The Hematologist Acknowledges a Year of Noteworthy Articles

Compiled by The Hematologist Editorial Board

At our Editorial Board Meeting last December, a suggestion was made that, moving forward, we should compile an end-of-the-year list of journal articles that we felt merited recognition. This idea was well received by the Board of Contributing Editors, and we made plans to incorporate this concept into a feature for publication in the November/December issue of The Hematologist. What we had in mind was that each member of the Board would nominate an article, published in the preceding 12 months, that he/she felt deserved recognition. The Contributing Editors had complete leeway in deciding which article to chose. For example, the article might be chosen because the reported observations were practice-changing or because the article reported a particularly surprising or long-anticipated discovery or because the science was especially innovative. Each citation was to be accompanied by an annotation that focused on why the Contributing Editor chose to acknowledge the paper. We felt that our readers would receive such a feature enthusiastically as it provides a highly select group of papers for review. We also liked the idea of formally recognizing outstanding work both as a bow to our colleagues who have committed so much energy, effort, insight, and imagination to investigation and as a way of encouraging those who are currently invested in tackling an important, challenging problem to press on. Please turn to page 14 to see this year’s list.

(Cont. on page 14)

Plasma Sampling Provides a Window for Real-Time Detection of Evolving Chemotherapy Drug Resistance


One of the major challenges in cancer treatment is tumor progression due to chemotherapy-induced drug resistance that emerges as a consequence of clonal selection. The underlying pathobiology of this process can be seen as a form of Darwinian evolution in which the selection pressure is applied by the chemotherapy. Tumor cells that have acquired resistance through somatic mutation are selected for; thus, they then become dominant and disease progression ensues. To improve clinical outcomes, it would be advantageous to be able to track the acquired mutations that confer drug resistance so that treatment could be promptly and appropriately modified. For hematologic malignancies, such as leukemias, in which the malignant cells are circulating in abundance in the peripheral blood, obtaining samples for analysis is a simple process. However, for some hematologic malignancies (e.g., lymphomas and myelomas/plasmacytomas), and for all solid tumors, tissue sampling is often problematic. To obtain tissue for mutational analysis by repeated biopsies is a cumbersome process that puts the patient at risk for procedure-related morbidity, and the results may prove uninformative if the sample is not representative of the parts of the tumor that have acquired drug resistance.

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Now, however, this barrier to repeat sampling of tumor DNA appears to have been circumvented by a powerful new technology, the isolation and analysis of plasma circulating tumor DNA (ctDNA). (Figure) In the current proof of concept study, Murtaza and colleagues from Cambridge University report that this futuristic approach produces results representative of the entire tumor genomic DNA, including sequence variants derived from different clones. By subjecting ctDNA to deep-sequencing technology, the molecular details of chemotherapy-induced clonal selection were characterized. (Figure) Murtaza et al. reported the identification of mutations associated with acquisition of drug resistance by exome sequencing of DNA isolated from serial plasma samples obtained over one to two years during chemotherapy treatment of six patients with advanced cancer (breast, ovarian, or lung). Mutations emerged during the course of treatment as represented by the following examples: 1) an activating mutation E545K in PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) shown to promote chemotherapy resistance in mammary epithelial cells following treatment with paclitaxel and a G56V mutation in FANCD2 (involved in DNA crosslink repair) after ipilimumab treatment in the same patients; 2) a truncating mutation in RB1 (the tumor suppressor gene, retinoblastoma 1) following treatment with cisplatin; 3) a truncating mutation in MEDI (mediator complex subunit 1, an estrogen receptor co-activator) that confers tamoxifen resistance following treatment with tamoxifen and trastuzumab; and following subsequent treatment of the same patient with lapatinib and capcitabine, a sparing mutation in growth arrest-specific 6 (GAS6), a ligand for the AXL tyrosine kinase receptor implicated in chemotherapy resistance of non-small cell lung cancer and breast cancer cell lines; and 4) a resistance-conferring mutation (T790M) in epidermal growth factor receptor (EGFR) as well as a Y163C mutation in p53 following treatment with gefitinib. Changes in the allele frequencies of these mutations were noted over time, suggesting that the mutant clone had expanded as a result of an acquired proliferative/survival advantage. Simultaneous samples of tissue biopsy of metastatic disease and plasma DNA were available for analysis in two of the patients. One case of breast cancer exhibited a total of 151 mutations, 83 were present in both the tissue and plasma, with a higher allele frequency of the mutations seen in the plasma, and a positive correlation coefficient of 0.71 for mutations in the ctDNA and the tissue biopsy DNA. The other case (ovarian cancer), in which simultaneous parallel samples were available, exhibited a total of 895 mutations, of which 172 mutations were found in both tissue and plasma, with a correlation coefficient of 0.72.

This remarkable technical advance of plasma ctDNA mutation analysis will allow real-time detection of evolving cancer clones that possess the genetic changes that confer drug resistance. As these mutations are catalogued and subjected to sophisticated informatics analysis, it will be possible to devise treatment algorithms that can identify chemotherapy combinations that can be used to eradicate emerging drug resistant mutant clones before significant tumor progression has occurred.

DIFFUSION

Study design for identification of treatment-associated mutations from exome sequencing of serial plasma samples. Plasma was collected before and at multiple time points during and after treatment of cancer patients. Mutations were identified across the plasma samples and increases in relative abundance were noted. Cataloguing of similar data across large cohorts could identify genes or pathways with recurrent mutations that arise under the selective pressure of specific treatments.

Throughout our professional lives we rely on “partners.” We collaborate with other researchers in the lab, we consult with colleagues on challenging cases, and we mentor trainees during the early stages of their careers. ASH also has had several critical partners, and I’d like to use this column — my last as ASH president — to focus on our extraordinary collaboration with the Wallace H. Coulter Foundation (WHCF) and its profound impact on our Society.

The ASH-WHCF partnership began in 2003 when the WHCF provided “seed” money to launch the ASH Clinical Research Training Institute (CRTC). Since that time, more than 200 young investigators have received rigorous training on how to conduct patient-based clinical research, resulting in more than 1,000 peer-reviewed publications, scores of grants and foundation awards, corporate funding for more than 40 CRTC-developed clinical trials, and many long-term professional and personal relationships.

CRTC’s success inspired the launch of the Translational Research Training in Hematology (TRTH) program in partnership with the European Hematology Association. This program has already taught nearly 80 promising translational investigators how to investigate the pathogenesis of hematologic disorders and design new diagnostic tests and therapies. Now in its fourth year, the TRTH program continues to bring together translational hematologists from around the world and to facilitate significant and lasting scientific collaborations.

Inscribed by Mr. Coulter’s desire to improve health-care worldwide, ASH took its Highlights of ASH® (HOA) program to Latin America and Asia. During these HOA events, internationally renowned hematologists highlight the major themes and breakthroughs presented at the antecedent ASH annual meeting. This program allows hematologists abroad — particularly those who are not able to attend the annual meeting — to network, review case studies, and meet one-on-one with leaders in the field. These meetings have created opportunities for international dialogue and have fostered meaningful collaborations between and among the hematology societies in each region — a special unanticipated outcome.

The legacy of Mr. Coulter also lives on within ASH through the Wallace H. Coulter Award for Lifetime Achievement in Hematology. Mr. Coulter discovered the Coulter Principle — that electrical charge could be used to determine the size and number of particles in a solution. This discovery led to the Coulter Counter® and then flow cytometry, which revolutionized the practice of hematology by allowing point of care CBC determinations, the classification of leukemias and lymphomas, and the tracking of minimal residual disease. This award is the Society’s highest honor and recognizes an individual who has demonstrated a lasting commitment to the field of hematology through outstanding contributions to education, research, or practice. I hope you will join me in New Orleans when we recognize Professor Sir David Weatherall, MD, the 2013 recipient of this award.

This year, to commemorate the 100th anniversary of Wallace Coulter’s birth, ASH will also honor Wallace Coulter’s innovativeness with a Special Symposium featuring two outstanding translational scientists, Dr. Stuart Orkin and Dr. Bruce Beutler, who will discuss the novel concepts and technologies that should revolutionize research and practice of hematology in the future.

In addition, to commemorate the 100th anniversary of Wallace Coulter’s birth, the WHCF will present ASH with a generous endowment that will benefit the field of hematology for years to come. The WHCF is dedicated to honoring the contributions of Wallace Coulter to science and society, including his unbridled efforts to help those whose quest for excellence was impeded by financial or geographic challenges. I am pleased by what we have been able to accomplish with the support and encouragement of the WHCF partnership. This support has been critical to our education, training, and outreach missions, and I look forward to ASH extending its international programs as we advance our mission of conquering blood diseases worldwide. The WHCF gave us the “risk capital” to think creatively, act globally, and support a new generation of hematology scientists and practitioners. We extend our gratitude to the WHCF — clearly this partnership will benefit the field of hematology for years to come. The WHCF is dedicated to honoring the contributions of Wallace Coulter to science and society, including his unbridled efforts to help those whose quest for excellence was impeded by financial or geographic challenges.

On a personal note, my year as president has also been characterized by many remarkable partnerships, including partnerships with the talented ASH staff and its executive leadership, Marty Liggett and Matt Gertzog. All are extraordinarily informed, proactive, dedicated, and collaborative. Similarly, the generosity, commitment, and volunteer effort by you, ASH members, is extraordinary. As a society, we are successful because of the quality and extensiveness of our collaborations.

Thank you for the privilege of serving as your 2013 president.

See you in New Orleans!
Bringing the Hematology Community Closer at This Year’s Annual Meeting

New features strive to facilitate communication and collaboration

The 55th Annual Meeting and Exposition is quickly approaching, and there are many new offerings this year including Scientific Spotlight Sessions, Continuing Conversations (formerly “Scientific Forums”), a Special Symposium on Innovation and the Future of Hematology (mentioned in Dr. Abkowitz’s President’s Column), two Featured Topic Discussions on novel targets in hemostasis and thrombosis and chimeric antigen receptors, and a Friday Scientific Workshop on Myeloid Development. In addition, ASH is keenly aware of the vast size of the Ernest N. Morial Convention Center and the number of attendees at the meeting. Thus, the Society has taken great strides to make the meeting feel more intimate by creating Hematology MeetUps, areas throughout the convention center furnished with tables and chairs so that attendees can gather for informal, impromptu conversations and small meetings. These MeetUp areas will be designated on the convention center maps to help attendees find the nearest location.

In addition, ASH Live: Remote Session Viewing Lounges located at each end of the convention centers will allow attendees to view sessions that are taking place at the opposite end of the convention center. Each remote session viewing lounge will have television monitors/screens displaying the slides from a selection of sessions. Participants will be provided with a set of headphones so they can listen to the session speaker. Please note that not all sessions will be broadcast into the remote session viewing lounges. Look for more specifics about this new feature in ASH News Daily and in the annual meeting app.

This year ASH received 6,024 abstracts—the second-highest number on record. To accommodate presentation of the large number of outstanding abstracts, this year’s poster viewing sessions will take place in two halls (E and G) in the convention center with the hall for the general session (Hall F) in between. The evening Poster Hall Receptions will have food available in both halls. The expansion of the poster viewing session into two halls will allow for additional lounge seating and round tables within each hall to encourage informal gathering and conversation among colleagues.

To alleviate confusion, there will be signage (including a color-coded map) in and around the Poster Halls showing the scientific categories of the posters being presented in each of the two halls, and information on the organization of the poster sessions will be published in the Program Book, Meeting Notebook, ASH News Daily, and in the annual meeting app.

The larger amount of space provided by using two halls will enable ASH to pilot two poster discussion sessions on featured topics. These sessions will take place in Hall E over lunch. Look for more information and updates about these sessions online and in the annual meeting app as the meeting approaches.

The staff and leadership look forward to greeting you at this year’s meeting. For more information, go to www.hematology.org/annualmeeting_info.

ASH Elects New Leadership

Vice President:
Charles Abrams, MD, Director of the Blood Center for Patient Care & Discovery of the University of Pennsylvania and Associate Chair for Patient Care & Discovery of the University of Pennsylvania School of Medicine

Dr. Abrams will serve a one-year term as vice president, followed by successive terms as president-elect and president.

Councillors:
Michelle Le Beau, PhD, Director of University of Chicago Comprehensive Cancer Center and the Arthur and Marian Edelman Professor of Hematology/Oncology at the University of Chicago

Dr. Le Beau will serve a four-year term as councillor.

Martin Tallman, MD, Chief of the Leukemia Service at Memorial Sloan-Kettering Cancer Center and Professor of Medicine at Weill Cornell Medical College in New York

Dr. Tallman will serve a four-year term as councillor.

ASH Foundation 3K Run/Walk

JOIN US DECEMBER 8

On Sunday, December 8, annual meeting attendees will have the opportunity to participate in a 3K Run/Walk to benefit the ASH Foundation. Registration is $50 per racer; trainees are eligible for a discounted rate of $35. In addition to the registration fee, attendees can donate funds in support of the ASH Foundation or sponsor one or more trainee registrants (by registering them and paying for their registration). One-hundred percent of the registration fees and donations will benefit the ASH Foundation. Participants can form a team or run/walk on their own.

Register at www.regonline.com/ASH2013. Online registration closes December 4. Check-in and on-site registration open at 7:00 a.m. on the day of the race; the race starts at 7:30 a.m. The course is along the Mississippi River.

This is a fun and healthy way to be a part of something big. See New Orleans in a different way, and interact with award recipients for programs supported by the ASH Foundation, such as ASH Scholars and Clinical Research Training Institute participants.

Learn more at www.hematology.org/Foundation.
The Question
What is your approach to the diagnosis and management of platelet alloimmunization?

My Response
Platelet refractoriness occurs in 5 to 15 percent of patients who receive chronic platelet transfusions. The patient’s size and the number of platelets transfused should be factored into the assessment of refractoriness. For example, one measure, the corrected count increment (CCI), is computed as follows: CCI = (platelet increment after transfusion/µl) x (body surface area in m²) / (platelet dose x 10¹¹). For the purposes of this calculation, assume that each single-donor apheresis unit contains 3 x 10¹¹ platelets or that each whole-blood-derived platelet concentrate contains 5.5 x 10¹¹ platelets. Using this formula, if the platelet count increased by 20,000/µl in a patient who had a body surface area of 2.0 m² and who received one apheresis unit of platelets, the CCI = 20,000/µl x 2 ÷ 3 = 13,333/µl.

Refractoriness is defined as a CCI value below 2,500 (the percentage of the population to which the patient has antibodies to platelet-specific antigens being much less than 1%). The TRAP study showed that refractoriness predominate.

Advantages Disadvantages

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<tr>
<th>HLA-matched platelets</th>
<th>HLA type may already be on file for allogenic SCT candidates</th>
<th>Testing takes up to 1 week to complete.</th>
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<td>One-time test per patient</td>
<td>Perfect matches are rare.</td>
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<td>Some haplotypes are relatively common such as A1 B8 in Caucasians.</td>
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<td>Grade B matches or HLA Matchmaker increase the number of donor possibilities.</td>
<td>Antibody testing takes several days.</td>
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Availability of known HLA-antibody specificities
Many more donor possibilities compared with HLA matching. Antibody testing should be repeated periodically.

Platelet cross-matching
Testing takes several hours Testing is repeated for each platelet search, labor-intensive unless automated.

Applicable to apheresis or pooled whole-blood-derived platelets Commercial kits have been temporarily unavailable in the United States.

Filtration/removal of leukocytes and ultraviolet B irradiation to inactivate leukocytes were equally effective in preventing the development of platelet transfusion refractoriness which occurred in 16 percent of control patients, compared with 7 to 10 percent of patients who received leuko-reduced or irradiated platelets. On the other hand, HLA antibodies developed in 45 percent of control patients compared with 17 to 21 percent of intervention patients. Outcomes were equivalent for filtered apheresis platelets and for filtered pooled platelets. In Canada, universal prestorage leuko-reduction of platelets has lessened the incidence of alloimmune platelet refractoriness from 14 to 4 percent.

Almost all apheresis platelet units and more than 80 percent of packed red blood cell units in the United States are leuko-reduced by filtration either at the time of collection or immediately prior to storage.

HLA typing and antibody testing are complementary approaches.
Platelets express only HLA Class I antigens. For patients who are candidates for allogenic stem cell transplantation, HLA typing results may already be known. Most HLA laboratories have adopted high-throughput molecular methods for genotyping HLA Class I and II antigens. A sequence-specific oligonucleotide probe method requires only small amounts of DNA and therefore can be performed on samples from neutrophil granulocytes. HLA-A and B typing (Class I antigens) is adequate for the management of platelet alloimmunization, while high-resolution typing, including sequencing, is reserved for HLA Class II typing to select stem cell donors.

HLA antibody detection can be performed using a variety of methods. Multiplex flow cytometric bead assays are more sensitive than traditional ELISA. In the former assay, patient serum or plasma is incubated with color-coded microbeads that are coated with HLA antigens. Fluorochrome-labeled anti-human IgG is added, and a flow analyzer is used to determine the color code of the reactive beads with a computer algorithm determining the specific antigens against which the antibody is reactive. The panel reactive antibody (PRA) represents the percent of HLA targets to which the patient has made antibodies. PRA can be determined by using the traditional lymphocytotoxic assay, by ELISA, or by fluorescence-based microbead method. Serial assays are useful in assessing candidates for organ transplantation, but less so for management of platelet-refractory patients because a numerical PRA result (the percentage of the population to which the patient has HLA antibodies) does not provide actionable information to guide platelet selection.

Strategies to select platelets for refractory patients include HLA-matching, avoidance of known HLA antibody specificities, and platelet cross-matching. For the purpose of platelet donor selection, a grading system (with designated categories A, B, C, D) is employed. A perfect four-antigen match (2 loci each at HLA-A and HLA-B) is grade A. In a B1 match, all of the donor’s HLA antigens are present in the recipient, but the donor lacks one of the recipient’s HLA antigens; in a B2-match, all of the donor’s HLA antigens are present in the recipient, but the donor lacks two of the recipient’s HLA antigens; in a C-match, the donor has one HLA antigen that is not present in the recipient; in a Donach, the donor has more than one HLA antigen that is not present in the recipient. High-grade matched donors (grade A, B1, or B2) are specifically recruited to donate platelets for a particular patient, but transfusion with grade C or D “matched” units is unlikely to produce a clinically meaningful incremental increase in the platelet count. This guiding approach to matching also allows for categorization of antigens into cross-reactive groups (CREG). For example, HLA-A1 and A36 are within the same CREG, so if a patient has the A36 antigen and no available donor platelet is A36 positive, then an A1 donor platelet – typically more prevalent in the Caucasian population – can be used in a grade B match. HLA Matchmaker is an epitope-based computer algorithm used in some centers to identify permissible donor platelets that are more likely to yield adequate platelet increment increases without being HLA matched. Despite the resources invested in the management of patients who are refractory to platelet transfusion, a recent review of the literature identified no studies that were adequately powered to detect an effect of transfusion of HLA-matched platelets on mortality or hemorrhage. Prospective studies utilizing current technology and examining clinical outcomes are needed to evaluate the effectiveness of HLA-matched platelet transfusion.

For management of the transfusion-refractory patient, available data argue that selection of donors with HLA antigens against which the recipient does not have antibodies is a better strategy than HLA matching. In one observational study involving 29 refractory patients and a database of more than 7,000 HLA-typed donors, a mean of 39 donors were HLA-A and B matched, but a mean of 1,426 donors were identified as permissible by antibody
Platelet cross-matching tests the patient’s serum against samples of available donor apheresis platelets using a solid-phase adherence assay or an ELISA. A recent study found a mean CCI of 7,000 at one hour in more than 400 cross-matched platelet doses transfused to 71 refractory patients. Platelet cross-matching can be done within a few hours, compared with several days for HLA testing, but it does involve frequent repeat testing because the shelf-life of platelets is five days. Automated platforms are invaluable in making this approach efficient and practical. A recent shortage of the commercial kits used for automated platelet typing in the United States is expected to be resolved by early 2014.

A comparison of some of the advantages and disadvantages of the three strategies for dealing with refractoriness to platelet transfusion is contained in the table. Choice of method depends on local resources, and communication between the hematologist and the transfusion service is critical to ensure that donor selection is appropriate and that valuable resources are not wasted or used inappropriately.

Anti-fibrinolytic agents can be a useful adjunct for mucosal bleeding. Other approaches to ameliorating the consequences of alloimmunized platelet refractoriness include infusion of IVIG, citric acid treatment of platelets to remove Class I HLA epitopes, and infusion of recombinant activated FVII in actively bleeding patients. Despite anecdotal reports of success, none of these approaches has been validated for clinical use. Use of family members as platelet donors for patients who are potential alloimmune SCT candidates is controversial, based on a theoretical concern for inducing alloimmunization that may jeopardize engraftment. Anti-fibrinolytic agents such as epsilon-aminocaproic acid and tranexamic acid, however, can be useful in platelet-refractory patients with oral mucosal bleeding.

Physicians Face 25 Percent Medicare Payment Cut January 1

Physicians are scheduled to receive a nearly 25 percent cut from Medicare beginning January 1, 2014, unless Congress takes legislative action to prevent it. ASH strongly opposes the proposed cuts to physician payments and has long advocated both for repeal of the sustainable growth rate (SGR) formula and for a transparent and stable physician payment system.

ASH strongly believes the solution to this physician payment problem is to permanently replace the current payment formula with a system that keeps pace with the cost of caring for our nation’s seniors and does not threaten the viability of physician practices. Continuing the practice of enacting temporary patches serves no purpose.

Physicians were scheduled over the summer on legislation to reform physician payment for Medicare services and repeal the SGR, further progress on the legislation stalled this fall. While policy experts remain hopeful that legislation to permanently reform the Medicare payment system may still pass Congress, the details are complicated and will take time to work out. Consequently, yet another short-term fix may be necessary before cuts take effect again.

All Members of Congress need to hear from their physician constituents about the need to avert the Payment Cut January 1, 2014, unless Congress takes legislative action to prevent it. ASH strongly opposes the proposed cuts to physician payments and has long advocated both for repeal of the sustainable growth rate (SGR) formula and for a transparent and stable physician payment system.

ASH was a sponsor and supporter of the September 17-18 Rally for Medical Research Congressional Reception and Hill Day in Washington, DC. The events, which included nearly 200 organizations, were another opportunity for the National Institutes of Health (NIH) advocacy community to join together to push for sustainable investments in medical research that will benefit patients. ASH leadership, along with hundreds of scientists, medical research advocates, health-care providers, and patients were in attendance to call on our nation’s policymakers to make funding for the NIH a priority. The considerable momentum that the campaign gained is expected to translate into increased investment in scientific research.
Ex-Vivo Expansion of Stem Cells: Inspiration From a Highly Specialized Biologic Niche

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Background

An increasingly detailed understanding of the hematopoietic stem cell (HSC) biology along with improved outcomes after transplantation of patients with malignant and non-malignant diseases has fueled interest in HSC expansion. Clinically, both the limited availability of suitable donors and the fact that cord blood units contain a suboptimal number of HSCs remain obstacles that contribute to transplant-related morbidity and mortality as a consequence of graft-versus-host disease, delayed engraftment, and graft rejection.1 Expanding allogeneic stem cell grafts in vitro prior to transplant is an attractive solution to overcome these barriers to successful transplantation. Moreover, such strategies may be useful for expanding autologous cells in the context of gene therapy for patients with heritable diseases, the field of regenerative medicine, the capacity to expand stem cells ex vivo could lead to the generation of novel off-the-shelf products such as universal donor erythrocytes ex vivo that was further enhanced in the setting of a 3D-synthetic niche. Here we review key aspects of HSC biology and highlight promising approaches to HSC expansion.

Stem Cell Biology

The unique capacity for both self-renewal and multi-lineage differentiation is a property of stem cells that maintains the supply of all mature blood cells throughout life (Figure 1). Ethical and practical concerns preclude extensive human research into the mechanisms that determine the direction of HSC division in vivo, but mouse models have been developed that offer insights into this remarkable process. In adults, under homoeostatic conditions, HSCs exist predominantly in a dormant state with self-renewal being achieved through asymmetric outcomes from cell division, in which an HSC divides and produces one daughter HSC and one lineage-committed early progenitor cell (Figure 1A). Under specialized circumstances such as during physiologic development in utero, symmetric division dominates. In this case, two identical daughter HSCs arise from each division resulting in a 38-fold increase in HSCs by mid-gestation (Figure 1B). Serial transplant studies further demonstrate the capacity of HSCs to expand through symmetric division. Such experiments have shown that a single HSC can expand 8,400-fold and multi-lineage reconstitution of hematopoietosis has been demonstrated in a xenograft model in which mice were infused with a single purified human HSC. In aggregate, these observations demonstrate the capacity of HSCs to expand and provide an experimental method by which to validate that HSCs that are expanded ex vivo retain the property of stemness by testing their capacity to repopulate the bone marrow in vivo.

Intrinsic Targets

Many early efforts targeting expansion of HSCs focused on manipulating genes involved in self-renewal. As an example, overexpression of the homeobox gene HoxD11 in murine HSCs via retroviral transduction yielded a 50-fold increase in HSC number over controls, boosting both short- and long-term repopulating capacity.1 Similarly, another transcription factor, SALL4, has been shown to regulate pluripotency and, when made constitutively active in human CD34-enriched cells by way of lentiviral transduction, yielded a 118-fold increase in HSCs with long-term repopulating capacity in a xenogenic setting.1 However, while providing strong proof of concept for feasibility, approaches to HSC expansion that rely on commonly used retroviral vectors generally lead to sustained and unregulated overexpression of the transduced gene that, together with concerns about insertional mutagenesis, raised the daunting specter of treatment-related leukemogenesis.1 Accordingly, alternative approaches designed to extrinsically regulate both the level and duration of target gene activation so as to achieve more physiologic HSC expansion have become the focus of investigation.

Targets of Extrinsic Activation

Constitutive activation of the Notch signaling pathway in murine HSCs was shown to give rise to immortalized pluripotent cells capable of multi-lineage repopulation in vivo.7 As expression is physiologically regulated through the extracellular-binding domain of one of four receptors, investigators turned their attention to ligand-mediated, reversible activation of Notch. Induction of endogenous Notch expression in human HSCs using an immobilized form of the Delta 1 ligand yielded a 200-fold increase in the number of CD34-positive progenitor cells, and the expanded cells were shown to efficiently repopulate hematopoiesis in a xenogenic transplantation assay. Results from the initial 10 patients enrolled in a phase 1 double cord unit transplantation trial showed a 164-fold Notch-mediated expansion of HSCs that resulted in a significant reduction in time to neutrophil engraftment. Cellular copper has been implicated in HSC regulation and proliferation,8 prompting investigation of copper as a target for extrinsic manipulation to expand HSCs ex vivo. Culturing human bone marrow cells with the copper chelator tetraethylporphyrinate (TEPA) yielded a 17-fold and 159-fold increase in immunophenotypically defined human HSCs at week three and week seven, respectively, and the cultured cells were shown to engraft in murine xenograft-transplantation assays. A phase I clinical trial utilizing TEPA-cultured cord blood cells demonstrated safety and feasibility and led to initiation of a phase II/III multicenter international trial, the results of which are not yet published.

Cytokines

Almost uniformly, the above studies utilized supportive cytokines during ex vivo culture. For example, the effect of SALL4 was dependent on culture with stem cell factor (SCF), Flt-3 ligand, and thrombopoietin (TPO).9 Likewise, efficient Notch activation required the presence of various cytokines, including SCF, IL-3, IL-6, IL-11, Flt-3 ligand, and TPO.10 Not surprisingly, angiopoietins and cytokines, alone and in combination, have been investigated as potential mediators of HSC expansion. Human cord blood cells expanded with SCF, TPO, and granulocyte colony-stimulating factor (G-CSF) were found to be safe when infused in patients, but treatment yielded only modest expansion and provided no advantage in the time to neutrophil engraftment.11 Moreover, combinations of insulin-like growth factor 2 (IGF-2) or IGF-binding protein 2 (IGFBP-2) with angiopoietin-like proteins (Agft1), including Agft2, Agft3, Agft5, and Agft7, have been found to enhance ex vivo expansion of stem cells up to 38-fold.12 The observation that cytokines and growth factors that are produced by a variety of cell types greatly influence stem cell physiology suggests the importance of intercellular crosstalk and supports investigation of approaches to HSC expansion that mimic the HSC environment.

Stem Cell Niche

Elegant studies have explored the intricate relationship between stem cells and their specialized regulatory microenvironment in the bone marrow (and to a lesser extent the fetal liver).13 Long-lived HSCs appear to reside primarily at the endosteal surface of the niche where their properties are influenced by signaling from a variety of bone marrow hematopoietic cells. Stem cell–niche interactions, as components of the intercellular matrix, have also been implicated in the regulation of the stem cell niche. In particular, co-cultures of human HSCs with human ECs results in a 400-fold expansion of immunophenotypic stem and progenitor cells.13 Mesenchymal stem cells (MSCs) have also been found to enhance expansion of HSCs and inhibit differentiation during ex vivo co-culture, findings that were dependent on cell-cell contact.14 Clinically approved protocols for extraction and propagation of MSCs exist, and investigators are now utilizing HSC-MSC co-cultures for clinical ex vivo expansion of cord blood-derived and peripheral blood stem cells. Reports suggest that this strategy can provide an approximate 40-fold expansion of immunophenotypic HSCs while decreasing the time to neutrophil engraftment. Interestingly, the role of the microenvironment is not limited to cellular interactions, as components of the extracellular matrix have also been implicated in the regulation of the stem cell niche. In particular, the ECM protein fibronectin appeared to support the expansion of cord blood HSCs, an effect that was further enhanced in the setting of a 3D-synthetic scaffold.15 Similar findings with other extracellular matrix components have led to the development of synthetic biosubstrates modified with adhesion molecules in an attempt to mimic the structure of the stem cell niche.

Future Directions

Technologic advances in the development of high throughput screens of small molecule libraries have led to the discovery of several compounds with apparent HSC regulatory potential. For example, the HSC regulatory properties of prostaglandin E2 (PGE2) were first recognized as a result of high-throughput screening in a zebrafish model. Subsequent studies showed that PGE2 expanded long-term repopulating HSCs in a murine transplantation model,16 and PGE2 is currently being investigated in clinical trials of ex vivo HSC expansion in humans. A different high-throughput screen identified an aryl hydrocarbon receptor antagonist (AhR) that yielded a 174-fold increase in cells with repopulating capacity in a mouse transplantation assay.17 This novel small molecule, non-matched as Stemcellin (SR1), is undergoing clinical trials in humans. In many cases, the mechanisms by which the identified candidate molecules modulate stem cell behavior are unknown,
Figure 2

The hematopoietic stem cell niche and some of its key regulatory components relevant to HSC expansion. HSC = hematopoietic stem cell. HPC = hematopoietic progenitor cell with further lineage commitment. Endo = endothelial cell lining a blood vessel containing erythrocytes. MSC = Mesenchymal stem cell. Ost = Osteoblast.

However, the discovery efficiency offered by high-throughput screening makes this technique an attractive tool for identifying molecules with stem cell regulatory potential that can be functionally validated subsequently using conventional culture assays. The future of stem cell expansion will likely incorporate biologic and bioengineering systems approaches that topologically mimic the stem cell microenvironment. Here microscopy and imaging are invaluable tools for guiding in vitro reproduction of the architecture of the stem cell niche. Clearly, understanding the molecular pathways that mediate HSC expansion through self-renewal without lineage commitment is central to harnessing the therapeutic potential of HSC expansion. Progress in the area of HSC expansion continues to accelerate, and success in the clinic will only succeed the field of HSC transplantation, but will also fuel progress in gene therapy and regenerative medicine.

Many students of medical education were attracted to hematology as a career because of its intimate connection to discovery. Great role-model hematologists took us to the microscope to discover the cause of the disease affecting our patients and opened our eyes to a new world in which careful analysis of cell size, shape, and color could not only lead to a diagnosis, but could also help establish the patient's likely course and indicate the best therapy. We then tasted our own independent thrill of discovery late at night in the “housestaff lab” or the hospital hematology lab when we were the first to discover what was wrong with our newly admitted patient by analyzing a blood smear, a sputum stain, or a wet prep of urine sediment. Some of us chose to continue along the path of clinical discovery while others opted to try their hand at more basic discovery, and others sought discovery through clinical trials, epidemiology, or health services research connected to public health and public policy. The first group wrote fascinating case reports, whereas the latter groups communicated their discoveries through papers in specialty and general journals, but we all subliminally internalized the belief that all physicians are discoverers.

A number of things over the past 40 years have eroded the model of the physician-discoverer, including: 1) The unfortunate designation of observational science as “descriptive” and case reports as “anecdotal” in favor of statistically robust large clinical trials that contribute to “evidence-based medicine.” This has likely discouraged astute clinicians from sharing their important observations personally or in writing, but also has been valued by the scientific community. 2) The demise of the housestaff laboratory, a victim of the well-intentioned Clinical Laboratory Improvement Act (CLIA), designed to insure the quality of all clinical lab studies. 3) The increasing automation of clinical pathology testing. 4) The impact of educational debt on physician-discoverers. 5) The increasing number of PhD investigators in biomedical science, giving and informatics burdens to maintain clinical privileges whom have not participated in discovery-based medicine, combined with a diminished presence of physician/scientists in clinical activities as both the competition for research funding and the regulatory, administrative, and informatics burdens to maintain clinical privileges have increased dramatically. 6) The growth in the number of NIH investigators in biomedical science, giving the false impression that they are a separate group to whom research can be “outsourced.” 7) The increasing acceptance of the insidious notion that physicians only need to know science so that they can apply it to their patients rather than needing to know it because they have a professional obligation to contribute to medical knowledge. 8) The impact of educational debt on clinicians who would like to participate in discovery and the time and administrative demands of current medical business models. 9) The unfortunate cynicism to distort the welcome recent focus on the humanistic aspects of medicine as implying a reduced need to advance medical science or, worse yet, a dualism between humanism and science.

One of the challenges in sustaining the physician–discoverer model is the need for individuals to be comfortable while toggling between the culture of clinical medicine and that of scientific discovery. As I have previously noted, there are fundamental cultural differences between clinical medicine and basic science investigation, rooted in how each discipline educates and acculturates its trainees. The table below highlights a number of these differences. It is important to recognize them, since they frame each group’s world view, and understanding them makes it easier to appreciate how each group is likely to perceive the other. This is particularly important when clinicians with little research training first enter a basic science lab and when basic investigators begin to develop collaborations with clinicians.

The task before us is not to pine for some past time that we have undoubtedly romanticized, but rather to maximize the advantages conferred by the new opportunities available to us today to best capture the positive features of the past. I suggest the following ideas to excite a new generation of physician-discoverers to experience the satisfaction and sheer joy that accompanies being the first person in the world to know something that has the power to heal.

1) Define the physician-discoverer as the goal of medical education and add this clause to the Hippocratic Oath: “I will preserve a curiosity to seek out observations about my patients and share my discoveries with my colleagues so that all patients will benefit.” The commitment to discovery should be made on the first day of medical school by formally charging the incoming class of medical students to develop a four-year-long project in which they define a gap in our understanding of medical education and then, with the assistance of senior faculty members, design and conduct a randomized study on themselves to test their hypothesis. The results should be seen as gift to future medical school classes and, more broadly, medical students everywhere. 2) Build “wet lab” experiences into medical school hematology pathophysiology courses, emphasizing how blood smears and bone marrow samples are prepared and analyzed and how hematology and blood bank laboratory tests are performed. Hematology/pathology conferences are especially valuable when they involve the presentation of new, interesting cases.

| Table. Comparison of Some Cultural Aspects of Clinical Medicine and Basic Science Investigation |
|---------------------------------|---------------------------------|
| **Clinical Medicine** | **Basic Science** |
| Timely action required regardless of certainty | Reserve judgment until all evidence is compelling |
| Focus on what is unique | Focus on the reproducible and generalizable |
| Many uncontrolled variables | All variables identified and controlled |
| Follow practice guidelines and standard of care | Be bold and take risks |
| Error may impede someone's life and create malpractice liability | Error is expected and valuable in framing new hypotheses |
| Physicians apply new knowledge | Scientists discover new knowledge |
| Provide care to a steady flow of patients | Need to generate new ideas |
| Respect for expert opinion | Suspicion of expert opinion |
| Oath | No oath |
| Suit and tie | Jeans and T-shirt |

Dr. Storm and Dr. Kurre indicated no relevant conflicts of interest.
New Studies Connect Coagulation and Inflammation to the Pathophysiology of Cerebral Malaria Through a Common Mechanism


Childhood malaria accounts for more than a million deaths annually, and survivors are often left with severe neurologic and cognitive impairment as a consequence of cerebral involvement that may complicate the disease. Advances in mosquito control, new anti-malarials, and vaccines have tempered this plague. Nonetheless, malaria remains a dominant public health problem in sub-Saharan Africa, as the offending parasite, *Plasmodium falciparum*, has developed, through thousands of years of co-evolution with humans, elaborate mechanisms for utilizing host properties to assure its survival. Vascular sequestration of infected RBCs, a process that is mediated by binding of members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family to receptors on the endothelial lining of blood vessels, plays a central role in the pathobiology of severe malaria. Each *P. falciparum* parasite genome contains approximately 60 var genes that encode different PfEMP1 types, enabling the parasite to attach infected erythrocytes to a number of different receptors that are normally expressed by vascular endothelial cells. Despite a high rate of var gene recombination, many tandem domain arrangements, called domain cassettes (DCs), have been maintained through evolution and are therefore thought to be of functional importance. An example is DC2, which mediates binding of infected erythrocytes within the placenta in patients with the malaria of pregnancy. Severe malaria in children is associated with expression of a subset of PfEMP1s, DC8 and DC13, but prior to the studies of Turner and colleagues, the endothelial receptor for these proteins had remained enigmatic.

To identify the receptor, Turner et al. generated recombinant, full-length DC8-PfEMP1 and used it to screen an array of 2,505 full-length human plasma membrane proteins. One specific hit identified the endothelial protein C receptor (EPCR) as a potential binding partner. *P. falciparum*-infected RBCs bound EPCR on brain microvascular endothelial cells, and binding was inhibited by anti-EPCR antibodies. To show functional relevance to activation of protein C, the authors demonstrated that domains derived from both DC8 and DC13 competed with activated protein C (APC) for the same binding site, and an antibody that blocks APC binding to EPCR also prevented parasite binding to endothelial cells. Soluble EPCR shed from endothelial cells by treatment with the metalloproteinase TNF-α converting enzyme was shown to block parasite attachment to the vessel wall cells. Together, these studies establish the EPCR as the binding site for DC8 and DC13 and suggest that hijacking of the receptor for APC contributes to the pathobiology of severe childhood malaria (Figure).

The studies of Turner and colleagues dovetail with the recent findings of Moxon and colleagues who examined the brains of 10 children who had died of cerebral malaria (CM). They observed sequestration of infected erythrocyte (IE) within the cerebral vasculature and showed significantly more fibrin deposition and microvasculature hemorrhage in those samples when compared with the brains of malaria-infected children without evidence of cerebral vascular IE sequestration who died of non-malarial causes based on autopsy findings. The brains of those with CM demonstrated endothelial loss of anticoagulant receptors EPCR and thrombomodulin (TM) that are critical components of the protein C pathway of anticoagulation (Figure). As previously reported, TM was not seen in control brain neuroendothelium, however, TM is expressed in the vasculature of the subcutaneous fat tissue, and examination of such tissue samples from children with CM showed evidence of IE sequestration and reduced expression of both EPCR and TM. Turner et al. also reported that soluble EPCR and TM were increased in the CSF of CM patients. Retinopathy is a sensitive marker of IE sequestration, and retinopathy-positive CM patients had evidence of activation of coagulation with higher concentrations of thrombin-anti-thrombin III complexes, longer prothrombin times, and elevated systemic APC levels.

The protein C anticoagulant system also has anti-inflammatory and cytoprotective activity. APC binding to EPCR activates PAR1 G-protein signaling in the endothelium (Figure), decreasing vascular barrier permeability through sphingosine-1-phosphate signaling. Thus, *P. falciparum*-infected RBCs can selectively bind to EPCR on neuroendothelium that are constitutively lacking in TM causing shedding of EPCR and loss of both APC’s cytoprotective and anti-thrombotic properties, leading to vascular microthromboses and hemorrhage in CM (Figure). The latter process may be responsible for the long-term neurologic and cognitive defects experienced by survivors of CM.

Van der Poll nicely summarizes the potential mechanisms by which *P. falciparum* interactions with EPCR contribute to the pathophysiologic of CM (Figure). The data suggest that recombinant APC or TM infusions or therapies that target the PAR1/sphingosine-1-phosphate anti-inflammatory signaling pathway may limit intracranial bleeding and tissue damage and thereby reduce the morbidity and mortality of CM.


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**Figure**

**Role of EPCR in cerebral malaria.**

A) During normal homeostasis, excessive thrombin is bound by thrombomodulin expressed by endothelial cells. The thrombomodulin-thrombin complex activates protein C to APC, a process that is strongly accelerated by APC. APC exerts the anticoagulant effects when it becomes detached from EPCR by inactivating clotting factor Va and VIIIa. In addition, APC bound to EPCR has cytoprotective properties by activating PAR1. B) (Upper) In malaria, parasite-infected erythrocytes induce the loss of EPCR and thrombomodulin from the endothelial cell surface at least in part by shedding of these receptors. As a consequence, the capacity to produce APC is greatly impaired, resulting in enhanced coagulation. The resulting high thrombin levels can induce proinflammatory-barrier disruptive effects on blood vessels via PAR1. (Lower) Plasmodium-infected erythrocytes transport PIEMPI to their membrane, which can bind EPCR in the same region as APC. As a result, APC is less capable of inducing cytoprotective effects via PAR1.

Figure from van der Poll. Blood. 2013;122:625

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**References**

**Decreased Erythropoiesis in Iron Deficiency: A Key Role for IRP1 Regulation of HIF2α**


Intracellular iron content is post-transcriptionally controlled by two iron regulatory proteins (IRP1 and IRP2) that bind to iron-responsive elements (IREs), which are specific RNA nucleotide sequences forming stem-loop structures in 5' and 3'-untranslated regions (UTRs) of mRNAs encoding proteins involved in cellular iron import, export, and storage. In iron-replete cells, IRP1 contains an iron-sulfur cluster at its active site and has cytosolic aconitase activity. In iron-deficient cells, IRP1 loses its iron-sulfur cluster and assumes the configuration that binds IREs. IRP2 is stable and binds IREs during iron deficiency but undergoes rapid degradation under iron-replete conditions. Examples of IREs can be found in the 5' UTR of mRNAs encoding feritin and ferritin where IRP binding inhibits translation, thereby decreasing iron storage and export, respectively, and in the 3' UTR of transferrin receptor mRNAs where IRP binding stabilizes and enhances mRNA translation, thereby increasing transferrin receptors and iron importation.

IREs are also found in mRNAs that encode proteins related to iron utilization, including heme biosynthesis, energy metabolism, and response to hypoxia. Among these mRNAs are those encoding HIF2α, the hypoxia-responsive component of the transcription factor complex that binds the hypoxia-responsive elements of many genes, including erythropoietin (EPO) (Figure). Under normoxic conditions, two prolines of HIF2α are rapidly hydroxylated by prolyl-hydroxylases that contain iron in their active site and use molecular oxygen, thereby targeting HIF2α for polyubiquitination by the von Hippel-Lindau protein and subsequent proteasomal degradation. This oxygen-dependent HIF2α hydroxylation process maintains low EPO production under normoxic conditions. Under hypoxic conditions, such as anemia, decreased oxygen availability limits HIF2α hydroxylation, resulting in greater HIF2α stability and activity, with the end result being rapid transcription, translation, and secretion of EPO by renal interstitial fibroblasts, the major source of EPO.

Tight feedback control of EPO production by this oxygen-regulated system is consistent with the absence of polycythemia overshoot in association with periods of increased erythropoiesis, such as following the period of rapid growth in post-natal mice or following acute blood loss. However, Ghosh et al., and the authors of two other recent publications,1,2 report that following the post-natal growth period, IRP1 knockout (lp1-/lp1-) mice develop increased hematocrit/hemoglobin (Hct/Hgb), serum EPO, and HIF2α in renal interstitial EPO-producing cells. These findings suggest an oxygen-independent mechanism in which HIF2α activity is controlled by IRP1 (Figure). In addition to renal interstitial cells, Ghosh et al. demonstrated that IRP1 is the prevalent IRP in pulmonary endothelium, and that lp1-/lp1- mice have increased pulmonary hypertension associated with increased lung HIF2α and endothelin-1, the protein of another HIF2α regulated gene. Thus, lp1-/lp1- mice provide a model for diseases with polycythemia and/or pulmonary hypertension due to increased HIF2α, such as Chuvash polycythemia, von Hippel-Lindau disease, and activating HIF2α mutations.

When fed a low-iron diet, lp1-/lp1- mice have decreased splenic iron, serum iron, and transferrin saturation compared with lp1+/lp1- mice fed a normal diet. Compared with lp1+/lp1- mice fed a normal diet or wild-type mice fed either type of diet, iron-starved lp1-/lp1- mice have increased renal HIF2α, serum EPO, Hct/Hgb, and extramedullary erythropoiesis, but they have lower MCV and MCH. Many iron-starved lp1-/lp1- mice die suddenly as a result of intra-abdominal hemorrhage. Thus, iron-starved lp1-/lp1- mice have a phenotype like some polycythemia vera patients i.e., elevated Hct/Hgb with hypochromic, macrocytic indices, and increased vascular accidents.) In polycythemia vera, erythropoiesis increases despite limited iron availability because mutant JAK2 kinase associated with EPO receptors increases erythropoietic responses when EPO levels are low. In lp1-/lp1- mice, elevated HIF2α, EPO, and erythropoiesis as a consequence of derepression of HIF2α mRNA translation are increased further with iron starvation. In this situation, iron starvation most likely inhibits HIF2α hydroxylation by iron-containing prolyl-hydroxylases (Figure). Thus, Ghosh et al. demonstrate that IRP1 suppression of HIF2α mRNA translation during iron deficiency inhibits EPO production, thereby restricting erythropoiesis. The IRP1--induced inhibition of erythropoiesis is crucial for development of anemia during iron deficiency and treatment of polycythemia vera by phlebotomy-induced, iron-restricted erythropoiesis.


**Figure**

**Iron Replete, Normal**

**Iron Deficient, Normal**

**Iron Deficient, IRP1 Knockout**

**IRP1 Regulation of Erythropoietin (EPO) Production**

The production of EPO in renal interstitial fibroblasts is shown for normal individuals under iron-replete conditions, and for normal and IRP1 knockout individuals under iron-deficient conditions. Under iron-replete conditions (left panel), IRP1 contains an iron-sulfur cluster and has cytosolic aconitase activity, but it does not bind the iron responsive element (IRE) of HIF2α. Under conditions of iron deficiency, IRP1 lacks the iron-sulfur cluster, and in this state, binds the IRE of HIF2α mRNA (center panel). HIF2α is a component of the transcription factor complex that controls EPO production. Relative amounts of HIF2α (pink), hydroxylated HIF2α (white), and EPO (red) are indicated by the size of rectangles. In iron-deficient individuals (center panel), IRP1 binding to the 9' IRE of HIF2α mRNA inhibits translation, thereby reducing the availability of HIF2α, ultimately restricting EPO production by renal interstitial cells and thereby contributing to the anemia of iron deficiency. When oxygen delivery to the kidneys is normal, HIF2α is rapidly hydroxylated by iron-containing prolyl-hydroxylases (blue oval) and undergoes proteasomal degradation so that renal HIF2α is low and only a few cells produce EPO under either iron-replete (left panel) or iron-deficient (middle panel) conditions. Under conditions of renal hypoxia, as occurs with anemia, less oxygen is available to support the prolyl-hydroxylase reaction. Consequently, degradation of HIF2α is limited, resulting in increased renal HIF2α and greater production of EPO compared with normal normoxia. This process contributes to the increase in EPO production that is observed in response to anemia. In normal individuals with intact IRP1 function, iron deficiency reduces the amount of HIF2α formed and, therefore, restricts the amount of EPO produced, even under hypoxic conditions (middle panel). Therefore, renal HIF2α and EPO production are decreased in iron deficiency as compared with iron-replete conditions. In the IRP1 knockout condition (right panel), basal HIF2α and EPO production are increased under normal conditions, and iron deficiency exacerbates these already elevated levels, presumably by decreasing the activity of iron-containing prolyl-hydroxylase (smaller blue oval), even when the kidney is normoxic.

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Dr. Koury indicated no relevant conflicts of interest.
Follicular Lymphoma Cells Spin a Web of Immunosuppression


There is a good deal of circumstantial evidence to suggest that follicular lymphoma is potentially immunogenic, including occasional spontaneous regressions and its strikingly long latency. Supporting information is derived from studies of gene-expression profiling showing that the signature of infiltrating T cells and macrophages appears to correlate with clinical outcomes. However, it is not clear how these relationships work, and the published literature can be confusing. The situation is made worse by the confounding effects of treatment; if patients receive chemotherapy, the presence of an inflammatory infiltrate is more likely to be seen as favorable, whereas for those receiving an anti-CD20 antibody it may be helpful. Similar to other tumours, it seems likely that follicular lymphoma can exert a locally immunosuppressive effect (Figure), but unlike epithelial cancers, nodal lymphoma arises in a tissue already consisting of a mixed infiltrate of T cells with which the neoplastic B cells normally have multiple cognate interactions (Figure). This study from Kaij and colleagues at Saint Bartholomew’s Hospital in London provides some interesting mechanistic data about the differences between normal and malignant lymph nodes, and the interplay of B and T cells within them.

Gene-expression profiles in purified tumor-infiltrating T cells (TILs) taken from lymphoma biopsies were compared with T cells taken from reactive tonsils, and a number of genes were identified as deregulated in both CD4+ and CD8+ cells. For certain specific genes (PMCH, ETV1, CD200, and NAMPT), the findings from the microarray analysis were confirmed by RT-PCR and by immunohistochemistry. The phenotypic changes in TILs could be reproduced in normal T cells by co-culture with malignant lymphoma B cells, although some genes (PMCH, ETV1) were upregulated in transwell cultures that keep the B and T cells physically separate, while others (CAV1) required direct cell-cell contact. One of the most strongly down-regulated genes in lymphoma TILs was ACTN1, which prompted the group to examine the changes in B-cell motility in vitro. They showed that co-culture of healthy T cells with lymphoma B cells significantly reduced their motility, suggesting that greater motility of follicular lymphoma cells as a result of inhibition of T-cell-derived production of alpha-granules may contribute to the neoplastic phenotype. Finally, Kaij and colleagues examined the prognostic significance of three of the upregulated T-cell genes and showed that levels of PMCH, ETV1, and NAMPT measured by immunohistochemistry correlated with overall survival and time to transformation, although the relationships were complex, depending on the specific location of the T cells within the node (interfollicular or intrafollicular). It was notable that the genes identified in this study were distinct from those determined to be prognostic in previous gene-expression array experiments that used whole-nodal RNA preparations.

Normal nodal physiology involves a dense set of interactions between B and T cells, and even malignant B cells from follicular lymphoma require additional signals from their microenvironment to maintain their viability. It is clear from this study and others that the signals go strongly in the other direction too, with malignant B cells having the capacity to produce important phenotypic changes in TILs, specifically immunosuppressive effects. Although our understanding of this process is still in the early stages, characterization of the immune regulatory properties of tumor cells is becoming increasingly important as immunomodulating approaches to treatment such as induction of innate immune responses by CpG, Toll-like modulation with anti-PD-1 or anti-PDL-1 antibodies, or adoptive cellular therapy with modified chimeric antigen-receptor-bearing T cells enter the clinic arena. The striking clinical results seen with the combination of rituximab and lenalidomide support the idea that future therapeutic approaches are likely to involve combination immunotherapy.

Elucidating the mechanisms that underlie lymphoma’s immune escape strategies will become increasingly relevant as development of novel therapeutics that target their reversal is now feasible.


The Consequences of Failing to Obey the Traffic Rules


The genetic basis of the multitude of disorders associated with neutrophil function reflects the interdependence of the numerous intracellular systems required to maintain a vigilant defense against infection and to maintain the integrity of the tissue microenvironment. Different cell lineages or organ systems are also affected by a particular mutation.

Now, two groups of investigators have identified an immunodeficiency syndrome linked to mutation of VPS45, one of the endocytic pathway vacuolar-sorting proteins found in neutrophils. VPS45 is a member of the Sec1/Munc18 protein family that regulates the assembly of SNARE complexes that mediate membrane traffic through the endocytic-lysosomal pathway. The phenotype of VPS45-associated human disease consists of congenital neutropenia, recurrent infection, progressive bone marrow failure, nephromegaly, thrombasthenia, and recurrent, life-threatening infections. In both case series, patients presented during infancy and were identified as the offspring of consanguineous marriages from several unrelated Palestinian families. Homozygosity for the Thr224Aun mutation (c.671 C→A) within exon 7 of VPS45 segregated with affected individuals and followed an autosomal recessive pattern of inheritance. The mutated codon was highly conserved throughout evolution and was not found in almost 400 Palestinian controls or in a panel of other healthy individuals. In a Moroccan pedigree, affected siblings exhibited a different homozygous mutation (c.712G→A; p.Glu238lys). Affected infants with mutant VPS45 frequently exhibited transfusion dependence, life-threatening thrombocytopenia, and G-CSF-resistant neutropenia.

Outcomes were determined primarily by susceptibility to infection that resulted in death either before or as a complication of hematopoietic stem cell transplantation. Laboratory and histopathologic findings included the following: normocytic anemia with reticulocytopenia, anisocytosis, and karyotypic abnormalities were not identified. Significant reticulin (and sometimes collagen) fibrosis was found in all patients. Kidney biopsies revealed nephromegaly that was due to extramedullary hematopoiesis.

Ultra-structural and functional studies were undertaken in selected patients to assess the biologic effects of mutant VPS45. Compared with age-matched controls, electron microscopy demonstrated immature neutrophils with fewer azurophilic granules, increased mitochondria, more developed Golgi and endoplasmic reticulum, and less condensed chromatin and cytoplasm.

Lymphocytes were depleted in cultured fibroblasts as well as in platelets, which also exhibited decreased alpha-granule content. Expression of VPS45 and its binding partners rabenosyn-5 and syntaxin-16 were decreased in circulating neutrophils and/or fibroblasts from an affected patient. Similar reduction of syntaxin-16 was observed with loss of VPS45 function in yeast. Migration of fibroblasts was impaired in an in vitro wound assay in which a scratch was introduced across a field of cells in culture. VPS45-mutant neutrophils were found to express less β1 integrin, a cell surface protein critical for motility. In addition, markers of apoptosis were significantly increased in fibroblasts and in bone marrow biopsy specimens from patients with the mutation. In zebrasfish, knockdown of VPS45 resulted in a marked reduction in myeloperoxidase staining, consistent with decreased numbers of mature neutrophils. Transfection of mutant fibroblasts with non-mutated VPS45 cDNA was capable of correcting the abnormal phenotype.

Mutation of VPS45 highlights the pathogenetic consequences of altering the integrity of lysosomal traffic. Further dissection of how mutation of VPS45 leads to disruption of this essential step and shipment of intracellular cargoes containing bioactive cytokines should provide mechanistic insights into neutrophil-related immunodeficiency, thrombocytopenia, and infection.
Immune Whispering: Safely Harnessing the GVL Effect Using Vaccines


For many advanced hematologic malignancies, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only therapy that is potentially curative. The initial strategy of allo-HSCT for treatment of leukemia was associated with significant clinical trials that eliminated many patients with the bone marrow (inevitably accompanied by eradication of normal marrow elements) by delivering myeloablative doses of radiation and chemotherapy. The ablated marrow would then be rescued by transplant of donor stem cells. Quickly (and surprisingly), however, investigators came to realize that an important component of the therapeutic efficacy of allo-HSCT was initiation of a robust and sustained anti-tumor effect mediated by the developing donor-derived immune system. Awareness of the importance of the graft-versus-tumor effect came in part from observations that treatment failure was greater if the source of donor stem cells was a syngeneic twin or if the donor sample was depleted of T cells ex vivo. Investigation into the mechanisms involved in the graft-versus-leukemia (GVL) effect has provided important insights into the basis of the power of cancer immunotherapy, and the notion of harnessing the curative potential of the immune system has fueled the field of tumor immunology for decades. A major challenge that has plagued the field is avoidance or minimization of the toxic effects of graft-versus-host disease (GVHD) while preserving the GVL effect. Although some would argue that allo-HSCT has been limited by toxicity, the use of donor lymphocyte infusion, manipulation of T regulatory cells, and inhibition of suppression of anti-tumor T cells by antibodies that target CTLA-4 and PD-1 as examples of how therapies derived from allo-HSCT have immunology have been translated into clinically applicable treatment modalities. Also because of lessons learned from allo-HSCT about the untoward consequences of GVHD, interest has focused on development of treatment approaches that more directly target the tumor cell. Exciting examples of this strategy include development of both chimeric antigen receptor (CAR) T cells and bi-specific T-cell engager (BiTE) compounds.

In another example of a strategy that has arisen from the study of tumor immunology, Ute Burkhardt and colleagues in the laboratory of Catherine Wu from Dana-Farber Cancer Institute in Boston set out to safely and effectively harness the GVL effect. To drive expansion of leukemia-specific T cells and thereby induce GVL immunity, the team of investigators used a well-characterized bystander-cell vaccine strategy in which non-sensitized cells that secrete large amounts of GM-CSF are co-injected with irradiated tumor cells obtained from the patient prior to allo-HSCT. This vaccine, injected half subcutaneously and half intradermally, delivers a broad range of antigens including patient-specific tumor targets to antigen-presenting cells within the context of a GM-CSF driven pro-inflammatory environment.1 This strategy has previously been investigated in solid and liquid tumors, but despite inducing detectable anti-tumor immunity, the array of immunosuppressive strategies that exist within the tumor environment including those mediated by PD-1 and CTLA4 thwarted investigators using this approach.

In the present studies, Burkhardt et al. focused on patients with chronic lymphocytic leukemia (CLL) in whom reduced-intensity conditioning (RIC) was used as the preparative regimen prior to allo-HSCT. The benefit of RIC is that the treatment is less toxic than standard ablative regimens, resulting in a favorable therapeutic index for patients that would otherwise be unsuitable for allo-HSCT.1 This advantage of RIC is dependence upon GVL for the elimination of residual leukemia cells. Given the unpredictable nature of GVL and our inability to specifically promote it, disease relapse has become a primary cause of treatment failure in patients whose treatment regimen includes a RIC-preparative regimen.

In this prospective, phase I study, 18 patients with a B/B HLA-matched, related or unrelated donor received 5×10⁸ normal cells and 45×10⁸ post-transplant. The authors reported an increase in leukemia-specific immune responses in patients who received the vaccine as compared with unvaccinated patients. Moreover, in the plurality of cases, ex-vivo testing revealed that this T-cell-driven response was capable of differentiating between tumor cells and non-malignant host tissues. Overall, after a median follow-up of 2.9 years (range, 1-4 years), the progression-free survival of vaccinated subjects was 82 percent.

This study is important because it documents the safety and potential efficacy of a vaccine strategy to harness allogeneic stem cell transplantation setting. The authors cited a number of factors that could have contributed to the success of their study; most notable, however, was the vaccine’s capacity to drive a specific GVL response despite GVHD prophylaxis and incomplete immune reconstitution. It will be exciting to watch the continued clinical development of this strategy. Perhaps this paper will stand as a milestone marking an approach to safely harnessing GVL, arguably the most effective means of initiating and sustaining potentially curative anti-tumor immunity.


What is the Optimal Minimal...


The availability of highly effective combination chemotherapeutic regimens has raised the question of whether treatment with high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) benefits patients with multiple myeloma. To address this question, clinical trials have been initiated in which patients between patients receiving induction with combination chemotherapy followed by treatment with a myeloma-specific regimen together with ASCT and those receiving the same induction regimen but without the subsequent myeloma-specific regimen in combination with ASCT. In some cases, the studies are also designed to test the value of maintenance therapy in the two treatment arms. The large enrolment of these multi-institutional (and in some cases multinational) studies provides opportunities to address other issues that might impact on management of patients with multiple myeloma. For example, within each treatment arm, establishment of parameters that predict outcome would allow identification of subcategories of patients who might benefit from alternative treatment modalities. One measure that has been used prognostically is complete response (CR) (achieved when the M-protein becomes undetectable by immunofluorescent electrophoresis). CR is a valuable, but imperfect predictor of outcome, leading investigators to seek other parameters, including assessment of minimal residual disease (MRD), which might have greater predictive value. Multiple myeloma especially lends itself to measurement of MRD as the malignant cells have both a distinct immunophenotype and specific clonal genetic markers. Thus, both flow-cytometric and polymerase chain reaction (PCR)-based techniques can be used to assess MRD in patients with multiple myeloma.

The current paper reports the results of analysis of MRD in patients with multiple myeloma treated on the British Medical Research Council Myeloma IX trial. Multiparameter flow cytometry (MFC) was used to assess MRD after completion of induction therapy (n=178) and at day 100 after ASCT (n=245) in patients (those who underwent induction therapy followed by treatment with high-dose chemotherapy in combination with ASCT) and at the end of induction therapy in the non-intensive-pathway patients (n=245) (those who underwent treatment with the same induction regimen but who did not go on to receive high-dose chemotherapy in combination with ASCT). For the intensive-pathway patients, absence of MRD at day 100 after ASCT was predictive of a favorable outcome (P<0.001 for progression free survival; P=0.018 for overall survival) and was demonstrable in patients with either favorable or adverse cytogenetics and also in patients who achieved an immunofixation-negative CR (P=0.0068 for progression free survival). The study also demonstrated that maintenance therapy could improve MRD status, as 28 percent of MRD-positive patients who received maintenance thalidomide subsequently became MRD-negative. MRD-positive patients who did not receive maintenance therapy had the shortest PFS, while MRD-negative patients who received maintenance thalidomide had the longest PFS. Notably, MRD status after induction therapy in the non-intensive-pathway patients was not predictive of outcome (PFS, P=0.1).

The results of the studies of Rawston and colleagues suggest that MRD status has prognostic significance for patients with myeloma who have undergone treatment with myeloblastic chemotherapy followed by ASCT, and the technique used in the current study can identify among patients who achieve a immunofixation-negative CR a subgroup of patients (i.e., those with a non-MRD negative) with a more favorable outcome as measured by progression free survival. Thus, an argument can be made that assessment of MRD should be included among the prognostic parameters used to evaluate response to treatment in patients with myeloma who receive the regimen of high-dose chemotherapy followed by autologous stem cell rescue. However, there are challenges to the routine incorporation of MRD assessment in the management of multiple myeloma including identifying the most appropriate assay and determining the most meaningful time points at which to measure it. 

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XAVIER LELEU, MD
Dr. Leleu indicated no relevant conflicts of interest.
We’ll Get Practice Pronouncing Schinzel–Giedion: Somatic SETBP1 Mutations in Myeloid Neoplasms


The key findings from the new studies are that SETBP1 mutations are present in 17 percent of secondary AML (sAML) and 15 percent of CMMML cases in the Makishima series and in 16 percent of patients with the pediatric MDS/MPN-juvenile myelomonocytic leukemia (JMML) in the Sakaguchi cohort. Other groups in the United States and Europe have presented data about recurrent SETBP1 mutations in their patients with MDS (2.2–4.3% mutation frequency), primary myelofibrosis and other MPNs (2.5–4.5%), MDS/MPN overlap syndromes including CMLL (6.2–9.4%), and sAML (1.7%).* Mutations in SETBP1 in these series have been associated with disease progression, higher white counts, higher-risk karyotypes such as monosomy 7, and poorer clinical outcomes.

Discovery of recurrent SETBP1 mutations in CMLL, MDS/MPN, and sAML has no immediate therapeutic implications, but it is a new piece of the myeloid neoplasia molecular pathology jigsaw puzzle, and does appear to be a marker of higher-risk disease. Further insight into the function of both wild-type and mutant SETBP1 should aid in developing new targeted treatments for myeloid neoplasms.


4-Panel Assay May Reverse “LAC” of Antiphospholipid Antibody Detection


The pathophysiology of recurrent pregnancy loss is incompletely understood, but testing for lupus anticoagulant (LAC)/phospholipid-dependent antibodies (aPL) is commonly performed to determine if an underlying prothrombotic disorder might contribute to disease pathogenesis in an individual case and, if detected, suggest a therapeutic approach (i.e., anticoagulation) to reduce the risk of spontaneous abortion. aPLs are acquired autoantibodies against phospholipids such as cardiolipin and against phospholipid-binding proteins such as β2-glycoproteins I, prothrombin, and annexin A5. In the clinical setting, they are detected by ELISAs for IgG and IgM anti-cardiolipin antibodies and for IgG and IgM anti-β2-glycoprotein I antibodies. Functional coagulation assays are used to identify the LAC. These assays are specially designed to take advantage of the requirement for phospholipids in in vitro coagulation assays. For example, anti-phospholipid antibodies prolong the clotting endpoint in the dilute Russell viper venom time (dRVVT) assay, in a modified, sensitive version of the activated partial thromboplastin time assay (PTT-LA), in the kaolin clotting time (KCT) assay, and in the dilute prothrombin time (dPT) assay. The International Society of Thrombosis and Haemostasis (ISTH) recommends the dRVVT and the PTT-LA for routine clinical use. Although there is support for diagnostic guidelines such as the Sapporo criteria, definitive establishment of the presence or absence of anti-phospholipid antibodies remains problematic because interpretation of testing is compromised by both false-positive and false-negative results, by temporal inconsistencies in test results, and by assay variability.

Some of the issues that bedevil the field are underscored in the report of Clark and colleagues from the University of Toronto. The investigators participate in a tertiary clinic that annually enrolls ~250 new patients with recurrent pregnancy loss, in addition to patients with systemic lupus erythematosus (SLE) and/or antiphospholipid syndrome (APS). In a retrospective analysis that covered a six-year period, 2,257 women with recurrent pregnancy loss, SLE, and/or APS who had been screened for the presence of the LAC were characterized. Each patient in the study had samples tested using each of four different LAC assays (dRVVT, PTT-LA, KCT, and dPT) that had been in operation in the clinic for 20 years. The LAC was considered present when the clotting times were prolonged in any of the four screening assays. Positive results of the screening assays were validated using both mixing studies and an assay that confirmed phospholipid dependence. To be counted as positive in the study, LAC had to be present on more than two occasions, not less than 12 weeks apart, and within a period of six years. LAC-negative patients were defined as those testing negative for LAC on all four tests. All patients also underwent immunologic testing for both IgG and IgM anti-cardiolipin antibodies.

Of the 2,257 females studied, 138 patients (6.0%) were initially LAC-positive, with 62 (2.7%) testing positive for LAC on at least two occasions (43/136 patients who were initially LAC-negative were lost to follow-up and were not tested on more than one occasion). Compared with LAC-negative subjects, LAC-positive patients were significantly more likely to have a history of thromboembolism (TE) (idiopathic or with a risk factor), stillbirth, intrauterine growth retardation, and HELLP syndrome; but they were significantly less likely to have a history of recurrent pregnancy loss or a history of two or more early spontaneous abortions. Notably, only 5 patients (0.2%) had a history of recurrent miscarriages were LAC-positive. No significant difference was observed between the LAC-positive and LAC-negative group for a history of preeclampsia, a history of at least one venous thromboembolism (VTE), a history of at least one arterial thromboembolism (ATE), or a history of antiphospholipid syndrome (APS). In a retrospective analysis that covered a six-year period, 2,257 women with recurrent pregnancy loss were characterized. Each patient in the study had samples tested using each of four LAC assays (dRVVT, PTT-LA, KCT, and dPT) that had been in operation in the clinic for 20 years. The LAC was considered present when the clotting times were prolonged in any of the four screening assays. Positive results of the screening assays were validated using both mixing studies and an assay that confirmed phospholipid dependence. To be counted as positive in the study, LAC had to be present on more than two occasions, not less than 12 weeks apart, and within a period of six years. LAC-negative patients were defined as those testing negative for LAC on all four tests. All patients also underwent immunologic testing for both IgG and IgM anti-cardiolipin antibodies.

Discovery of recurrent SETBP1 mutations in CMLL, MDS/MPN, and sAML has no immediate therapeutic implications, but it is a new piece of the myeloid neoplasia molecular pathology jigsaw puzzle, and does appear to be a marker of higher-risk disease. Further insight into the function of both wild-type and mutant SETBP1 should aid in developing new targeted treatments for myeloid neoplasms.


David P. Steensma, MD
Dr. Steensma indicated no relevant conflicts of interest.
CLINICAL TRIALs: GOv IDENTIFIER: NCT01425307
SPONSOR: Cincinnati Children’s Hospital Medical Center
FUNDING: National Heart, Lung, and Blood Institute (NHLBI)
PARTICIPATING CENTERS: This is a national trial that includes 25 participating centers.
ACCRUAL GOAL: 160 patients
STUDY DESIGN: This is a phase III, randomized, non-inferiority trial. To be eligible, children ages 4 to 15.99 must have sickle cell anemia (Hb SS) or sickleβ-thalassemia (Hb Sβ0) and have been on a chronic transfusion program for at least 12 months for primary stroke prophylaxis due to abnormally transcranial Doppler (TCD) velocities (≥200 cm/sec). The main exclusion criteria include prior overt stroke, transient ischemic attack, and severe cerebral vasculopathy or moyamoya disease. Participants are randomized to 24 months of alternative therapy (hydroxyurea) or standard therapy (transfusions). The primary endpoint is the maximum TCD velocity on the index side, which is the cerebral hemisphere with the higher velocity. The non-inferiority margin is 15 cm/sec.

RATIONALE: Without prevention, by 18 years of age, overt stroke occurs in 11 percent of children with Hb SS. Children with Hb SS who have the highest risk of stroke have also had abnormally increased cerebral arterial blood flow velocities, which can be measured by TCD. An abnormal TCD examination (≥200 cm/sec) confers an approximately 10 percent yearly risk of stroke for the three years following the test. The Stroke Prevention Trial (STOP) in sickle cell anemia, published in 1998, showed that chronic red blood cell transfusions decreased the rate of first stroke in children with an abnormal TCD by 92 percent compared with no transfusions (observation) (Adams RJ et al. N Engl J Med 1998;338:811). Using TCD, most pediatric sickle cell disease programs in the United States now screen children with Hb SS or Hb Sβ0 (usually 2-16 years of age) and initiate chronic transfusions for high-risk children. This strategy has proved effective, as the incidence of hospitalization for overt stroke in children with Hb SS in the United States has decreased by 45 percent in the decade following publication of the STOP trial (McCauley TL et al. Pediatr Blood Cancer. 2013;60:823-827). Although this management approach represents an important advance, most children with abnormal TCD velocities would not have a stroke even if untreated, and chronic transfusion therapy is burdensome, is indefinite in duration, and quickly necessitates iron chelation therapy. Accordingly, alternative management strategies are desirable and continue to be evaluated.

One such alternative is to simply stop transfusions after several years, in the hope that the high-risk period for stroke has passed. However, the STOP 2 trial showed that discontinuation of transfusions after ≥30 months resulted in a high rate of both reversion to abnormal TCD velocities and risk of stroke (Adams RJ et al. N Engl J Med 2005;353:2769-2778). So, truncation of transfusion is not a reasonable strategy for most children. Another alternative is the possibility that hydroxyurea can be given instead of chronic transfusions for primary stroke prevention; this alternative is now being tested in the TWiTCH trial. The TWiTCH trial builds upon the recently completed SWITCH trial, which compared alternative treatment (daily hydroxyurea for stroke prevention with monthly phlebotomy to manage iron overload) and standard treatment (chronic transfusions for stroke prevention and daily chelation for iron overload) for secondary stroke prevention. The SWITCH trial was stopped early due to futility of reaching its composite primary endpoint, and there was an excess of recurrent strokes in the hydroxyurea/phlebotomy arm (Ware RE et al. Blood. 2012;119.3925-3932). The severity of baseline cerebral vasculopathy in patients enrolled in the SWITCH trial (the study enrolled subjects with sickle cell anemia, previous stroke, and a 18 months of transfusions with documented iron overload) may have reduced the effectiveness of hydroxyurea for secondary stroke prevention. In TWiTCH, primary stroke prevention, an arguably different goal, is being studied, and severe cerebral vasculopathy is an exclusion criterion.

COMMENT: TWiTCH is not designed to determine whether hydroxyurea is better than chronic transfusions as a clinical superiority trial would. Nor is TWiTCH designed to determine whether hydroxyurea is not unacceptably different than chronic transfusions as an equivalence trial would. Rather, as a non-inferiority trial, TWiTCH is designed to determine whether hydroxyurea is not unexpectedly worse (even possibly less efficacious, but within limits) than chronic transfusions for primary stroke prevention. What, then, is the rationale for conducting a trial to test a therapy that the investigators realize may not work as well? In an excellent review of non-inferiority trials, Schumi and Wittes write: “A non-inferiority trial is reasonable when a new treatment has some property sufficiently favorable that physicians, and their patients, would be willing to sacrifice some degree of benefit relative to ...” (currently accepted therapy). The advantage could be reduced costs, extended use of existing dose, or an improved safety profile. The benefit given up in exchange for these advantages, however, should not be so large that patients and physicians are not willing to use the new...[treatment].”1

Hydroxyurea is the “new” treatment in this clinical setting has such favorable criteria. It is less costly, easier to use, and probably safer than lifelong chronic transfusions. What about the benefit given up? TCD velocity, which is a surrogate for risk of stroke, will increase for many patients when switching from chronic transfusions to hydroxyurea, in large part because the hemoglobin concentration will decrease once chronic transfusions are stopped. Hemoglobin concentration is a major determinant of TCD velocity. The TWiTCH investigators set as their non-inferiority margin an increase in TCD velocity of up to 15 cm/sec. That is, as long as the mean TCD velocity in the hydroxyurea arm is no higher than 15 cm/sec above the mean of the transfusion arm (measured pre-transfusion) at the end of the 24-month treatment period, hydroxyurea will be declared non-inferior to chronic transfusions. The margin of 15 cm/sec was chosen because it is the within-subject standard deviation of TCD velocities from past studies. The TWiTCH investigators argue that a change of this magnitude is within the limits of precision for TCD measurements, so the additional risk of stroke would be negligible.

One limitation of the trial’s primary endpoint, TCD velocity, is that it is a surrogate outcome. The outcome we are really interested in, however challenging to study in a clinical trial, is stroke. The predictive capacity of TCD velocity for overt stroke is well characterized in untreated Hb SS patients, but the meaning (e.g., the positive or negative predictive value for overt stroke) of any particular TCD velocity in a patient on hydroxyurea or chronic transfusions is not well characterized, and its absolute value will be lower than the untreated patient. So, when the TWiTCH study results are finally available, we need to remember that changes in TCD velocity do not always predict the risk of stroke. Hopefully, any sacrifice in benefit (stroke prevention) using hydroxyurea will be small compared with its many practical advantages over indefinite transfusions.


Dr. Quinn indicated no relevant conflicts of interest.
The Hematologist Acknowledges a Year of Noteworthy Articles


This article is the culmination of the massive effort of dozens of researchers to unravel the mystery of the genetic aberrations of acute myeloid leukemia (AML). Two hundred adult cases were analyzed by DNA sequencing, RNA expression, and epigenetic profiling. On average, each type of leukemia has 13 mutations, only five of which are recurrently mutated genes. About 260 genes were mutated in more than one patient. Extensive analysis reveals extraordinary complexity of the interrelationships between mutations, gene expression and DNA methylation. These data will serve as the basis for unraveling the pathogenesis of AML and the key to unlocking its vulnerability.

John Byrd

This article is one of the most important papers in 2013 because it identifies Bruton’s tyrosine kinase (BTK) as a valid therapeutic target for mantle cell lymphoma (MCL). This study, performed in relapsed and refractory MCL, demonstrated a high response rate and durable remissions with the first-in-class available BTK inhibitor, ibrutinib. The toxicity of ibrutinib was manageable and allowed continuous dosing for an extended period of time. While it is clear that ibrutinib will not cure mantle cell lymphoma, ibrutinib does provide a new tool to work with to improve outcome for patients with this aggressive, often fatal disease.

Robert Flamenhaft

In 2006, Andrew Fire, PhD, and Craig Mello, PhD, shared the Nobel Prize in Physiology or Medicine for their work on RNA interference (RNAi) in C. elegans. RNAi has become a valuable research tool that has allowed for the study of the function of specific genes in cell culture and in model organisms. The application of RNAi to treating human diseases, including cancer, has been stymied by many challenges, most notably the development of a safe and effective delivery method. The paper by Coelho and colleagues provides proof of concept that anti-thrombin siRNA encapsulated lipid nanoparticles delivered to human liver can modulate gene expression of mutant and non-mutant thrombin. These data hold promise not only for treatment of this subtype of amyloidosis, but also for other hematologic conditions such as beta-thalassemia and iron-overload disorders, hemophilia, acute intermittent porphyria, and complement-mediated disorders. The application of RNAi to treating human diseases, notably the development of a safe and effective delivery mechanism, demonstrates that RNAi therapy for transthyretin amyloidosis. N Engl J Med. 2013;369:438-447.

Despite recent progress in disease management, multiple myeloma-related morbidity and mortality remain high. Conceivably, unfavorably prognosis may correlate with disease burden. The idea to treat earlier is not new, but observation, without myeloma-specific therapy, has remained the standard of care for management of patients with smoldering myeloma. In the paper by Mateos and colleagues, smoldering myeloma patients with high-risk features that were treated with lenalidomide and dexamethasone were found to have a survival advantage compared with the observation group. The results of this study compel us to redefine or subclassify smoldering myeloma so that those patients with high-risk features can be identified and treated.

Pete Lollar

I selected this paper as my favorite Blood paper in the last year for two reasons: first, it describes an interesting new approach in the ongoing effort to develop novel antithrombotic agents; and second, it highlights methods developed by Steven Olson and colleagues that represent unprecedented biochemical rigor in the characterization of interactions involving proteinase inhibitors.

Charles Quinn

Atypical hemolytic uremic syndrome (aHUS) is an uncommon, life-threatening thrombotic microangiopathy. Most forms of primary aHUS are caused by disorders regula

therapy improves hematologic parameters, renal function, and quality of life in patients with aHUS — and these beneficial effects are sustained over time while receiving maintenance eculizumab. The toxicity profile is generally acceptable, and no meningococcic disease occurred in these vaccinated patients. In summary, eculizumab is an effective, long-term treatment for aHUS, and it should be started as early as possible after the diagnosis of aHUS is made.

Margaret Ragni

I chose this outstanding article by Young et al. because of the growing importance of global assays of hemostasis in the current and future management of patients with bleeding disorders as long-acting proteins and novel therapeutics come to market. With evidence that global assays (e.g., thrombin generation) are more accurate predictors of bleeding than PT- and APTT-based assays, there is urgency to bring global assays to the clinic. Countless clinical scenarios provide objective data to guide application of global tests to everyday management of patients.

This was the subject of Dr. Ragni’s Diffusion article in the September/October 2013 issue.

David Steensma

Drugs, even major therapeutic advances like imatinib, are only useful until something better comes along. Diagnostic tests also tend to have a vogue: the chest x-ray and complete blood count have had remarkable staying power, but who orders a plasma cell labeling index or a leukocyte alkaline phosphatase score anymore? In contrast to the evolution of enzymology will last forever — or at least as long as humans are around to suffer those diseases. That’s why the article I chose to highlight is the acute myeloid leukemia (AML) genomics study by The Cancer Genome Atlas Research Network, in which the investigators analyzed the genomes of 200 clinically annotated adult cases of de novo AML, using whole-genome and whole-exome sequencing, RNA and microRNA sequencing, and DNA methylation analysis. Of course, much more work needs to be done in AML, including assessment of the genome in special disease subtypes, functional characterization of the discovered mutations, and discovery of uncommon yet informative anomalies. And AML genomics work has yet to benefit patients; we leukemia specialists continue to prescribe 40-year-old cytotoxic chemotherapy regimens for want of anything better. But the work provides a small-scale map for investigators and will be the foundation of future studies into AML pathology and treatment.

Gregory Vercellotti

This study enrolled a total of 921 patients with severe acute upper gastrointestinal bleeding. Of these, 461 were assigned to a restrictive transfusion strategy (RBC transfusion when the hemoglobin level fell below 7 g per deciliter) and 460 were assigned to a liberal strategy (transfusion when the hemoglobin fell below 9 g per deciliter). Survival was better in the restrictive group, and the risk of re-bleeding was also less. This paper underscores the overuse of RBC transfusions in medicine today and reminds clinicians that RBC transfusions may, in some instances, do more harm than good. Unfavorable events related to battlefield resuscitation, the potential dangers of transfusion of aged red blood cells, the risk of transfusion-related infection, and transfusion-induced immunosuppression or immunocompromise are examples of how transfusion can promote disease progression through trafficking of exosomes, a recently discovered class of cell membrane-derived vesicles that are important both physiologically and pathophysiologically.
The Hematologist: rapid evolving practice of adoptive T-cell therapy using relevant finding reinforces the importance of rigorous transgenic TCR as the cause of the toxicity. This clinically expressed in cardiomyocytes that was recognized by the Linette and colleagues identified a titin-derived peptide presented in the context of human leukocyte antigen A1. Enhanced TCR specific for the MAGE-A3 tumor antigen cardiovascular toxicity occurring in two patients treated and specificity for target antigens has generated While the successful genetic engineering of T cells augments 2013;122:863-871. The use of high-resolution computed tomography (CT) scans to detect pulmonary embolism (PE) has led to a marked increase in diagnosis of small subsegmental PEs, causing speculation that the greater sensitivity of the scans might be leading to overdiagnosis and overtreatment of patients. In the August 15 issue of Blood, den Exter and colleagues address this question in a review of more than 3,700 cases of suspected PE evaluated by high-resolution CT scans. They demonstrate that patients with subsegmental PE have comparable risk and outcomes compared to those with more proximal PE. This observation suggests that all patients with PE, regardless of the location of the clot, should be anticoagulated.

AUGUST 15, 2013


While the successful genetic engineering of T cells to express T-cell receptors (TCRs) with high affinity and specificity for target antigens has generated much enthusiasm in the budding field of cancer immunotherapy, toxicities associated with this procedure are still being uncovered. This manuscript describes fatal cardiovascular toxicity occurring in two patients treated with lymphocytes engineered to express an affinity-enhanced TCR specific for the MAGE-A3 tumor antigen presented in the context of human leukocyte antigen A1. Linette and colleagues identified a titin-derived peptide expressed in cardiomyocytes that was recognized by the transgenic TCR as the cause of the toxicity. This clinically relevant finding reinforces the importance of rigorous investigation and reporting of toxicity associated with the rapidly evolving practice of adoptive T-cell therapy using genetically engineered lymphocytes.

AUGUST 8, 2013


Recommendations from the European LeukemiaNet have been influential worldwide in providing guidance for management of patients with chronic myeloid leukemia (CML). This eagerly anticipated update, published in the August 8 issue of Blood, takes into account data dealing with the impact of second-generation tyrosine kinase inhibitors and provides recommendations for when to change therapy based on metrics established to quantify drug response.


In this study, investigators report on the largest number of children to date (485 cases) with thalassemia major or sickle cell disease who have undergone sibling-matched hematopoietic stem cell transplantation. Eurocord registry data and Oakland registry data were combined to provide a detailed look at both transplant and disease outcomes in children who have received either sibling-matched bone marrow or sibling-matched cord blood as the hematopoietic stem cell source. The data, collected during the last 20 years, demonstrate six-year overall survival results of 85 percent with a median follow-up of 70 months after matched-sibling transplants, regardless of the stem cell source. The six-year disease-free survival rates for the various subgroups varied between 80 percent and 92 percent. The data underscore the favorable outcome of transplantation of children with either thalassemia major or sickle cell disease using either bone marrow or cord blood from an HLA-matched sibling as the donor source.


Cerebral malaria is the most common cause of death in sub-Saharan African children; however the pathophysiology of the neurologic toxicity is incompletely understood. In this study, Moxon and colleagues provide insight into this important issue, reporting that malaria-infected erythrocytes cause loss of protein C receptors thereby inducing a microvascular coagulopathy. Using a novel technique for visualizing microvessels, the investigators demonstrate the loss of protein C receptors at sites of adherence of infected erythrocytes resulting in disruption of endothelial protection against fibrin clot formation. Because protein C receptors are expressed at low levels in the cerebral vasculature, the investigators postulate that the brain is unusually sensitive to this type of malaria-induced injury.

This paper and another dealing with similar issues are the subject of a Dilight article by Dr. Greg Vercelli (see page 8). Two more Blood Hubs – centralized places for readers to find content – are now accessible on the Blood website. They focus on multiple myeloma and sickle cell disease. There are a total of four Blood Hubs; the others focus on thrombocytopenia and pediatric hematology. Go to http://bloodjournal.hematologylibrary.org for more information.

The Hematologist Discoverer (Cont. from page 7)

slides are reviewed jointly because they provide an opportunity for sharing thoughts across disciplines and for generating sparks of ideas for further evaluation. 3) Reunite the clinical specialties with pathology by reconvening the joint teaching clinical and anatomic pathology, joint clinic-pathologic and patient management conferences, reciprocal trainee rotations, and facilitated access for medical students, house staff, and fellows to the clinical pathology laboratory. 4) Help students and house staff share in the initial discovery of disorders affecting their patients and recognize the power and beauty of analyzing blood cell morphology by encouraging them to review blood smears and bone marrow specimens day or night with hematology fellows, faculty, and/or technicians committed to teaching. I have, for example, reviewed blood smears with internal medicine students and residents for many years at Mount Sinai School of Medicine in a format in which I have to create a clinical scenario to explain the morphology without knowing anything about the patient’s history, physical, or laboratory findings. This exercise is followed by comparing the scenario to the actual history, followed by a more general discussion of pathophysiology and patient management. 5) Make the FDA package inserts for all laboratory tests being conducted in the clinical pathology lab available electronically to the hospital staff, including the laboratory, the laboratory and the clinician, the laboratory and referring physicians. 6) Use the new tools of social media to empower every hematologist in the United States to participate in discovery by creating an ASH website titled “N=1” in which members can upload an interesting case report and other members can add their comments on the case, including suggestions for research follow-up and offers to volunteer to conduct additional studies. I encourage ASH members to add other creative ideas to this list.

I hope that some or all of these suggestions will help light the flame of discovery in a new generation of medical students and trainees who come to see the intellectual excitement and beauty in hematology and the great opportunity to contribute to the health of patients today and in the future.

ASH has launched a new Web-based registry tool, PQRSpro, to facilitate the aggregation and submission of quality measure data to the Centers for Medicare & Medicaid Services Physician Quality Reporting System (PQRS). PQRSpro will validate that your reporting is incentive eligible and that you avoid impending payment adjustments.

In the 2013 PQRS reporting year, a 0.5 percent incentive is still available for those who successfully report on quality measures; additionally, non-participation in 2013 will result in -1.5 percent “payment adjustment” on all reimbursements in 2015.

For more information, please visit www.hematology.org/PQRSpro. In addition, ASH held a webinar on this subject in early October. Go to www.hematology.org/Meetings/Webinars/11282.aspx to download the slides and to peruse the questions that were posed during the live presentation. Detailed responses are included below the questions.

Look for information on the ASH website about downloading the 2013 annual meeting app in mid-November.