them to their own model systems it is likely that the HSC niche will continue to be parsed into smaller and smaller subsets of locales and cells, perhaps with differing regulatory properties.

Short-Term Treatment Strategies for Recurrent Venous Thromboembolism


E ven with optimal anticoagulant therapy, as many as 8 percent of cancer patients experience recurrent venous thromboembolism (VTE) within six months of their index event. Unfortunately, there is no high-quality evidence to guide the management of anticoagulation failure in such patients. Using an observational study design, Dr. Sam Schulman and colleagues provide us with more information about how cancer patients are treated after a breakthrough VTE, and how frequently various anticoagulation strategies are associated with outcomes such as (second) recurrent VTE and major bleeding.

The authors included data on 212 patients who both had cancer and experienced VTE despite anticoagulant therapy. Data from approximately one-third of the patients were gathered retrospectively; the other two-thirds of included patients were studied in a prospective fashion. During the three months after their breakthrough event, the treatment used for each patient was recorded, and all patients were followed for VTE events, major bleeding episodes, residual thrombotic symptoms, and changes in anticoagulation therapy. Event rates associated with the different treatment strategies were compared using Cox proportional hazards regression. Within this cohort of patients who had failed some sort of anticoagulant, 59 percent had adenocarcinoma and 73 percent had known metastases. When they experienced their breakthrough event, 70 percent of patients were using low-molecular-weight heparin (LMWH), while 27 percent were on a vitamin K antagonist (VKAs). While insufficiently aggressive anticoagulation probably explained at least some of the treatment failures, 70 percent of included patients were known to be receiving a therapeutic or supratherapeutic dose at the time they experienced a qualifying event. The treatment used by the local physician after the index event was unchanged anticoagulant (and unchanged, therapeutic dose) in 33 percent, unchanged anticoagulant (but dose increased) in 31 percent, and a different anticoagulant in 24 percent (most of these patients switched from VKA to LMWH).

During the subsequent three months, 8 percent had major bleeding, 11 percent had another VTE, and 27 percent died. Additional VTE recurrence was less common with LMWH than with a VKA (hazard ratio [HR], 0.29; 95% confidence interval [CI], 0.11-0.70) but was similar with unchanged or increased anticoagulant intensity (HR, 1.09; 95% CI, 0.45-2.63).

Although the observational nature of this study is limited by the authors’ inability to adjust for potential confounding variables, Dr. Schulman and colleagues have provided important, new information that may help both clinicians and investigators charged with defining the management of cancer patients who experience VTE recurrence despite anticoagulation. First, anticoagulation failure in cancer patients is associated with a guarded prognosis. More than one quarter of the persons included in this registry died within three months of their breakthrough event. Second, the present findings support the widespread practice of switching to LMWH when (or other) patient has recurrent thrombosis while taking VKA. Unfortunately, clinicians are still left without definitive evidence about how best to treat the cancer patient who experiences new VTE while on LMWH. Many physicians recommend increasing the LMWH dose (by 20%-30%) in this situation, and, the possibility of selection bias notwithstanding, the findings of Dr. Schulman and colleagues suggest that increasing the dose of LMWH is not prohibitively risky. On the other hand, the observational nature and small size of this registry leave us uncertain about whether (or to what extent) this dose escalation strategy is beneficial.

D A V I D  A. G A R C I A, M D
Dr. Garcia indicated no relevant conflicts of interest.

Mutated Calreticulin Stimulates the Thrombopoietin Receptor to Drive the Development of MPN


T he major BCR-ABL1-negative myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These chronic myeloid malignancies are caused by constitutive activation of the JAK-STAT pathway producing elevated blood counts, most often through JAK2 V617F mutations.1 Mutations in the thrombopoietin receptor (TPO or MPL) are also found in patients with ET and PMF.2-4 In 2013, mutations in exon 9 of the C-terminal domain of the calreticulin (CALR) gene were identified in a significant subset of ET and PMF, but not PV.1,5 The mutations uniformly result in a 1-6 base pair (bp) frameshift which in turn leads to loss of the endoplasmic reticulum retention sequence, or KDEL. Two major subtypes of CALR-mutations have been identified, which account for the vast majority of mutations. The 52 bp deletion (del52; type 2) is more commonly associated with myelofibrosis than the 5 bp insertion (ins5; type 2).6 The mechanism by which CALR mutations lead a MPN phenotype has puzzled investigators since their discovery.

To examine the role of CALR and its interactions with cytokine receptors, Drs. Ilyas Chachoua and Stephan Constantinescu and colleagues employed retroviruses to infect cell lines with mutant CALR. The cytokine-dependent BaF3 cell line was infected with retroplasm containing CALR-wild type, del52, or ins5 sequences, as well as one of several cytokine receptors including MPL, the EPO receptor (EpoR), and G-CSF receptor (GCSFR). These cells were induced to have autonomous growth with mutant CALR and MPL, and, to a limited extent, GCSFR. These findings showed that mutant CALR requires MPL and does not interact with EpoR.

To explore the role of JAK2 in MPL activation, the JAK2-deficient 32D cell line was used. These cells could not induce MPL activation in the presence of mutant CALR, indicating the requirement of JAK2. Further emphasizing this point, the JAK2 inhibitor ruxolitinib was successful in inhibiting proliferation of Ba/F3 cells with mutant CALR. Interestingly, the addition of a MEK/ERK inhibitor, but not a PI3-K inhibitor, provided a synergistic effect.

Alteration of MPL N-glycosylation sites blocked mutant CALR from activating MPL. This activation was independent of Tpo, as demonstrated by Tpo-binding deficient MPL, which could still be activated by mutant CALR. The authors hypothesize that CALR mutants may stabilize one of the active interfaces of MPL in addition to directly interacting with MPL. This could explain why CALR mutants do not interact with other N-glycosylated mutated receptors, and why the delt2s and insd have different clinical phenotypes. Finally, this group verified the importance of the MPL/JAK2 signaling pathway in primary cells from CALR-mutated ET patients. Short hairpin RNAs targeting MPL or JAK2 were successful in inhibiting Tpo-independent colony formation.

Similar findings were presented at the 2015 ASH Annual Meeting by Dr. Shannan EI and colleagues from the laboratory of Dr. Ann Mullally. By overexpressing CALR-del52 in the cytokine dependent BaF3 cell line, IL-6 independent growth was achieved in cells expressing MPL, but not in cells expressing EpoR. These cells exhibited increased phosphorylation of MPL, JAK2, and STAT3, and the JAK1/JAK2 inhibitor ruxolitinib could block STAT3 activation in a phospho-flow cytometry assay. A variety of mutant CALR proteins were studied, and it was found that the positive charge of the C-terminus was critical for its transforming capacity. This mutant CALR physically interacts with MPL, as shown by co-immunoprecipitation. These findings are in agreement with Dr. Chachoua and colleagues by showing that mutant CALR interacts with MPL and signals through the JAK-STAT pathway.

This research provides important insights into the pathophysiology of CALR-mutated MPN and proposes an intriguing mechanism of a mutated chaperone protein inducing cytokine activation. These findings suggest a novel signaling paradigm whereby a mutated CALR undergoes a different cellular localization from the wild-type protein and induces activation of MPL at the N-glycosylation site through JAK2, independent of Tpo. The observation that different mutations in CALR may stabilize different clinical interfaces of MPL provides a possible explanation for different clinical phenotypes. Importantly, by demonstrating a JAK-STAT signaling pathway that is different from the JAK2 V617F driven neoplasms, these data offer hope for new therapeutic opportunities (Figure; available online only).

D A V I D T. L Y N C H, M D; T R A C Y J. G O R D O N E, M D
Dr. Lynch and Dr. George indicated no relevant conflicts of interest.
What Lies Beneath? Prevalence of Heritable Mutations in Pediatric Cancer


In 1971, Dr. Alfred G. Knudson proposed his “two-hit” hypothesis to explain the role of recessive tumor suppressor genes in dominantly inherited cancer syndromes. He postulated that the first hit was inherited, and the second hit was acquired and triggered tumorigenesis. This hypothesis was subsequently confirmed by the demonstration of a loss of heterozygosity at 13q14 in retinoblastomas and the cloning of the first tumor suppressor gene Rb1. Since then, numerous genes associated with inherited cancer predisposition syndromes have been discovered. Mutations in these genes lead to disease through several mechanisms, including, but not limited to, inactivation of tumor suppressor genes. The frequency of germline mutations in cancer predisposition genes in children and adolescent patients had formerly not been determined across a broad range of tumor types.

To determine this frequency, Dr. Jinghui Zhang and colleagues performed whole-genome and/or whole-exome sequencing of constitutional DNA purified from blood samples from 1,120 children with a variety of cancers. The median age of the patients was 6.9 years (range, 8 days to 19.7 years). Using a candidate-genome analysis approach that largely focused on the known autosomal-dominant or 29 autosomal-recessive cancer-predisposition genes, they found that 8.5 percent of the patients (95 of 1,120) harbored pathogenic or likely pathogenic mutations in their candidate genes with 1.1 percent (19 of 1,120) cancer-predisposing mutations. They illustrated that sequencing allowed identification of mutations that would have been missed by standard Sanger sequencing, including the identification of mutations in noncoding regions of the genome that is limited, and thus pathogenic mutations in these regions could have been missed.

This is the most comprehensive study to date of the genetics of childhood cancer predisposition. It demonstrates the clinical utility of next-generation sequencing to identify inherited cancer predisposition in pediatric and young adult patients. The quality and depth of sequencing allowed identification of mutations that would have been missed by standard Sanger sequencing, including the identification of germline mosaicism. This broad genetic approach to diagnosis highlights how patients with inherited cancer predisposition may present with atypical findings, that is, cancers not commonly expected within the phenotypic spectrum of their disorder and in the absence of a strong family history of cancer. This work also raises a number of important questions, including, but not limited to, inactivation of tumor suppressor genes. The frequency of germline mutations in cancer predisposition genes in children and adolescent patients had formerly not been determined across a broad range of tumor types.

One Step Closer to Functionally Characterize the Human Genome: Genomic Screens Identify Essential Genes and Liabilities in Cancer Cells


More than 15 years ago, the Human Genome Project provided a comprehensive sequence of our genome’s gene-containing regions. We now have the ability to directly edit DNA in a systematic manner using a variety of techniques, allowing the comprehensive study of the function of all genes.

Three independent studies have recently furthered our understanding of those genes that are indispensable for viability of humans.” Each of those studies, by Dr. Tim Wang and colleagues, utilized a near-haploid human chronic myelogenous leukemia (CML) cell line called KBM7 (Bcr-Abl: 25, XY, +8, Ph+). To disrupt DNA coding sequences throughout the genome and uncover which genes are essential to the survival of these cells. Using a CRISPR/Cas9-based gene-editing strategy with pools of lentiviruses containing small-guide RNA (sgRNAs), they targeted every protein-coding gene in the genome (Figure A). Comparison of sgRNA abundances between the initial infected cell population and 14 population doublings then identified the sgRNAs and the corresponding genes, which were depleted in the final population. In an orthogonal approach, they also utilized retroviral gene-trap mutagenesis to disrupt the coding sequence of all protein-coding genes. Integration of a gene into the genome occurs at one, nonrandom position into coding orientation within the coding region, resulting in a frameshift in gene inactivation while in the retrovirus, thereby blocking its effect. The proportion of gene-trap insertions in the activating versus inactivating orientation for each gene in the genome thereby allows for deduction of the function of genes, which have an essential function. A similar methodology of gene-trap insertional mutagenesis in KBM7 cells and an additional cell line derived from KBM7 cells, was also published by Dr. Virgil A. Hart and colleagues in the same issue of Science magazine. Finally, Dr. Traver Hart and colleagues utilized a genetic screening approach utilizing CRISPR negative-selection screens across five human cancer cell lines to identify core fitness genes (Figure B).

The results from each of the three mentioned studies were enlightening and highly analogous in their findings. First, all three made the surprising finding that approximately 1,000 genes (nearly 10% of the genes in the human genome) are essential. The majority of essential genes have similar attributes that reflect their critical roles: they are often conserved amongst species, evolve slowly, rarely have paralogs, are highly expressed, tend to be rich in protein-protein interactions, and are rarely affected by nonsynonymous mutations. More surprisingly, roughly 300 of the essential genes in our genome have no previously studied function. The discovery of these newly identified essential genes was made possible through the systemic use of techniques to directly mutagenize DNA, identifying fourfold to fivefold more essential genes compared with prior efforts using RNA interference.

While the studies by Dr. Wang and Dr. Hart and their colleagues begin to define genes that appear to be essential in the context of specific cancer ecologies [such as BCR-ABL1 and KRAS G12D], these represent only the begining of such efforts. For example, the work by Dr. Birsoy and colleagues revealed that those genes that are nonessential on their own may actually result in cell death when co-deleted with another nonessential gene. Systematic efforts to define similar synthetic lethal interactions between pairs of nonessential genes may be especially important in identifying novel targets for cancer therapy. Indeed, it is estimated that each nonessential human gene may have approximately 20 synthetic lethal partner genes. Moreover, identification of nonessential genes, which are synthetic lethal in the context of specific cancer drugs, may provide further therapeutic insights. Future efforts utilizing the techniques, tools, and knockoout cells created in these studies may provide insight into novel functions of our genome and new therapeutic vulnerabilities to target cancer.

CD-19–specific CARs is a promising immunotherapeutic approach for the treatment of B-cell malignancies. However, MM remains an incurable malignancy. Novel immunotherapies, such as chimeric antigen receptor (CAR) T cells, B-cell–specific monoclonal antibodies, and checkpoint inhibitors are promising therapies that are helping to advance the field.

As reported by Dr. Marcela V. Maus and colleagues, transduction of autologous T cells to express chimeric antigen receptor (CAR) T cells, B-cell–specific monoclonal antibodies, and checkpoint inhibitors are promising therapies that are helping to advance the field.

In a late-breaking abstract presented during the 2015 ASH Annual Meeting, Dr. Syed Abbas Ali and colleagues presented the first in-human clinical trial of T cells expressing anti-CD19 CARs. Patients had advanced MM with a median of six prior lines of therapy. All patients received fludarabine to enhance activity of the CAR T cells. Two patients were treated at the highest dose level: one attained a stringent complete response, and the other had a very good partial response. Both patients were treated at the highest dose level and experienced signs of cytokine release syndrome, including fever, tachycardia, hypotension, hypoxia, and coagulopathy. The toxicities were similar to those seen in leukemia patients treated with anti-CD19 CAR T cells. This trial suggests that there may be strong anti-MM activity for CAR T cells targeting BCMA.

Humanized monoclonal antibodies against BCMA are another area of investigation showing great promise in the field.11 The success of these trials and future clinical trials will depend on the development of more patient-specific CAR T-cell approaches, which may yield better long-term disease control. The challenges of targeting BCMA-directed monoclonal antibodies are the ease of access and potential lack of toxicity. However, with the use of tocilizumab, a humanized monoclonal antibody directed against interleukin-6 receptor, to treat CAR T-cell–related cytokine release syndrome, and with better supportive care strategies, cellular therapies may also become better tolerated overtime.

Additional immune strategies are currently under investigation. For example, checkpoint inhibitors against PD-L1 and PD-L2 in pembrolizumab, a humanized monoclonal antibody of the IgG4/k, isotype against the ligands 1 and 2 (PD-1 and PD-L1), respectively. As an oligoclonal B-cell lymphoma that is unique compared with other subtypes of DLBCL, PD-1 inhibitors are actively being studied in Hodgkin lymphoma as well as multiple subtypes of non-Hodgkin lymphoma. Studies in other tumor types suggest that these drugs have activity in primary and secondary tumors involving the CNS, including melanoma and glioblastoma. Therefore, evaluation of these agents in PCNSL and PTL is warranted. Additionally, IM-8400, an antagonist to TLR 7, 8, and 9, is currently being evaluated in MYD88–mutated DLBCL. Whether these agents will have activity in PNL and PTL is yet to be determined.

These findings provide insight into oncogenic pathways in primary CNS lymphoma and primary testicular lymphoma that are unique compared with other subtypes of DLBCL. PD-1 inhibitors are actively being studied in Hodgkin lymphoma as well as multiple subtypes of non-Hodgkin lymphoma. Studies in other tumor types suggest that these drugs have activity in primary and secondary tumors involving the CNS, including melanoma and glioblastoma. Therefore, evaluation of these agents in PCNSL and PTL is warranted. Additionally, IM-8400, an antagonist to TLR 7, 8, and 9, is currently being evaluated in MYD88–mutated DLBCL. Whether these agents will have activity in PNL and PTL is yet to be determined, but rational approaches based on this work provide hope for improving treatment beyond high-dose chemotherapy.

**Novel Targets in Primary Central Nervous System Lymphoma**


Lymphoma involving the central nervous system (CNS) and the testes has long posed a therapeutic challenge given the paucity of active systemic chemotherapy achieving pharmacologic levels in these sanctuary sites. Since the 1950s, methotrexate has been used as an antineoplastic drug, and high-dose methotrexate remains the cornerstone of treatment for CNS lymphoma, although durable remission is extremely rare. As the majority of patients affected by recurrent primary CNS lymphoma (PCNSL) and primary testicular lymphoma (PTL) are elderly and not appropriate candidates for high-dose chemotherapy with stem cell rescue, novel therapeutic approaches for these diseases are desperately needed.

Dr. Bjorn Chapuy and colleagues recently performed a comprehensive analysis of the genetic features of PCNSL (Epstein Barr virus negative) and PTL by analyzing copy number alterations, chromosomal rearrangements, and recurrent somatic mutations. These findings establish a genetic signature of the two diseases, clearly distinct from that of primary mediastinal B-cell lymphoma (PMBL) and diffuse large B-cell lymphoma (DLBCL; Figure). Their findings establish a basis for clinical investigation of novel targeted approaches.

Overall, PCNSL and PTL have a unique set of abnormalities characterized by genomic instability, abnormal signaling through the TOLL-like receptor (TLR) – often in combination with activation of the B-cell receptor (BCR) pathway, and upregulation of programmed cell death-1 (PD-1) ligands. Unlike DLBCL, in which p53 and related cell cycle proteins are dysregulated, frequent copy number alterations were found, including bi-allelic loss of CDKN2A, which lies upstream of p53. In terms of mutations, nearly all cases of both PCNSL and PTL harbored MYD88 L265P mutations. MYD88 is a protein used by TLRs to activate transcription through NF-kB. This mutation is found in the vast majority of cases of Waldenstrom macroglobulinemia and in approximately 30 percent of the activated B-cell subtype of DLBCL. Copy gains of NFkB2, which also regulates NF-kB signaling, were frequently identified, as were alterations in the B-cell receptor component CD79B. Lastly, copy gains of B2M1, which includes the genes for programmed death-1 (PDL1 and PDL2) were found in more than 50 percent of cases of PCNSL and PTL.

These findings provide insight into oncogenic pathways in primary CNS lymphoma and primary testicular lymphoma that are unique compared with other subtypes of DLBCL. PD-1 inhibitors are actively being studied in Hodgkin lymphoma as well as multiple subtypes of non-Hodgkin lymphoma. Studies in other tumor types suggest that these drugs have activity in primary and secondary tumors involving the CNS, including melanoma and glioblastoma. Therefore, evaluation of these agents in PCNSL and PTL is warranted. Additionally, IM-8400, an antagonist to TLR 7, 8, and 9, is currently being evaluated in MYD88–mutated DLBCL. Whether these agents will have activity in PNL and PTL is yet to be determined, but rational approaches based on this work provide hope for improving treatment beyond high-dose chemotherapy.

**Oncogenic TLR and BCCR Signaling**

**PD-1 Ligand Deregulation**

There are no significant differences in survival between patients with PD-L1 and PD-L2 expression.

**Unique combinations of structure alterations in discrete LBCL subtypes.** The table notes frequency of specific genetic alterations modulating “genomic instability,” “oncogenic TLR and BCCR signaling,” and “PD-1 ligand deregulation” in all DLBCL, ABC-type DLBCL, PTL, EBV PCNSL, and PMBL. Used with permission.
Antithymocyte Globulin Proves a Promising Ally against GVHD


Since the development of allogeneic stem cell transplantation in the 1970s, the question of how to optimize the number and activity of donor T cells in the early transplant period has dominated the development of transplant regimens. Given the exquisite sensitivity of the immune system and the substantial genetic differences between siblings, it is no surprise that the donor immune system recognizes the patient as “foreign” and mounts a vigorous immune response. However, this alloreactive response is both damaging, in that it mediates graft-versus-host disease (GVHD), and beneficial, as it underlies the graft-versus-leukemia effect that helps to reduce disease relapse. As with many aspects of life, this balance has proven difficult to achieve, and interventions such as vigorous T-cell depletion, or the early infusion of additional T cells, have both led to clinical problems.

The administration of antithymocyte globulin (ATG) to patients around the time of transplantation is one of several approaches that have been introduced for T-cell depletion. Compared with the sophistication of modern targeted drugs, ATG has an almost slacker-like quality, as it is made from the purified immunoglobulin of rabbits that have been immunized with a human thymocyte cell line. In their article, Dr. Nicole Krögner and colleagues performed an open-label study in which ATG was administered in the three days before transplantation in a cohort of 168 patients with a primary diagnosis of acute leukemia who were undergoing myeloablative transplantation from a sibling donor. Clinical trials have been challenging to perform in the transplantation setting, and the delivery of this study is therefore a significant achievement in its own right.

The major outcome of the study is that the incidence of chronic GVHD was more than halved, from 68.7 percent in the control group to 32.2 percent in the treatment arm. Chronic GVHD is a poorly understood and often debilitating condition with a wide range of clinical manifestations, and this reduction is therefore of great significance for improving the quality of life for patients. At this stage in reading the article, transplant physicians would probably turn to the information on disease relapse and might anticipate that this benefit of reduced GVHD would be offset by an increase in disease relapse. But the remarkable finding of this study is that there was no statistically significant decrease in two-year relapse-free survival associated with the use of ATG. Indeed, at this point of follow-up, the percentage of patients who had not relapsed and who remained free of chronic GVHD more than doubled from 16.8 percent to 36.6 percent.

ATG is a three-day treatment course given prior to transplantation, and the results of this study suggest that it has an important role in this setting. It should be noted that reduced-intensity-conditioned transplantation is now a common transplant regimen, and the role of ATG in this setting remains uncertain. This is an important incremental advance for clinical transplantation. However, the observation that only 57 percent of patients remained free of both disease relapse and chronic GVHD at two years after transplantation indicates that further opportunities remain to increase the efficacy and tolerability of this extraordinary procedure.

Expanding the Mutational Spectrum in Juvenile Myelomonocytic Leukemia


Juvenile myelomonocytic leukemia (JMML) is a rare and unique form of childhood leukemia with both myelodysplastic and myeloproliferative features and clinical and biological similarities to chronic myelomonocytic leukemia (CMML) and chronic myelogenous leukemia (CML).1 The hallmark of this disease is hyperactive Ras signaling. While JMML often arises within the context of an inherited syndrome, de novo cases occur as well. Hematopoietic stem cell transplantation is presently the sole curative treatment option, but this has a success rate of only approximately 50 percent overall. There is great variability in the clinical course of JMML, and one of the most significant challenges in managing this disease is distinguishing children who will have favorable versus unfavorable outcomes.

To address these challenges, Dr. Elliot Stiegllitz and colleagues performed a comprehensive genomic characterization of primarily nonsyndromic JMML, with the goal of identifying new mutations to refine outcome prediction and to aid in the development of new therapies. The authors first performed whole exome sequencing in 29 cases of JMML, distinguishing paired germline and diagnostic tumor tissue. Tumor samples from progression or relapse were analyzed in a subset of patients as well. The authors applied an innovativeinformatics algorithm to accurately distinguish somatic and germline mutations, which was of critical importance because approximately 25 percent of patients are known to have inherited syndromes that predispose to JMML.

Key findings from this analysis were the discovery of 10 new mutations in known oncogenes and tumor suppressor genes to add to the list of mutations in five canonical Ras pathway genes (NF1, KRAS, NRAS, PTPN11 and CBL), which have been shown to be implicated in the pathogenesis of JMML. These newly discovered genes are involved in diverse processes, including signal transduction, splicing, transcription, and epigenetic regulation, and they help introduce the possibility of treating JMML with existing targeted agents, such as Janus kinase (JAK) inhibitors or epigenetic agents. All of the identified pathogenic mutations were validated in an independent cohort of 71 patient samples using targeted deep sequencing.

Additional key findings in this report included the discovery of mutations in SH2B3 in a subset of JMML patients. Mutations in this tumor suppressor gene lead to JAK-STAT pathway activation. While mutations in SH2B3 have been observed in adult myeloproliferative neoplasms and lymphoid malignancies, this was the first report in JMML. The genomic complexity of JMML was further highlighted by the observation that coexisting mutations in Ras pathway and other genes were identified in 11 percent of patients, so not all Ras pathway alterations are mutually exclusive. Furthermore, mutations in epigenetic modifier genes were observed in 14 percent of patients and led to global hypermethylation in those studied. Finally, the authors illustrated several examples of the acquisition of secondary genetic events at the time of disease progression or relapse.

The authors next analyzed the prognostic contribution of genetic mutational profile. Notably, they observed that the number of somatic alterations at diagnosis rather than the specific mutation, determined prognosis (Figure). Overall survival rates at 10 years were significantly better for patients with zero or one somatic alteration compared with two or more somatic alterations (log-rank P = .002) in the 65 percent of patients with zero or one somatic alterations at diagnosis, compared with the 35 percent of patients who had two or more mutations. Moreover, in a multivariate analysis, the number of somatic alterations at diagnosis was the most significant predictor of outcome, exceeding the prognostic impact of traditional clinical risk factors.

There is great heterogeneity in the clinical course of JMML, with some children experiencing spontaneous disease regression while others exhibit an aggressive and rapidly progressive course. Some of the greatest challenges in the management of children with JMML include this unpredictability in the clinical course as well as the limited number of effective treatment options. This study by Dr. Stiegllitz and colleagues sheds important new light on the pathogenesis of juvenile myelomonocytic leukemia and offers opportunities for both refinements in outcome prediction as well as the potential for development of novel combinatorial therapies, targeting both driver and acquired secondary mutations.

Discussions of cross-cutting science (CCS) have been percolating for quite some time. However, in the field of hematology, there have been few opportunities to fully implement a collaborative, interdisciplinary approach to solving problems, using diverse, unique perspectives. The Division of Blood Diseases and Resources (DBDR) capitalize on an opportunity to give a lofty concept some real-world meaning, in the form of a division-wide reorganization.

Dr. W. Keith Hoots, director of DBDR, spoke with The Hematologist and described how cross-cutting perspectives are making a positive impact throughout the National Institutes of Health (NIH) and NHLBI, and how they could lead to a new paradigm within the field for accelerating the pace of scientific discovery.

Can you describe the nature of the reorganization of DBDR and what is prompting the change?

DBDR reorganized its structure to better align itself with the research process — from laboratory investigations to clinical trials to population studies, and back-to-basic investigations, with the various translational steps in between these areas. With this reorganization, DBDR is moving away from organizing itself by disease category.

The new structure was established to permit DBDR to adapt to a new reality in blood science — the growing number of shared research pathways among scientists from diverse disciplines. This new landscape is providing opportunities for investigators to work in teams, and particularly for hematologists to extend their reach into disease areas not traditionally examined with the aid of their knowledge and skills.

DBDR’s decision to reorganize is in line with current activities of NIH and NHLBI in that both have initiated new programs to optimize their research enterprises in ways that cut across areas of expertise. At NHLBI, this is referred to as strategic visioning. The goal is to catalyze the development and implementation of bold, new approaches that would be difficult for any individual researcher or organization to undertake alone.

DBDR’s previous, more disease-focused branches were the Hemostasis and Thrombosis Branch, Blood Diseases Branch, and Transfusion Medicine and Cell Therapies Branch. The new branches are Molecular, Cellular, and Systems Blood Science (MCSB); Translational Blood Science and Resources (TBSR); and Blood Epidemiology and Clinical Therapeutics (BECT). Together, the new branches create a scientific research loop from basic discovery to translational clinical trials to population implementation and back to discovery (see Table).

How do you define cross-cutting science, and how is this concept being incorporated into DBDR’s mandate?

Cross-cutting science (CCS) serves as a primary rationale for undertaking this reorganization. It is inextricably linked to the need for team science since no single scientist or lab can master all the nuances of multiorgan pathogenesis. Teams of diverse individuals or groups approaching a scientific question from alternative perspectives enable a more integrated investigation that may increase both the rate and the depth of discovery. Fostering these collaborations is essential to our responsibilities as a national research program charged with enabling blood science.

In the context of our reorganization, we define CCS in at least three ways: First, we define it in terms of science that falls in the “gray zone” between the three branches, requiring ongoing conversations between two or all three branches. To expedite this essential dialogue, we are creating project groups to develop new scientific initiatives that do not fail neatly or exclusively into our organizational niches. An example would be developing a pathway for a newly developed drug to progress from first-in-human safety studies to phase II efficacy studies, toward a pivotal licensure trial. At a minimum, members from TBSR and BECT would need to collaborate to chart the funding course forward.

A second definition of CCS includes the essential collaborations across NHLBI. These CCS efforts can be institute-wide or bilateral efforts between DBDR and either the Division of Lung Diseases or the Division of Cardiovascular Sciences. With regard to the former, one recent success has been the development of a sickle cell disease (SCD) trans-institute collaborative. It will test strategies to improve the incorporation of proven therapies within the adolescent and adult SCD community. Other examples of bilateral work between divisions include the co-sponsoring of scientific initiatives in the pathophysiology of sepsis/pneumonia with our colleagues in NHLBI’s Lung Division and shared efforts with the Cardiovascular Sciences Division to determine the role of blood cells in cardiovascular biology across the arterial-microvasculature-venous continuum.

A third and very important CCS strategy for us entails cross-institute (NIH) or cross-agency (e.g., the U.S. Department of Defense [DoD]) collaborations. Not only do such efforts often enable us to leverage shared funding for common research endeavors, but they often allow entities that are chartered to target research in diverse organ loci to collaborate to undertake essential research at interfaces between the organ-centric areas.

One example we are pursuing is a collaboration with the National Institute of Neurological Disorders and Stroke (NINDS) to engender new research into the blood-brain barrier based on recent research showing the complex interplay between the cells and circulating proteins of the systemic circulation and their messaging to critical cells in the central nervous system. In addition, we work closely with our colleagues in the DoD to support research in the coagulopathy of trauma and its treatment.

Addition CCS enterprises that we are aggressively supporting focus on the molecular and cellular infrastructure that defines regenerative capacity in human injury states. Efforts to define this process entail CCS such as “organ on a chip,” induced pluripotent stem cell development, and propagation and cell-cell cross-talk. Organ-specific efforts are ongoing in multiple Institutes at NIH. Yet the inter-institute collaborations being promoted by the National Center of Appointed Sciences (NCATS) are playing an essential role in assuring that CCS scientific initiatives will benefit from an integrated effort at NIH.

Can you describe the structure of DBDR’s new branches?

Table. New DBDR Branch Structure

<table>
<thead>
<tr>
<th>Branch Name</th>
<th>Mission</th>
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<tbody>
<tr>
<td>Molecular, Cellular, and Systems Blood Science (MCSB)</td>
<td>The mission of MCSB is to advance basic research in blood science by stimulating, supporting, and overseeing: 1) basic research in normal hematology and blood disorders; 2) technology development related to blood research in academia and by small businesses; and 3) workforce training in basic and early translational blood science. More specifically, MCSB is prioritizing the following:</td>
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<td>Facilitating the mechanistic understanding of immune tolerance to therapeutic proteins or gene transfer vectors</td>
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<tr>
<td>Promoting stem and progenitor cell research and enabling early translation to cell therapy applications</td>
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<tr>
<td>Supporting innovative next-generation technology for systems and precision medicine</td>
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<tr>
<td>Providing opportunities to sustain and increase the discovery workforce in nonmalignant hematology</td>
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<tr>
<td>Translational Blood Science and Resources (TBSR)</td>
<td>The mission of TBSR is to advance translational research in all areas of blood science. This will be accomplished by supporting and stimulating development of blood-focused therapeutics and the manufacture thereof. This will require a focus on extending discovery from bench to first-in-human studies. Furthermore, this branch will play a particularly important role in coordinating training programs and in supporting small business research and development. Future priorities for TBSR include the following:</td>
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<td>Developing and maintaining strategic blood research resources (e.g., assay development and preclinical product characterization capability)</td>
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<td>Facilitating team science to develop the next generation of animal models</td>
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<tr>
<td>Developing the next generation of scientists with expertise in research translation</td>
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<tr>
<td>Working within NHLBI and across NIH to assist investigators with regulatory requirements and commercialization potential</td>
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<tr>
<td>Facilitating improved design and execution of early phase clinical trials</td>
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<tr>
<td>Blood Epidemiology and Clinical Therapeutics (BECT)</td>
<td>The advancement of clinical research throughout the spectrum of blood science is the mission of BECT. To achieve this aim, staff will oversee, support, and stimulate epidemiologic, health services, and observational research. They will promote the design and execution of therapeutic and intervention trials. The efficacious outcomes from the latter will generate comparative effectiveness and implementation research trials to measure effectiveness on population health. It is expected that these aims will result in enhancement of innovative approaches for prevention and therapeutic trials for rare diseases, and completion of societally important implementation trials of proven therapies. In addition, expected outcomes include integrated clinical trials across the lifespan, optimized transfusion and cell therapy products across broad populations, innovative methods to integrate and analyze data from population and cohort research, and “reverse translation” from the community and bedside, back to the bench.</td>
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What do you see as the potential impact of CCS on the next wave of discovery and progress within the hematologic community?

CCS will likely expand and expedite scientific discovery. Through CCS and the team science that goes with it, scientific discoveries in one area can more quickly catalyze discovery in another area. Without CCS and team-based science, such beneficial connections between areas of expertise might take years to happen, if they happen at all. By hematologists working with infectious disease experts, for example, more may be learned and faster about blood-borne parasitic infections such as malaria, and particularly, emerging blood-borne pathogens.

In addition to the intergovernmental collaborations noted above, progress within the hematologic community will depend on creative public-private partnerships dedicated to CCS. For example, with the National Cancer Center, we are creating a myelodysplastic syndrome (MDS) resource that will permit longitudinal assessment of genomic and epigenomic transcriptomic changes within hematopoietic progenitor cells in a cohort of patients with MDS as compared with a contemporaneous cohort of age-matched individuals with unexplained anemia/thrombocytopenia (e.g., idiopathic cytopenias of undetermined significance [ICUS]). We hope that this will provide molecular insight into what drives MDS and its not-invariable progression to acute myeloid leukemia.

The future of CCS will be dependent on how well we cultivate the future research workforce... Attention to every aspect along the educational continuum, including enhancing resources, will be required.

Given the ASH Agenda for Hematology Research and DBDR’s restructuring, how do you see our professional organization and your agency working together to synergize efforts?

The 2015 ASH Agenda for Hematology Research identified six areas of priority for research support (including dedicated resources from funding agencies): 1) genomic profiling and chemical biology, 2) immunologic treatments of hematologic malignancies, 3) genomic and gene therapy, 4) stem cell biology and regenerative medicine, 5) epigenetic mechanisms, and 6) venous thromboembolic disease.

Aside from the second priority focused on hematologic malignancies and immunotherapy (more appropriately the province of the National Cancer Institute), DBDR shares these priorities. As I have discussed above, for example, regenerative medicine and stem cell biology are a focus of both ongoing research support as well as our cross-cutting emphases for future scientific emphasis. We are presently supporting such efforts both through investigator-initiated research project grants as well as targeted initiatives such as the RFA for basic stem cell biologic approaches to blood “pharming” (R01) and the technological applied research “pharming” (R01) and the technological applied research and development (STTR) programs.

With regard to genomic profiling, we are presently participating in the NHLBI TOPMED (Trans-Omics for Precision Medicine) program, which is underwriting whole genome sequencing for existing NHLBI cohorts. For DBDR, this translates to a commitment to sequence DNA from patient cohorts with SCD, hemophilia, platelet disorders, and venous thromboembolism (VTE). (SCD was the ASH priority for 2014.) All of the hematologic cohorts to undergo DNA sequencing will have extensive phenotyping performed longitudinally over several years. This will permit a careful scrutiny of yet-to-be characterized genes influencing the monogenic diseases such as SCD and will allow an exomic and intronic examination of the “acquired” polygenic diseases such as VTE. Furthermore, in relation to the ASH priority of deciphering epigenetic mechanisms, the long-term strategy for TOPMED is to supplement the whole genome sequencing with targeted epigenetic sequencing. Ultimately, these efforts should provide an important foundation for precision medicine for patients with both common and rare hematologic diseases.

Gene transfer has been a mainstay of DBDR translational research efforts for nearly two decades. We have provided vector production support for multiple phase I trials on hemophilia B, for example. More recently, investigators we support by both R01 grants and targeted RFAs are doing seminal work in gene editing for SCD and severe combined immune deficiencies. We have supported work utilizing editing strategies for zinc-finger nucleases and the TALENS and CRISPR-Cas9 enzymes for SCD and thalassemia. Under the reorganization, the TBSR Branch is developing a long-term strategy for scientific and resource support for advanced gene transfer and gene editing to move toward a cure for multiple monogenic hematologic diseases.

Finally, with regard to VTE, DBDR is exploring with our colleagues at the National Cancer Institute (NCI) strategies to understand more extensively how cancer predisposes patients to pathogenic clotting — a collaborative approach that will likely engage all three of our new branches. We think that combining the oncologic expertise of investigators funded by NCI and the cohorts they have assembled with the basic, translational, and clinical coagulation expertise of DBDR-funded thrombosis investigators offers unique opportunities to extend our knowledge in this important clinical area.

Which of DBDR’s accomplishments are you most proud of during your tenure there and what is an example of a concrete endpoint you’d like to see DBDR achieve with the reorganization?

I am most proud of the team that we have assembled to accomplish the DBDR reorganization. It consists of some very experienced and dedicated scientists who have shepherded hematologic research over several decades and some newer arrivals who have brought new enthusiasm and new expertise that will enable DBDR to pursue the long-term goals discussed above. I am particularly indebted to the DBDR Deputy Donna DiMichele and the Branch Chiefs of the reorganized Branches (the DBDR Leadership Team) who have helped to engineer the process and spread the word across the “Heme” community about the how, why, and when of the reorganization. Already, we are seeing both new avenues of science (for DBDR) being pursued and broad resources being leveraged to enhance capacity.

We are very proud of the new SCD initiative for implementation science and the successful collaboration of DBDR with DoD and the NHLBI Division of Cardiovascular Sciences to complete the “PROPPRE” (Pragmatic Randomized Optimal Platelet and Plasma Ratios) trial of transfusion components for severe trauma. We are also proud of the DoD/DBDR Trans-Agency Collaboration in Trauma-Induced Coagulopathy (TACTIC), the substantial translational initiatives in both hemoglobinopathies and hematosis/thrombosis, and the other programs cited above. Additionally, we are proud of the work we have done to enhance opportunities for the next generation of researchers in blood science and are determined to further enhance efforts in this area.

A particularly important example of what would constitute a concrete endpoint for us is to increase the number of investigators nationally who research blood science.

Achieving this will require close work with many other stakeholders including ASH. We must turn the curve on the declining research workforce in blood science. Multiple strategies across many organizations and agencies will be required. The pursuit of great science is, we believe, worth the effort.

Dr. Hoots indicated no relevant conflicts of interest.
The therapy of chronic lymphocytic leukemia (CLL) has been transformed over the last several years by the introduction of novel therapeutic regimens. In this week’s Blood, Dr. Susan O’Brien and colleagues present the first trial of up-front therapy with the PD-1 inhibitor idelalisib in older patients with chronic lymphocytic leukemia. They show a remarkable 97 percent response rate, with excellent progression-free survival, albeit with significant toxicity.

In their plenary paper in this week’s Blood, Dr. Laurie Menger and colleagues report a novel gene editing strategy that disrupts the glucocorticoid receptor and renders cytomegalovirus (CMV)-specific T cells resistant to steroids while retaining antiviral functions. This study addresses an unmet clinical need: how to infuse CMV-specific T cells in transplantation patients receiving steroids.

In a pioneering contribution, Dr. Long Zhou and colleagues report their remarkable long-term results for collagen recognition by the immune receptor OSCAR. Their findings for collagen recognition by the immune receptor OSCAR-collagen interactions and create a foundation for potential therapies for a variety of diseases.

In their plenary paper, Dr. Karen Birdle and coauthors report the safety and efficacy results of a unique, prospective multi-institutional trial using sirolimus as long-term monotherapy in 30 patients with a variety of treatment-refractory autoimmune hematocytopenias, with highly encouraging results. They show particularly remarkable long-term efficacy in the 12 children with autoimmune thrombocytopenic purpura.

Chemotherapy as a Double-Edged Sword: Figuring out Who Pays the Price Later

**STUDY TITLE:** N-PhenoGENICS: Neurocognitive-Phenome, Genome, Epigenome and Nutriome in Childhood Leukemia Survivors

**CLINICALTRIALS.GOV IDENTIFIER:** NCT01913093

**SPONSOR:** The Hospital for Sick Children, Toronto, Ontario, Canada

**COLLABORATING CENTERS:** Canadian Institutes of Health Research (CIHR), Canadian Cancer Society Research Institute (CCSI), CI7 Council, Garrison Family Cancer Center at the hospital for Sick Children, Pediatric Oncology Group of Ontario

**ACCRUAL GOAL:** 500 patients, estimated at 100 patients per year for five years from 2013 to 2018

**STUDY DESIGN:** The N-PhenoGENICS trial is a prospective observational case control study with a primary outcome measure to define neurocognitive and behavioral phenotypes of childhood leukemia survivors. The two cohorts will be leukemia survivors aged between eight to 20 years with or without treatment-related adverse neurocognitive or behavioral side-effects. The trial will focus on children with a past diagnosis of acute lymphoblastic leukemia (ALL) who had received their last treatment two years prior to enrolling on this study and who are in continuous complete remission. Patients who have undergone bone marrow transplantation or who had a Down Syndrome diagnosis are not eligible.

**RATIONALE:** Chemotherapy for childhood ALL has been effective, but in some cases, it may lead to long-term adverse side effects including abnormal neurocognitive function and behavioral problems, collectively classified as the “TRANCE” phenotype, in some survivors. The hypothesis underlying this study is that individual genetic variations in folate pathways and the metabolism of methotrexate are associated with the TRANCE phenotype. To explore these aspects, the study investigators will characterize the folate and vitamin B12 intakes of these children to establish whether there are significant differences that may influence folate-dependent pathways. To identify possible epigenetic mechanisms underlying this phenotype, DNA samples will be obtained from both study cohorts and analyzed for methylation patterns.

**COMMENT:** In the past few decades, the overall survival rate for children’s cancers has increased from 10 percent to nearly 90 percent, but long-term follow-up studies have revealed that this success has come with a price. Approximately 60 percent of children who remain in complete remission suffer devastating late effects such as secondary cancers, muscular difficulties, infertility, and neurocognitive abnormalities. This raises the question as to why only some children develop these adverse effects. Are there polymorphisms in critical genes that confer a protective effect? Could epigenetic phenomena play a role? What is the contribution of important nutrients such as folic acid and vitamin B12? To answer these questions, a new phase of research is required. The investigators in this study have chosen children who have survived ALL as a model to try and explain the selective development of detrimental side effects. They are embarking on the initial steps to identify differences between children with adverse events and those who have not developed any symptoms to uncover the underlying molecular characteristics driving this susceptibility. Their ultimate goal will be to find target molecules or pathways that may be amenable to therapeutic intervention. This will be the first step towards improving the long-term quality of life for children who have survived cancer.

—Theresa Coetzee, PhD

Dr. Coetzee indicated no relevant conflicts of interest.
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