In 2008, we met in San Francisco to celebrate the Society’s 50th anniversary. That was a special occasion, but whenever we meet in “The City”, it’s a cause for celebration. ASH and San Francisco seem to be kindred in spirit as the beauty and vitality of the city together with the enthusiasm and creative energy of the Society’s members and guests synergize to create a sense that anything is possible. Make plans to join colleagues at the 56th Annual Meeting, and keep in mind the opportunity to enjoy the advantages of this special venue. Take a few extra days to cross the Golden Gate Bridge into the wine country of Napa Valley or travel the majestic Pacific Coast Highway south to Carmel and on to Big Sur. As Nietzsche reminded us, “Is life not a thousand times too short for us to bore ourselves”?

As we look forward to the annual meeting, we asked the current chair of the Committee on Training Programs and the 2014 co-chairs of the Education and Scientific Programs to give their perspectives on this year’s annual meeting program.

Trainee Program
The Committee on Training is responsible for presenting educational programs specifically designed for physicians and researchers who are either in training or who are in an early phase of their academic careers. These events, supported by the Trainee Council, include Trainee Day, Career-Development Lunch Sessions, Trainee Simultaneous Didactic Sessions, and the Trainee Welcome Reception. A workshop is also held during the annual meeting for training program directors and for hematology course directors.

(Cont. on page 6)

**OP-ED: “ASK AND YE SHALL RECEIVE” – WOMEN, STAND UP AND BE COUNTED!** – Dr. Cynthia Dunbar, Dr. Janis Abkowitz, Dr. Nancy Berliner, and Dr. Susan Shurin discuss the concerted grassroots efforts to nominate women for ASH awards.

**ASK THE HEMATOLOGISTS** – Dr. Deepa Suryanarayan and Dr. David Garcia share their approach to the management of patients with anti-phospholipid antibodies who are pregnant or considering pregnancy.

**LOOKING BACK – LOOKING FORWARD: 40 YEARS IN HEMOPHILIA**

Research – Dr. Gilbert White chronicles his involvement in the HIV and HCV epidemics that devastated the hemophilia population in the 1980s, highlighting the power of science and the resilience of patients, physicians, and scientists, who faced adversity and triumphed.

**DEPARTMENTS**

2 PRESIDENT’S COLUMN

3 NEWS AND REPORTS

7 THE HEMATOLOGIST ADVOCATE

8 DIFFUSION

13 EDITORS’ CHOICE

15 CLINICAL TRIALS CORNER

16 MARK YOUR CALENDAR

**FEATURES**

2 Fox Drives CAR Cells in Niche


One marrow haematopoietic niches are highly organized microenvironments that are composed of non-haematopoietic cells and an extracellular matrix that together support the generation and maintenance of vertebrate haematopoietic stem cells (HSCs) and progenitor cells. Areas of the bone marrow that are not involved in haematopoiesis are mainly composed of adipose tissue that serves as an inhibitory microenvironment by restricting hematopoietic progenitor development.1 Marrow haematopoietic niches have osteogenic and endothelial components with interposed cells of various types, including autonomic neurons, glial cells, adipocytes, and macrophages; and, in concert, these elements activate or suppress the function of mesenchymal stem cells (MSCs) and their progeny, specifically chondroblasts, osteoblasts, and adipocytes. Highly specialized subsets of MSCs secrete ligands that bind to surface receptors that mediate induction of quiescence, self-renewal, and differentiation of HSCs, as well as proliferation and differentiation of hematopoietic progenitors. In the bone marrow, these MSC subsets produce the chemokine ligand CXCL12 (stromal cell factor-1 (CXCL12)) and the cytokine Kit-ligand/stem cell factor (SCF), which activate their respective receptors, CXCR4 and Kit, on HSCs and hematopoietic progenitors. CXCL12-abundant reticular (CAR) cells comprise one such MSC subset that physically associates with HSCs, produces CXCL12 and SCF, and has both osteogenic and adipogenic differentiation potential.2 Now investigators from the laboratory of Dr. Takashi Nagasawa at the Institute for Frontier Medical Sciences at Kyoto University in Japan, where CAR cells were first identified,3 report in a paper in the Blood book box CXCL12 (Foxc1) transcription factor is a key regulator of the hematopoietic niche through its effect on CAR cell function.

In the current study, Dr. Yoshih Omatsu and colleagues showed that conditional knockout of Foxc1 in all mesenchymal cells of the developing limb led to a marked reduction in CAR cells, HSCs, progenitors of all lineages of hematopoiesis, and circulating blood cells. But while the marrow hematopoietic tissue was found to be reduced in the conditional knockouts, an increase in marrow adipose cells was observed. The residual CAR cells in the marrow were characterized by decreased expression of CXCL12 and SCF with an increased expression of lipid markers and the accumulation of lipid inclusions indicative of differentiation into preadipocytes. Knockout of Foxc1 that was restricted to the majority of CAR cells in young mice was associated with a similar loss of bone marrow CAR and hematopoietic cells with induction of adipocyte development, demonstrating that the phenotype of hematopoietic failure in bone marrow was mainly due to loss of Foxc1 activity in CAR cells. Conversely, in vitro experiments that used forced expression of Foxc1 in marrow-derived preadipocyte cells showed suppression of adipocyte differentiation in conjunction with increased CXCL12 and SCF expression in CAR cells. Inducible knockout of Foxc1 in adult mice caused hematopoietic marrow failure without adipogenesis. Notably, the experimental group was found to have an increase in HSCs and erythropoiesis in the spleen, indicating that extramedullary hematopoiesis was not affected by knockout of Foxc1.

Dr. Omatsu and colleagues demonstrated that Foxc1 expression in bone marrow plays a crucial role in the establishment and maintenance of the hematopoietic niche by orchestrating CAR cell development and CAR cell fate during differentiation while restricting adipocyte development. By fostering the hematopoietic niche in the bone marrow, Foxc1 has actions similar to those of Foxn1, another member of the Fox transcription factor family that is required by thymic epithelium for all T-lymphocyte cell development.4 Understanding how these niche-inducing transcription factors establish and maintain hematopoietic microenvironments has relevance to clinical hematology, where potential manipulation of Foxc1 might be used to treat marrow-based disorders. For example, in normal aging, marrow adipose tissue increases while hematopoietic reserves decrease, resulting in an increased sensitivity to chemotherapy in older adults that limits treatment of malignant diseases. If Foxc1 activity could be enhanced in this setting, then increased hematopoietic reserve might improve chemotherapy tolerance and treatment outcomes in older patients. Likewise, enhanced Foxc1 activity has the potential to increase blood cell production in patients with inherited bone marrow failure syndromes, acquired aplastic anemia, and cytopenias after stem cell transplantation. Conversely, decreasing Foxc1 in patients with myeloproliferative disorders may help control excessive hematopoiesis and associated increases in peripheral blood cell counts.


**DIFFUSION**

Need Research Support? Think ASH!

**CYNTHIA E. DUNBAR, MD; JANIS L. ABRKOWITZ, MD; NANCY BERLINER, MD; AND SUSAN B. SHURIN, MD**

1. Head, Malignant Hematopoiesis Section, Hematology Branch, at the National Heart, Lung, and Blood Institute, Bethesda, MD
2. Clement A. Finch Professor of Medicine, Head of the Division of Hematology at University of Washington, Seattle, WA
3. Division Chief and Senior Physician, Hematology Division, Department of Medicine at Brigham and Women's Hospital; Professor of Medicine, Harvard Medical School
4. Former Deputy Director, National Heart, Lung, and Blood Institute, Bethesda, MD

At the ASH annual meeting last December in New Orleans, attendees were inspired by the accomplishments of Nancy Andrews, MD, PhD (winner of the Henry Stratton Medal for basic research); Elaine Jaffe, MD (winner of the Henry Stratton Medal for clinical/translational research); Katherine High, MD (E. Donnall Thomas Lecturer and Prize winner); and Clara Camaschella, MD (Ham-Wasserman Lecturer) that led to their being chosen by the Awards Committee about the situation, we were informed that the ASH Executive Committee has allocated up to $1 million for additional Scholar Awards for the coming year.

So what accounted for the dearth of award nominations for female Society members? The “aha” moment came when several committee members pointed out that plenty of men either nominated themselves or orchestrated the process led by the Trainee Council and distinct from the honorific awards, but reflecting recognition of a different type of achievement. Gender parity in U.S. medical schools and biomedical doctoral programs was reached in the past decade, and graduating classes have been more than 40 percent female for at least an additional decade. Based on American Council for Graduate Medical Education data, in 2010, almost 50 percent of hematology-oncology fellows were women, and currently, approximately one-third of ASH members are women. With so many women involved in the field, surely more than 5 to 10 percent of major contributions to hematology have come from women.

Have the ASH Awards and Executive Committees really demonstrated a long history of gender bias? The answer to this question is a resounding “no” as representation of women in leadership positions at ASH over at least the past two decades has been halting. When we asked colleagues who had served on the Awards Committee about the situation, we were informed that the problem was that few women had been nominated. And they acknowledged that, given the pool of deserving woman hematologists worldwide who had never received an ASH award, the lack of female nominees was a major source of frustration for the committee.

Our 2013 award nomination effort was spurred by the observation that, since 1970, only 11 percent of ASH honorific awards had gone to women (including several instances of women receiving an award together with their spouse, also a hematologist). Perhaps more troubling, no significant improvement in this paucity rate of representation has been observed since 2000. Even the ASH Mentor Awards, initiated in 2006 and chosen by a process led by the Trainee Council and distinct from the honorific awards, went to women only 18 percent of the time, progress perhaps in comparison with the other awards, but reflecting recognition of a different type of achievement. Gender parity in U.S. medical schools and biomedical doctoral programs was reached in the past decade, and graduating classes have been more than 40 percent female for at least an additional decade. Based on American Council for Graduate Medical Education data, in 2010, almost 50 percent of hematology-oncology fellows were women, and currently, approximately one-third of ASH members are women. With so many women involved in the field, surely more than 5 to 10 percent of major contributions to hematology have come from women.

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**Publications:**

**Key Dates for the 2014 Annual Meeting in San Francisco**

- **July 24,** at 11:00 a.m. EDT
  - Advance member registration and housing opens
- **August 5,** at 11:59 p.m. PDT
  - Abstract submission deadline
- **August 13,** at 11:00 a.m. EDT
  - Advance registration for non-members opens
- **November 5,** at 11:59 p.m. EST
  - Advance registration deadline

**Attend the First ASH Meeting on Lymphoma Biology**

There is still time to register for the new ASH Meeting on Lymphoma Biology, which is exclusively dedicated to research in lymphoma and lymphoid malignancies. This three-day forum will be held August 10-13 in Colorado Springs, Colorado. Keynote speaker Klaus Rajewsky, MD, will discuss immune regulation and cancer, and attendees will have the opportunity to learn about breakthroughs in basic and translational lymphoma research, share their findings with colleagues, and exchange ideas with experts on how to move the field forward. Register today to attend. Advance registration closes August 7. Go to www.hematology.org/Lymphoma-Biology for more information.

**Find Solutions to Challenging Patient Cases Through Interactive, Case-Based Discussions at the 2014 State-of-the-Art Symposium**

Registration is open for the 2014 ASH State-of-the-Art Symposium: Clinical Hematology Updates with a Focus on Lymphoid Malignancies. The meeting will be offered in two locations: in Chicago, Illinois, September 12-13, and in Baltimore, Maryland, October 17-18. This meeting is designed to offer the same high-quality educational content for which the ASH annual meeting is known, providing clinicians with the latest updates in hematology and hematology-oncology. This year’s program will focus on clinical hematology updates with a focus on lymphoid malignancies. The same presentations and speakers will be featured in each city. Visit www.hematology.org/SAS for information about the program schedule, hotel, and online registration.

In addition to State-of-the-Art Symposium, ASH is offering three Consultative Hematology Courses in 2014, one focused on malignant hematology and two focused on non-malignant hematology. The malignant-focused course will take place on September 12 in Chicago (on the morning prior to the first 2014 State-of-the-Art Symposium). The non-malignant-focused courses will be conducted in Baltimore on October 17 (on the morning prior to the second State-of-the-Art Symposium) and on December 8 (during the 56th ASH Annual Meeting in San Francisco). The courses are intended to be “refreshers” for clinicians who see patients with hematologic disorders and who wish to update their core knowledge of hematology. Lectures will cover commonly encountered clinical problems that require the expertise of a hematologist, and the forum will feature case-based presentations and interactive discussions. Visit www.hematology.org/CHC for more details about the program and to register.
Antiphospholipid antibody syndrome (APS) is a heterogeneous autoimmune disorder characterized by arterial and venous thromboembolic events and obstetric complications (especially recurrent fetal losses) in association with persistent laboratory evidence of anti-phospholipid antibodies (aPL). The aPL associated with the disease include the lupus anticoagulant (LA), anti-cardiolipin (a-CL), and anti-β2 glycoprotein-1 (anti-β2 GP-1). APS and aPL can occur in isolation (i.e., primary) or in association with other disease processes (i.e., secondary) including connective tissue diseases, most commonly systemic lupus erythematosus (SLE).

Diagnostic Criteria for APS

The presence of aPL alone does not constitute APS. The international classification criteria for APS (the Sapporo criteria) were introduced in 1998 and revised in 2006.1 To make a definitive diagnosis of APS, the presence of at least one clinical feature (thrombosis or pregnancy morbidity) and one laboratory abnormality must be observed, and the laboratory abnormality must be present on two or more occasions at least 12 weeks apart. Laboratory parameters are as follows: IgG and/or IgM a-CL or anti-β2 GP-1 in moderate to high titre (i.e., a titre > 40 for IgG or for IgM or a titre > the 99th percentile), or documentation of the LA. Further details about the diagnostic criteria for APS can be found in the Journal of Thrombosis and Haemostasis.1

Maternal and Fetal Morbidity

The pathogenesis of aPL-related pregnancy morbidity is incompletely understood. For a pregnant woman, APS (and perhaps the presence of aPL) raises the possibility that she is at increased risk for thrombotic events and/or obstetric complications such as recurrent pregnancy losses, preeclampsia, eclampsia, intra-uterine growth restriction, and hemolysis, Elevated Liver enzymes, Low Platelets (HELLP) syndrome. In a European referral center cohort of 1,000 patients with APS (many of whom had a connective tissue disease), 188 pregnancies were documented over 10 years; among patients in this group, early pregnancy loss (16.5%), intra-uterine growth restriction (26.3%), and prematurity (48.2%) were common.2 A systematic review found that lupus anticoagulant was associated with preeclampsia (OR 2.34), intra-uterine growth restriction (OR 4.65), and late (after 10 weeks of gestation) fetal loss (OR 4.73); however, numerous case-control studies were included in the analysis. When the analysis was restricted to cohort studies, late fetal loss was the only outcome associated with the aPL.2 Catastrophic APS (CAPS), a rare but devastating form of this syndrome, has been associated with pregnancy and the peripuerum with 8.9 percent of the pregnancies from the aforementioned cohort being complicated by CAPS.2

Laboratory Diagnosis of aPL

The laboratory identification of aPL can be problematic because of variability in the sensitivity of assays and reagents, high false-negative and false-positive detection rates, lack of standardization of assays, and lack of adherence to established guidelines. To address these issues, guidelines for laboratory detection of aPL were published in 2009 by the Scientific Standardization Subcommittee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH) and by the British Committee on Standards in Haematology. The Clinical Laboratory Standards Institute (CLSI) has also prepared guidelines for laboratory testing for LA that are to be published soon. Before counseling a patient about risk or recommending therapy, we suggest that the hematologist ensure that aPL testing is not only confirmed on more than one occasion but also performed by a laboratory that follows approved aPL testing guidelines.

Pre-Pregnancy Counseling

Management of a woman with APS should start with pre-pregnancy counseling that focuses on the involved risks. Discussion should include risks of maternal thrombosis and fetal loss (both early- and late-term) as well as the increased risk of placenta-mediated complications such as preeclampsia, HELLP syndrome, preterm delivery, and intra-uterine growth restriction. Involvement by a multidisciplinary management team that includes a maternal-fetal medicine specialist and a hematologist experienced in the management of thrombophilic conditions is recommended. APS patients on chronic anticoagulation should be informed about the potential teratogenic effects of warfarin, and oral anticoagulation should be switched to a therapeutic dose of low-molecular-weight heparin (LMWH) either before or very shortly after conception. For all women with APS, the American College of Chest Physicians (ACCp) evidence-based guidelines3 recommend commencing low-dose aspirin on confirmation of pregnancy, not only for its antithrombotic effects but also to reduce the risk of preeclampsia.4 In practice, it would be reasonable for a woman with obstetric APS to begin aspirin during the time that she and her partner are trying to conceive, because the risk of ASA is low and the impact of a delay between conception and ASA use is unknown.

Pregnancy with aPL or APS – Clinical Management Scenarios

1. Pregnant women with persistent evidence of aPL who do not fulfill the diagnostic criteria for APS

There are no robust data to guide management of pregnant women in this cohort. Dr. Anne Lynch et al. measured aPLs in early pregnancy in 451 low-risk nulliparous women and found that 24.4 percent had aPLs.2 The rate of fetal loss in this cohort was higher than those without aPLs (15.8 vs. 6.5%), but the rate of adverse maternal outcomes was similar in both groups. We usually recommend low-dose ASA because it is easy to take and there is a reasonably high quality of evidence that it reduces the risk of preeclampsia; however, the significant uncertainty about the benefit of any antepartum, antithrombotic therapy in women who do not meet criteria for APS means that decisions should be individualized. The role of post-partum thromboprophylaxis in this patient group is not established.

2. Women with adverse obstetric outcomes plus aPL, but no history of thrombosis

In three meta-analyses of randomized trials in women with APS, the combination of ASA with prophylactic heparin significantly reduced pregnancy loss (RR 0.46)5 or first-trimester loss (OR 0.396) and increased live births (RR 2.5).7 These analyses, however, have several limitations, including a small number of trials from which to gather data, small sample size within trials, and low quality of trial design. Compared with women who have had recurrent pregnancy losses, but whose thrombophilia testing is negative, women with a history of obstetric APS are at increased risk for both arterial and venous thrombotic events.8 In the NOH-APS observational study among women with prior fetal loss, those treated with LMWH and low-dose aspirin had lower pregnancy loss but higher preeclampsia rates than other women. Therefore, the evidence that combined (LMWH + ASA) therapy improves outcomes is, at best, of modest quality. Nonetheless, we suggest combination LMWH + ASA for pregnant women with APS and adverse obstetric outcomes, not only because there is some evidence that this will decrease the likelihood of pregnancy complications, but also because LMWH + ASA should also reduce the risk of thrombosis during pregnancy. However, because the quality of evidence to support combination therapy over LMWH or ASA alone is not compelling, we suggest that the hematologist consider not only patient preferences, but also the recommendation of a maternal-fetal medicine specialist before choosing LMWH + ASA over LMWH or ASA alone. The optimal doses for LMWH and ASA have not been defined, but we suggest 75 to 100 mg of ASA per day plus prophylactic-dose LMWH (Table). For women with obstetric APS and no history of thrombosis, thromboprophylaxis during the postpartum period should be considered, but the net benefit is not well established.

3. Pregnant women with thrombotic APS

If a woman has APS and is anticoagulated for prior thrombosis, we suggest switching to therapeutic-dose LMWH before six weeks gestation. Full-intensity anticoagulation is important during the pregnancy and the postpartum period since these women have a significant, ongoing risk of thrombosis that is likely magnified by pregnancy. If a woman is on therapeutic-dose LMWH, delivery should be planned, and LMWH should be discontinued at least 24 hours prior to delivery. Because there is no high-quality evidence to define the optimal anticoagulant management of patients who desire regional anesthesia, communication with maternal-fetal medicine and anesthesiology is especially important. For a woman with prior thrombosis who has discontinued anticoagulation, we suggest LMWH be started upon confirmation of pregnancy; whether to use prophylactic/intermediate or therapeutic-dose LMWH would depend on the perceived risks of thrombosis and bleeding. An elevated risk of thrombosis persists for up to 12 weeks following delivery.9 The absolute risk for thrombosis beyond six weeks, however, is small. Thus, thromboprophylaxis beyond the six-week mark of the postpartum period should be reserved for those patients with an especially high baseline risk.
Management of pregnant women with APS is challenging. Despite the imperfect data that are currently available, pregnancy outcomes in women with APS who are managed in centers that use a multidisciplinary approach, which includes input from clinicians experienced in thrombophilia management and fetal-maternal medicine, is often favorable. Future studies that expand our understanding of this complex disease, that clearly define obstetric and thrombotic risks, and that identify optimal antithrombotic regimens are needed.

Conclusion

Management of pregnant women with APS is challenging. Despite the imperfect data that are currently available, pregnancy outcomes in women with APS who are managed in centers that use a multidisciplinary approach, which includes input from clinicians experienced in thrombophilia management and fetal-maternal medicine, is often favorable. Future studies that expand our understanding of this complex disease, that clearly define obstetric and thrombotic risks, and that identify optimal antithrombotic regimens are needed.

Table. Overview of the Therapeutic Options Used in Antiphospholipid Syndrome Pregnancy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
<th>Evidence</th>
<th>Safety in pregnancy</th>
<th>Safety in breast-feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Reduction of fetal loss Prevention of eclampsia Antiplatelet effect</td>
<td>No randomized controlled trials of aspirin for preventing VTE</td>
<td>Will cross the placenta; human data inconsistent, but risk is likely low</td>
<td>Does enter breast milk, but at low doses; should be safe</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Discontinue as soon as pregnancy is confirmed</td>
<td>Not recommended in pregnancy unless LMWH may be less effective (e.g., prosthetic heart valves)</td>
<td>Teratogenic between 6-12 weeks of gestation; switch to LMWH before 6 weeks of gestation</td>
<td>Not excreted in breast milk</td>
</tr>
<tr>
<td>Unfractionated heparin (UFH)</td>
<td>May be used in event of massive pulmonary embolism If rapid reversal of anticoagulation is needed during peripartum period or operative procedures</td>
<td>Most studies using UFH have been superseded by studies using LMWH.</td>
<td>Safe</td>
<td>Not excreted in breast milk</td>
</tr>
<tr>
<td>LMWH</td>
<td>Drug of choice for women on warfarin, women who have had VTE or arterial thrombosis during pregnancy, women with previous pregnancy complications, or women who require thromboprophylaxis</td>
<td>Evidence for its role in preventing first-trimester loss remains controversial</td>
<td>Does not cross placenta</td>
<td>Does enter breast milk, but of little concern due to low bioavailability</td>
</tr>
<tr>
<td>Steroids (e.g., prednisone)</td>
<td>Little evidence for benefit in APS; used in immune thrombocytopenia associated with APS or SLE</td>
<td>Minimal evidence of therapeutic benefit Benefit outweighed by its adverse effects (e.g., preeclampsia, gestational diabetes, increased risk of preterm deliveries)</td>
<td>Cleft palate reported with first-trimester use</td>
<td>Low concentrations in breast milk</td>
</tr>
<tr>
<td>Intravenous immunoglobulins</td>
<td>Used in a small number of women with APS as therapy for concomitant, immune-mediated thrombocytopenia</td>
<td>No additional benefit when added to conventional therapy with ASA and LMWH</td>
<td>Crosses placenta after 30 weeks of gestation</td>
<td>Excretion is unknown</td>
</tr>
<tr>
<td>Hydroxychloroquine (HQC)</td>
<td>Used when women have coexisting SLE</td>
<td>Mild antithrombotic effects Decreased risk of congenital heart block in women on HCO in case-controlled studies</td>
<td>No reports of fetal toxicity</td>
<td>Considered safe despite excretion in breast milk</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>Reserved for treatment of catastrophic APS</td>
<td>Limited to case reports</td>
<td>Rarely used in pregnancy</td>
<td>Unknown</td>
</tr>
</tbody>
</table>


Dr. Suryanarayan and Dr. Garcia indicated no relevant conflicts of interest.
to write award nomination letters as part of an organized process spearheaded by friends or institutional colleagues of male awardees. Senior trainees often feel more isolated within their institutions and may not have developed a professional or personal network as strong as that of their male colleagues.1 To remedy this situation, we initiated an effort that might deserve deserving for the 2013 awards, with the successful results being evident at the annual meeting in New Orleans.

Gender imbalance in ASH awards reflects a much larger issue that plagues academic medicine. The percentage of female full professors at U.S. biomedical institutions remains stubbornly below the 20 percent level.2 A 2008 analysis by Tim Ley and Barton Goldstein of the National Heart, Lung, and Blood Institute revealed that the lack of women in the upper echelons of business and academia may be explained, at least in part, by a lack of persistence in response to perceived failure. Claudia Goldin, a Harvard Business School economist, discovered that female undergraduates with a declared interest in economics were more likely to change their plans upon receiving Bs and Cs in introductory economics classes as compared with male classmates.3 Many studies, including those focusing on medical students and residents, demonstrate differences in self-assessment of competence or achievement, with men being more likely to overestimate their abilities.4 During Cynthia Gerson’s tenure as Editor-in-Chief of Blood, only a single instance of a female senior author rebutting a rejection decision occurred, in contrast to almost 100 direct rebuttals from male authors.5 To remedy this situation, we initiated an effort that might deserve deserving for the 2013 awards, with the successful results being evident at the annual meeting in New Orleans.

Diversity is Essential and Overdue

Accordingly, this year, within each session, a lecture will be devoted specifically to career counseling for PhD scientists and to medical students and residents who are interested in hematology fellowships. Several Didactic Sessions specifically aimed at trainees will be presented on Sunday, December 7, and Monday, December 8. On Sunday, a session on outcomes research in hematology will be presented. This is a new topic that was developed to provide trainees with insights into comparative effectiveness research, the use of large database resources, and the methods and resources that are required for large systematic reviews. A second didactic session on Sunday is titled “How to Transition from Trainee to Faculty.” This lecture will include discussion on how to find a job, how to negotiate an academic resource plan, and how to facilitate the transition from trainee to faculty member, with a focus on career goals, support, and service. Two more didactic sessions will be offered on Monday at 12:00 noon. The first session will focus on the essentials of managing personnel and how to foster teamwork, and the other session is intended to support the goals of PhD scientist interested in translational research.

Later in the meeting, the Committee will offer a Junior Faculty Education Session with the following three didactics focusing on the theme of mentorship: “Identifying a Mentor and Optimizing the Mentor-Mentee Relationship,” “The Warning Signs of a Problematic Mentoring Relationship and How to Fix It,” and “Transitioning from Being a Mentee to Becoming a Mentor.”

We hope that these presentations and sessions provide fellows, postdoctoral students, and junior faculty with the tools needed to find a job and to build a successful career.

Gary Schiller, MD, David Geffen School of Medicine at UCLA

Education Program

The 2014 ASH Education Program was developed not only to provide state-of-the-art clinical information but also to communicate to clinicians the scientific underpinnings upon which diagnostic and treatment recommendations are built. It is crucial to ensure that while hematology patients receive optimal care, clinicians are equipped to think innovatively about assessment and management issues. Accordingly, this year, within each session, a lecture will

Op-Ed (Cont. from page 2)

Annual Meeting (Cont. from page 1)

The schedule begins on Friday, December 5, with Trainee Day, and this year, the program will focus on negotiating and keeping a job and on how to fund a research career. The session kicks off with a noon luncheon during which there will be two simultaneous sessions. One is titled “Giving an Effective Presentation” and the other titled “The Hurdles of Translational Research.” The former session will be valuable to all trainees, and in the latter session, trainees will learn about the challenges unique to translational research, including how to balance time between the clinic and the lab, how to deal with local and governmental regulatory bodies, how to develop collaborations, and how to secure funding. After these presentations, the attendees will move into small breakout groups to discuss either how to negotiate a job offer or how to secure start-up funding and how to identify and apply for career-development awards. Next, the attendees will come together for a final didactic session, followed by the Trainee Welcome Reception where trainees meet with ASH leadership and learn more about the ASH meeting and trainee resources. The reception provides a unique opportunity for networking with fellow trainees and with senior members of the Society in a collegial, spirited atmosphere.

The Career-Development Lunch Program on Saturday, December 6, allows for an intimate opportunity to receive career counseling and guidance. Tables will be organized so that trainees can have lunch with ASH members who have a special interest in the topic. Career topics include adult hematology, clinical research, pediatric hematology/oncology, hospital-based careers with an emphasis on hemapheresis, hematopoietic stem cell transplantation, laboratory medicine, or hematology pathology; or in careers in industry, government, or private practice. And space within the session will be devoted specifically to career counseling for PhD scientists and to medical students and residents who are interested in hematology fellowships.

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ASH Committees Visit Congress, NIH in Support of Issues Affecting Hematology

Following the mid-May meeting in Washington, DC, members of the ASH Committee on Practice visited more than 30 congressional offices to advocate for patient access to affordable drugs, for repeal of the current Sustainable Growth Rate (SGR) formula, and for reform of Medicare payment for physician services.

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ASH advocacy efforts in support of hematology research continue. Also in May, members of the ASH Committee on Scientific Affairs traveled to the National Institutes of Health (NIH) campus in Bethesda, Maryland, to meet with leaders from the National Center for Advancing Translational Sciences; the National Heart, Lung, and Blood Institute; and the National Cancer Institute. These meetings provided ASH with an opportunity to highlight a number of promising areas of hematology research and to emphasize the need for sustained support in these areas.

These face-to-face meetings are an essential component of ASH’s advocacy efforts, providing an opportunity for NIH staff and Members of Congress and their staff to gain insight into issues of concern to hematologists. The strength in ASH’s advocacy, however, is the continued involvement of all members of the Society who, through their enthusiastic support, bring issues important to the future of hematology research and practice to the attention of the U.S. Congress and other governmental agencies. You can also participate in the Society’s advocacy efforts by visiting the ASH Advocacy Center or by joining the ASH Grassroots Network. Contact ASH Legislative Advocacy Manager Tracy Roardes at roardes@hematology.org, or visit www.hematology.org/Advocacy for additional information.

ASH Provides Resources to Help Practitioners Participate in PQRS

Bonuses for reporting quality measures in the Medicare program end on December 31, 2014. Those who successfully participate in the Centers for Medicare & Medicaid Services’ (CMS) Physician Quality Reporting System (PQRS) in 2014 receive a 6.5 percent bonus in 2015. Participating in PQRS is more important than ever because there are penalties associated with not reporting. Physicians who do not report will have payments reduced by 2 percent in 2016. There are many ways to meet PQRS reporting requirements, including the ASH PQRS Pro registry (https://ash.pqrspro.com), which is now available for reporting 2014 data. ASH has also created a guide for hematologists participating in PQRS program: go to www.hematology.org/Clinicians/GuidelinesQuality/PQRS/2835.aspx.

The PQRS program will also form the basis for quality measurement in the Medicare pay-for-performance program known as the value-based modifier. Physician practices with no PQRS participation will have payments reduced another 2 percent. Those who do participate can have payments adjusted based on performance. ASH has developed resources to explain the impact of this program on hematologists, including a graphic to show how the quality measures and cost measures are composited. For additional information on the PQRS program and to access ASH’s resources, visit www.hematology.org/PQRS.

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H E A D L I N E S  F R O M

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Members of the ASH Committee on Scientific Affairs in front of Building 1 on the NIH Campus in Bethesda, MD. From left: Dr. Willem E. Fibbe, Dr. Alex A. Rosmarin, Dr. Carolyn A. Felix, Dr. Charles Mullighan, Dr. Debra K. Newman, Dr. Emery E. Bruniac, Dr. Robert A. Hromas (Chair, ASH Committee on Scientific Affairs), Dr. David M. Weinstock, Dr. Grzegorz S. Nowakowski, and Dr. Michael J. Hulek.

– Jonathan W. Friedberg, MD, University of Rochester School of Medicine, Rochester, NY; and Margaret V. Ragni, MD, MPH, University of Pittsburgh Medical Center, Pittsburgh, PA

Scientific Program

At the core of the Scientific Program are the members of the Society’s 18 Scientific Committees. The chair of each committee is charged with organizing a session for inclusion in the program of the annual meeting that provides attendees with an update on research progress that falls within the purview of their particular committee. The result is a scientific program of unparalleled scope that features the breadth of hematological research presented by the leaders of their field.

In addition to the Scientific Committee Sessions, interactive learning opportunities in the form of “Continuing Conversations with the Speakers” will be a feature of the Scientific Program. Using a roundtable discussion format, these sessions are designed for attendees to meet with speakers subsequent to completion of a session so that information presented during the session can be expounded, with audience members driving the conversation.

We are particularly excited about two Special Scientific Symposia that will be held this year. Highlighting transformative studies with implications for both research and clinical practice, these symposia will focus on two emerging technologies. The symposium on RNA therapeutics will address the scientific basis of RNA therapeutics and spotlight examples of potential applications for treatment of hematologic disorders, including RNA-based therapeutic approaches to antithrombotic therapy and long non-coding RNA therapeutics for HIV and cancer. The symposium on chimeric antigen receptor T-cell therapy will provide attendees with an update on this rapidly developing field of immunotherapy, in which a patient’s T lymphocytes are engineered to target specific antigens. This approach has demonstrated dramatic efficacy in the treatment of hematologic malignancies, including refractory acute lymphocytic leukemia, and a wide range of potential applications are currently being explored.

For the second year, ASH will offer Scientific Spotlight Sessions. Here, speakers will discuss current challenges and controversies in a particular scientific field in a format that encourages audience participation. These 90-minute sessions will address the current state of knowledge, translational and clinical applications, and future directions for the following topics: lessons from mouse models of sickle cell disease, the best preclinical model for testing novel therapeutics, and the malignant bone marrow niche.

– Benjamin Ebert, MD, PhD, Brigham and Women’s Hospital, Harvard Medical School; and Steven Lentz, MD, PhD, The University of Iowa Carver College of Medicine, Iowa City, IA

ASH member registration opens on Thursday, July 24, at 11:00 a.m. (EDT) and housing reservation requests can be made at the same time. The abstract submission deadline is Tuesday, August 5, at 11:59 p.m. (PDT). More information is available online at www.hematology.org/AnnualMeeting.
Refining Anticoagulation with Dabigatran: Can We RE-LY on a No-Monitoring Paradigm?


In the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial, the safety and efficacy of unmonitored dabigatran in more than 18,000 patients was examined. The trial was designed to establish non-inferiority of two different dose levels of dabigatran compared with warfarin for prevention of stroke or systemic embolism in patients with nonvalvular atrial fibrillation and one additional risk factor for thrombosis. After a median follow-up of two years, patients receiving dabigatran, 110 mg twice daily, had similar rates of stroke or embolic events and lower rates of major hemorrhage compared with the warfarin-treated control group. At the higher dose level of 150 mg twice daily, the investigators found lower rates of stroke and systemic embolism and similar bleeding rates compared with the warfarin group. On the basis of these data, the FDA approved dabigatran for reduction of stroke and systemic embolism in nonvalvular atrial fibrillation in 2010, thereby providing patients and clinicians the first oral, unmonitored alternative to warfarin. At the same time, pharmacokinetic analyses revealed wide inter-individual variation in dabigatran plasma concentrations. While the RE-LY trial demonstrated the promise of unmonitored dabigatran, there remained a deficit of data on whether and how variability in plasma drug concentrations might impact clinical outcomes. In the present study, Dr. Paul Reilly and colleagues aimed to address this important issue.

The study examined the correlation between plasma concentrations of dabigatran and the clinical outcomes of ischemic stroke/systemic embolism and major bleeding among a subset of patients who had been enrolled in the RE-LY trial. Plasma dabigatran levels were measured using tandem mass spectrometry at peak (1 to 3 hours post-treatment) and trough (10 to 16 hours post-treatment) in subjects who had been taking dabigatran for one month. The impact of clinical and demographic variables was included in the investigation.

Using plasma samples from 9,183 patients, the authors found that dabigatran concentration was influenced by renal function, age, weight, and gender. Plasma concentrations showed greater than five-fold variation between the 10th and 90th percentile at both peak and trough for both the 110 mg and the 150 mg dose, and a large overlap in drug levels was observed between the two doses.

Trough dabigatran concentration was an independent predictor of both ischemic and hemorrhagic outcomes. Multiple logistic regression modeling showed that trough dabigatran levels were inversely related to the probability of an ischemic event (p=0.045) and directly related to the risk of major bleeding (p<0.0001). The best-fit model to predict major bleeding risk included trough dabigatran concentration, age, aspirin use, clopidogrel use, sex, and coronary artery disease. In multivariate analysis of ischemic stroke/systemic embolism risk, the best-fit regression model included trough plasma concentration, age, previous stroke/transient ischemic attack, and diabetes mellitus.

The figure illustrates the probability of major bleeding and ischemic events as a function of dabigatran trough plasma concentration and patient age. The probability of ischemic events flattened out at trough levels above 100 ng/mL but increased steeply with lower concentrations. In contrast, the probability of major bleeding continued to increase in a near linear fashion at trough concentrations above 100 ng/mL. These data suggest that in patients with relatively high trough dabigatran levels, decreasing the dose to target a concentration of ~100 ng/mL could reduce bleeding risk without appreciable loss of efficacy. Conversely, in patients with relatively low trough levels, increasing the dose to target a concentration of 100 ng/mL could reduce ischemic events at the potential cost of increased bleeding.

The relationship between trough dabigatran levels and ischemic and hemorrhagic events presented by Dr. Reilly and colleagues suggests a potential role for monitoring dabigatran to guide dosing and thereby optimize clinical outcomes. A randomized, controlled trial comparing dose-adjusted with standard unmonitored dabigatran is needed to test this hypothesis. Several major questions need to be tackled in designing such a trial. First, should all patients meeting RE-LY eligibility criteria be included, or should enrollment be restricted to patients with advanced age, renal dysfunction, or other characteristics that influence drug concentration? Second, should dabigatran levels be measured at just one time point (as in the study by Reilly et al.), or should they be measured more frequently (as with warfarin)? Third, should monitoring be carried out using tandem mass spectrometry (as in the study by Reilly et al.), or should a functional coagulation assay such as the dilute thrombin time, which may be more practical in a clinical setting, be used? Fourth, how would monitoring affect cost-effectiveness of dabigatran? Similar questions regarding the relationship between clinical outcomes and levels of the heretofore unmonitored oral factor Xa inhibitors, rivaroxaban, apixaban, and edoxaban, must also be asked. While we await answers to these questions, the data presented by Dr. Reilly and coworkers serve as a reminder that we still have much to learn about optimal clinical implementation of the novel oral anticoagulants. This class of drugs is likely to greatly simplify therapy for many patients, but a no-monitoring paradigm must be viewed with caution.


Reprinted from the Journal of the American College of Cardiology, Vol. 63, Reilly PA et al. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). Page 325, Copyright 2014, with permission from Elsevier.
NF-κB is Key in Mantle Cell Lymphoma


Mantle cell lymphoma (MCL) is an uncommon subtype of non-Hodgkin lymphoma that is incurable with standard chemoinmunotherapy. The recent approval of the Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib represents a major therapeutic breakthrough in MCL, as the overall response rate is nearly 70 percent in the relapsed/refractory setting. The majority of patients treated with ibrutinib, however, do not achieve complete remission, and the median progression-free survival is approximately 14 months. Thus, effective new treatment options are needed both for non-responders and for those patients who experience disease progression while taking ibrutinib.

Using pharmacologic and genomic profiling, Dr. Rami Rahal and colleagues at the Novartis Institutes for Biomedical Research in Cambridge, Massachusetts, recently reported the results of experiments that demonstrate the importance of the NF-κB pathway in the pathogenesis of MCL (Figure). After testing 119 leukemia and lymphoma cell lines with a variety of therapeutic agents that are active against known targets involved in the pathophysiology of hematologic malignancies, the authors chose for further study 10 MCL cell lines, four that were sensitive and six that were resistant to ibrutinib and sotrastaurin (STN), a pan inhibitor of protein kinase C (PKC).

In vitro experiments demonstrated that the cell lines that are responsive to ibrutinib and STN are dependent on signaling through the NF-κB pathway. PKC-δ, the primary isoform of PKC in MCL, is downstream of BTK and activates the CARD11-BCL10-MALT1 (CBM) complex, leading to cell proliferation through the classical NF-κB pathway (Figure). Cell lines that were sensitive to ibrutinib and STN had detectable cleavage of Rel-B, a marker of activity of the CBM complex. Following the administration of STN, Rel-B cleavage decreased, as did phosphorylation of IκBα, both results indicating suppression of the NF-κB pathway. In confirmatory experiments, short hairpin (sh) RNA-mediated inhibition of PKC-δ resulted in selective loss of growth in both ibrutinib and STN responsive cell lines. Subsequent gene set-enrichment analysis confirmed the up-regulation of BCR pathway components in sensitive cell lines and demonstrated the down-regulation of NF-κB pathway target genes in response to treatment with ibrutinib and STN. Further, the authors reported that a constitutively active allele of CARD11 restored the expression of NF-κB target genes in the presence of STN.

Upon further analysis of NF-κB signaling, both pharmacologic and shRNA-mediated inhibition of IKK-β was shown to suppress the growth of all MCL cell lines irrespective of response to ibrutinib and STN. To elucidate the mechanisms by which NF-κB signaling was important in the resistant cell lines, the authors subjected samples to RNA sequencing and identified a nonsense mutation in TRAF2 and biallelic deletion of TRAF3. In the alternative NF-κB pathway, TRAF2 and TRAF3 are negative regulators of NIK (also called mitogen-activated protein 3 kinase or MAP3K14), a kinase that phosphorylates p100, generating its active isoform, p52 (Figure). Levels of p52 were low in ibrutinib- and STN-sensitive cell lines and elevated in resistant cell lines. shRNA inhibition of NIK resulted in depleted p52 levels and prevented cell growth. In a xenograft mouse model using a resistant MCL cell line, knockdown of NIK resulted in decreased tumor growth and reduced expression of NF-κB target genes. Sequencing of key genes in the NF-κB pathway in 165 patient-derived MCL samples revealed mutations in TRAF2 and its downstream protein, BIRC3, in 6 percent and 10 percent of samples, respectively. Subsequent analysis showed that mutant BIRC3 had dominant negative properties that resulted in the loss of inhibition of NIK-NF-κB signaling (Figure).

The elegant work by Dr. Rahal and colleagues identifies the critical role of NF-κB signaling in MCL and provides key insights into the pathogenic mechanisms in ibrutinib-sensitive and -resistant MCL. Understanding these pathways may allow for the prediction of patient response to ibrutinib and lead to development of rational treatment combinations. In addition, the authors have identified NIK as a novel therapeutic target in patients who are resistant to ibrutinib.
adenosine is a purine nucleoside derived primarily from the catabolism of adenine nucleotides, ATP and ADP (Figure). The abundance of adenosine is determined by the action of a number of enzymes (Figure). In the extracellular compartment, the ectonucleotidases, and CD39 (ectonucleotide triphosphohydrolase 1) and CD73 (ecto-5’-nucleotidase), promote the formation of adenosine. Adenosine is degraded by adenosine deaminase (ADA), which can be present on cell surfaces in a complex with CD26. Adenosine signals through four G-protein coupled receptors, which mediate different actions. Recently, potential potential roles for adenosine in the pathophysiology of sickle cell disease (SCD) have been identified. In the May/June 2013 issue of The Hematologist, I reviewed a study that examined the therapeutic potential of agonism of the adenosine 2B receptor (A2B) by regadenoson to decrease the activation of invariant natural killer T (iNKT) cells and thereby ameliorate the sickness-reperfusion injury of SCD. Another function of adenosine, mediated in this case by signaling through A2BR, is stimulation of erythrocyte 2,3-bisphosphoglycerate (2,3-BPG) production, a process that enhances delivery of oxygen to ischemic tissues by decreasing the oxygen affinity of hemoglobin (Hb). While normally a beneficial compensation in the case of ischemia, decreasing Hb oxygen affinity in SCD favors the formation of deoxyhemoglobin S polymers and consequently promotes vaso-occlusion.2

Hydroxyurea (hydroxycarbamide, HU), an inhibitor of ribonucleotide reductase (Figure), has many salutary effects in SCD, most of which are mediated by the induction of fetal Hb (HbF) synthesis. However, clinical benefits can occur before a significant increase in HbF concentration is observed, so additional mechanisms of action have been postulated, including changes in cell-surface expression of adhesion molecules and greater bioavailability of nitric oxide.6 In the current paper by Dr. Ana C. Silva-Pinto and colleagues from the University of São Paulo, Brazil, reduction of adenosine activity was investigated as a novel mechanism of action of hydroxyurea. Dr. Silva-Pinto and coworkers studied 15 patients with SCD (HbSS) treated with HU, 17 patients with SCD who did not receive HU, and 10 healthy controls. The main findings were that monocytes in HU-treated patients expressed CD26, which forms a complex with ADA on cell surfaces (Figure), while monocytes from both untreated patients and controls did not. ADA transcripts were higher in monocytes of HU-treated patients than in untreated and control individuals, and HU-treated patients also had higher ADA activity. Together, these effects would serve to promote degradation of adenosine by ADA. Further, a lower percentage of T lymphocytes expressing CD39 was observed in HU-treated patients compared with untreated patients and controls, and there was a suggestion that CD39 expression might be lower in iNKT cells in HU-treated patients. Together, the effects of HU on CD26 and CD39 would be expected to reduce production of adenosine. In the HU-treated patients, several of the above findings were more pronounced in those with laboratory evidence of a good response to HU.

Dr. Silva-Pinto and colleagues have reported intriguing evidence for an alteration in adenosine metabolism in HU-treated patients, specifically a decreased potential for synthesis of adenosine (via reduction in expression of CD39 on T lymphocytes and CD73 in iNKT cells) and an increased potential for its catabolism (via increased expression of CD26 and co-localization with ADA on monocytes). It is unclear how HU might lead to those changes, although altered cell-surface expression of adhesion molecules on erythrocytes by HU was first reported nearly 20 years ago.3 Both CD39 and CD73 are hypoxia-responsive, so it is possible that HU could reduce their expression directly because it ameliorates anemia, consequently decreasing tissue hypoxia. Regardless of the pathways involved, lower levels of adenosine may decrease the detrimental (in SCD) signaling through A2BR, thus accentuating the benefits of HU. It is also possible that A2B receptor-specific and anti-inflammatory effects of signaling through A2BR (Figure) would also be decreased. One issue not considered by the investigators is that HU, as a ribonucleotide reductase inhibitor (Figure), may increase substrate (ADP) availability for the generation of adenosine, at least in the intracellular compartment. The greater abundance of adenosine in SCD, however, is proposed to occur in the extracellular compartment due to lysis of ATP- and ADP-containing erythrocytes. Much remains to be learned both about the role of adenosine in pathophysiology of SCD and about how the role of adenosine in the central dogma of DNA → RNA → protein has diversified largely in the past decades, and evidences novel parts to the major regulatory role of non-coding RNA (ncRNA). Recent technological advances have enabled in-depth analysis of transcriptomes that now show that most of the RNA transcribed from mammalian genomes does not code for proteins. Apart from ribosomal and transfer RNA, the ncRNAs are divided into two major classes depending on their length. Short ncRNAs are < 200 nucleotides and include microRNAs, whereas long ncRNAs (IncRNAs) are > 200 nucleotides in length and are classified according to their position of origin in the genome. The function of most IncRNAs is obscure, but available evidence implicates their involvement in a variety of cellular processes, including genomic imprinting, X chromosome inactivation, stem cell pluripotency, cell-cycle control, and pathogenesis of certain cancers and inherited disorders.

In an elegant study from the laboratory of Dr. Harvey Lodish at the Whitehead Institute/Massachusetts Institute of Technology in Cambridge, MA, the investigators reported a large number of IncRNAs involved in erythropoiesis was identified, and the functional role of a few selected samples was investigated. Dr. Alvarez-Dominguez and colleagues studied erythroid and non-erythroid cells derived from mouse fetal liver, erythroid progenitors at three stages of differentiation, and 30 murine cell lines of different tissue types. Analysis of the transcriptome from these various categories of samples revealed a vast array of erythroid-specific IncRNAs covering the whole spectrum of erythroid phenotypes. Several IncRNAs were differentially expressed during red cell development, and their expression levels correlated with specific chromatin signatures based on methylation status of selected histones that reflect epigenetic activation or repression of genes. To confirm that differentially expressed IncRNAs participated in erythropoiesis, the investigators demonstrated that the core erythroid transcription factors, GATA1, TAL1, and KLF1, bound to the promoters of these IncRNA genes. The binding peaks of the transcription factors coincided with DNase I hypersensitive sites, RNA polymerase II binding sites, and active chromatin marks, implying that the IncRNA genes are regulated by these factors.

To probe the erythroid-specific functions of IncRNAs, the investigators used a stringent strategy to select 12 candidates (all localized to the nucleus) and performed loss-of-function experiments. Knockdown experiments showed that the selected IncRNAs inhibited cell size reduction, enucleation, and erythrocyte maturation.

IncRNAs can regulate their target genes by acting in cis to influence a gene on the same allele from which it is transcribed, or they can act in trans on another chromosome. Of particular interest in the current study was an anti-sense to other genes, alncRNA-EC7, which is transcribed from an enhancer site to its site of transcription, to make contact with and activate the SLCA41 gene. This gene codes for band 3, the transmembrane anion exchanger of the red cell. It is synthesized early in erythropoiesis and serves as a focal point to recruit and stabilize the assembly of other cell membrane proteins, thus playing a crucial role in erythroid membrane biogenesis.

This study identifies a repertoire of erythroid IncRNAs and provides evidence of another layer of regulation in the complex process of erythropoiesis. It lays the foundation for exploiting these molecules as novel diagnostic tools and therapeutic targets and provides a framework for exploring the functions and mechanisms of action of these regulatory IncRNAs. It also highlights the integration of laboratory-based scientific advancements, computational analysis, and clinical insight to advance fundamental knowledge about red blood cell development.
Collective Action at the Cell Surface: Antibodies Club Together to Activate Complement


Binding and activation of complement is a key effector mechanism by which therapeutic IgG antibodies mediate their anti-tumor effects and, in some cases, their systemic toxicity. Previous work has shown that cell-bound IgG activates complement as the Fc tail of the immunoglobulin molecule has a low binding affinity for C1, the first component of the classical pathway cascade. In this study, an international research team led by Dr. Paul Parren of Genmab in the Netherlands has elucidated the molecular basis for this crucial antibody function, and their findings suggest an approach to enhancing the efficiency of complement activation by modifying the structure of IgG.

Building upon their prior crystallographic finding that anti-HIV IgG1 can pack as a hexameric ring, the investigators hypothesized that such a structure might match the six antibody-binding headpieces of C1q (the binding component of the multimeric C1 complex), thereby overcoming the low affinity of individual IgG molecules for C1q. They used a small peptide to block the key site in the Fc region that mediates interactions between multimeric IgG molecules and showed that this blockade caused a marked inhibition of complement-dependent cytotoxicity (CDC) produced by anti-CD20 and anti-CD38 IgG1 antibodies. Additional experiments used a genetic approach, introducing a variety of mutations in the multimerization interface in the Fc region of IgG to show that, at key points, single amino acid substitutions could substantially impair CDC, while antigen binding and C1q binding to IgG immobilized on a solid surface was unaffected. Most mutations outside the critical region were neutral in their effects on CDC, while a change at one site (E343R) enhanced CDC by 10-fold. This effect was observed for each of the four subtypes of IgG and was demonstrated for different antigen specificities including CD20 and CD38. Introducing three different enhancing mutations into IgG also resulted in hexamer formation in solution in equilibrium with the monomeric form. This molecule was capable of directly activating complement in human serum as demonstrated by C4d generation, a marker protein that is known to be released during complement activation.

The modeling suggested that one arm of the IgG would bind antibody, while the other protruded from the membrane. The presence of hexameric IgG on a membrane was modeled on liposomes using cryo-electron tomography, which showed an electron-density cluster consistent with a hexameric structure. When the gain-of-function E345R mutant IgG was used, the tomography studies also showed the presence of associated multimeric C1q, and crystal packing experiments in silico confirmed the potential docking of the two into a large membrane-bound complex with a ratio of IgG to C1q of 6:4. The modeling suggested that one arm of the IgG would bind antigen, while the other protruded from the membrane. This monovalent binding was confirmed by comparing the CDC potency of a bispecific antibody in which one Fab region had anti-C2D0 specificity and the other Fab region had irrelevant specificity. This bispecific antibody that was functionally monovalent (i.e., only one of the two Fab arms bound to cell-surface CD20) was found to be a more potent mediator of CDC than a bispecific antibody in which both Fab regions had anti-C2D0 specificity.

The conclusion from this sophisticated and highly technical study is that when monomeric IgG binds to antigens on the cell surface, a hexameric aggregate can form, thereby generating high-affinity binding sites for C1q resulting in activation of the classical pathway of complement. This model may help to explain why complement activation varies between antibodies of similar specificity, in that the epitope geometry of the combinations will accommodate hexamORIZATION much more readily for some than others, leading to differences in complement activation.

These findings have important implications for the design of antibodies that are aimed at activating complement. CDC remains one of several mechanisms of action for anti-lymphoma monoclonal antibodies such as rituximab and ofatumumab. While the exact contribution of CDC to the therapeutic efficacy of these and other antibodies is still incompletely understood, the work of Dr. Parren and colleagues suggests a genetic engineering approach by which complement activation can be modulated. In some instances, enhancing CDC may be beneficial, while in other instances, reducing complement activation could ameliorate treatment-associated toxicity.

The von Willebrand Factor Assembly Line Gets Longer


Von Willebrand factor (vWF) is a multifunctional protein produced by endothelial cells which is essential for hemostasis and plays a fundamental role in a variety of cellular events, including metabolism, immunity, and cellular repair. However, recently, macroautophagy also has been implicated in protein secretion. For example, deletion of a critical autophagy gene, Atg7, within pancreatic beta cells leads to impaired secretion of insulin.

Dr. Takehiro Torisu and colleagues in the laboratory of Dr. Toren Finkel at the Center for Molecular Medicine at the National Heart, Lung, and Blood Institute in Bethesda, Maryland, now find that macroautophagy is involved in the mechanisms that regulate expression of von Willebrand factor (vWF) by endothelial cells. vWF is the major component of Weibel–Palade bodies and is produced by a complex biosynthetic pathway that includes dimerization of pro-vWF monomers in the endoplasmic reticulum, furin-dependent cleavage of pro-vWF, and multimerization of mature vWF in the Golgi complex.

By use of immunoelectron and immunofluorescence microscopy, the authors observed that Weibel–Palade bodies and autophagosomes are located in cultured human umbilical vein endothelial cells (HUVECs) in closer proximity than expected by chance. Additionally, they observed that Weibel–Palade bodies and autophagosomes are occasionally fused together. Additional immunoelectron microscopy using anti-vWF antibodies revealed the presence of vWF in autophagosomes (Figure). Lentiviral shRNA-mediated knockdown of the autophagy genes, Atg7 and Atg5, in HUVECs produced several abnormalities on vWF expression. Although the intracellular concentration of vWF was not reduced, the ratio of pro-vWF to mature vWF was significantly increased in Atg7 or Atg5 knockdown cells. Stimulated release of vWF by histamine or vascular endothelial growth factor was reduced. Two inhibitors of autophagosome function, chloroquine and bafilomycin, blocked stimulated release of vWF from HUVECs. Additionally, the number and size of Weibel–Palade bodies was decreased in Atg7 and Atg5 knockdown cells, as well as in cells treated with chloroquine or bafilomycin. The rate of exocytosis of Weibel–Palade bodies from endothelial cells was also decreased in Atg7 knockdown cells compared with normal HUVECs.

The authors developed an in vivo model to assess the role of autophagy in vWF expression by endothelial cell-specific deletion of the Atg7 gene in mice using the Cre recombinase system. Vascular development and structure in these mice, called Atg7−/− mice, were normal, as judged by examination of skeletal muscle and retinal microvasculature and large vessel anatomy. However, cultured endothelial cells from Atg7−/− mice displayed reduced numbers of Weibel–Palade bodies. Although plasma vWF levels were normal in Atg7−/− mice in contrast to control mice, there was no epinephrine-induced increase in plasma vWF in Atg7−/− mice. Additionally, plasma vWF from Atg7−/− mice expressed lower levels of higher-molecular-weight vWF multimers compared with control mice. Consistent with this finding, the tail snip bleeding time was prolonged in Atg7−/− mice compared with control mice.

The results of this study provide additional evidence that autophagy is involved in the regulation of protein expression in certain specialized cell types, including endothelial cells. Exocytosis from endothelial cells, including release of vWF, provides a major defense mechanism after vascular injury. Elucidation of the role of autophagy in vWF expression adds a new dimension to the regulation of this complex protein and may have implications for the pathogenesis of von Willebrand disease.


PETE LOLLAR, MD
Dr. Lollar indicated no relevant conflicts of interest.
Epidemiologic studies conducted in many industrialized countries have shown a correlation between rising rates of obesity and an increasing incidence of endocrine and cardiovascular diseases, and obese individuals have been reported to be at greater than normal risk for developing cancer in general and leukemia in particular. Further, atopic and immune disorders appear to occur at a greater than normal rate in obese children. Until recently, however, little research has been directed toward understanding how Western diets and adiposity impact the function of hematopoietic stem and progenitor cells, which, after all, give rise to the immune system. Now, a study by Dr. Benjamin Adler and coworkers from Stony Brook University, Stony Brook, New York, is among several that are aimed at rigorously exploring these relationships.

Using a validated murine model, the authors investigated the effects of a high-fat diet (HFD) (60% calories from fat) on hematopoiesis at three time points (2 days, 1 week, and 6 weeks). Along with an increase in bone marrow adipocytes, they observed an early influx of macrophages and immature myeloid cells into the expanding fat deposits. The HFD cohorts were found to have an early, transient increase in lymphocytes and a transient gain in the bone marrow progenitor cells that gave way subsequently to a sustained net loss of both B- and T-cell populations and an expansion of myeloidopoiesis with peripheral blood counts and leukocyte subset distribution in the peripheral blood remained largely unaffected. Studies that focused on the lymphopoiesis showed that the B-cell population was reduced by 10% after one week and 25% after six weeks in the HFD-fed animals compared with those fed a normal diet. IL-7 secreted by supportive cells of the BM niche is a critical factor in early B-cell lineage development and is necessary for B-cell commitment. By one week, IL-7 expression fell by 19 percent in the HFD-cohort, and by week six, expression had fallen by 23 percent, at which time a 363 percent increase in adipose encroachment within the marrow space was observed.

The processes that mediate the effects of marrow adiposity on myeloid skewing and lymphopoiesis are incompletely understood, but an earlier report from the laboratory of Dr. George Daley suggested that local bone marrow adipocytes suppress hematopoiesis through release of paracrine factors. This hypothesis was supported by experiments that showed that pharmacologic or genetic interference with marrow adiposity improved several measures of hematopoietic function and compelled studies by others implicating adipocytes as predominantly negative regulators of the bone marrow microenvironment.

Additional studies are needed to characterize more completely the mechanisms that underlie the effects of a HFD on hematopoiesis and to delineate the long-term consequences of these effects. And it remains to be determined if the lymphopenia observed in animal models is likewise demonstrable in humans with atopic and autoimmune disorders who consume a high-fat diet. Nonetheless, the studies from Dr. Adler and colleagues support the concept that marrow as a dynamic organ whose function is significantly influenced by nutritional factors. On the backdrop of higher rates of infection and malignancy and impaired vaccination responses in obese individuals, additional research on the effects of bone marrow adiposity is warranted.


**Lymphoid Cancers Thwart Marrow Response to Therapeutic Antibodies, But Chemotherapy Triggers “ASAP” to Save the Day**


The tumor microenvironment contributes to malignant cell growth and drug resistance through diverse mechanisms including elaboration of stimulatory cytokines that promote tumor cell proliferation and neoangiogenesis; release by marrow mesenchymal stromal cells of exosomes containing IL6, fibronectin, and microRNAs that support growth of the neoplastic plasma cells; adhesion of malignant cells to cellular or matrix components of the microenvironment that confers chemotherapy resistance by upregulating survival and anti-apoptotic pathways; and specific genetic alterations in matrix components, such as deletion of Discl in osteoprogenitors, or an activating mutation of β-catenin in osteoblasts, that induce development of leukemia. Now Dr. Christian Pallasch and colleagues at the Massachusetts Institute of Technology in Cambridge, Massachusetts, and the University of Cologne in Germany, have identified a mechanism by which the microenvironment confers resistance to antibody therapy. But this story has a happy ending as the investigators have identified a novel way to overcome the resistance and thereby restore response to treatment.

Monoclonal antibodies, such as rituximab (anti-CD20) and alemtuzumab (anti-CD52), directed against antigens expressed on tumor cells are fundamental components of chemoimmunotherapy regimens used to treat lymphomas and some leukemias. The anti-tumor effects of antibodies are mediated by immunoglobulin activation of the classical pathway of complement and/or by antibody-dependent cell-mediated cytotoxicity (ADCC), which relies on recognition of the Fc portion of the immunoglobulin molecule by natural killer cells and macrophages.

Dr. Pallasch and colleagues developed a murine xenograft model of the aggressive “double-hit” form of non-Hodgkin lymphoma by transplanting human umbilical cord blood progenitor cells with the BCL2 and MYC genes., which has tumor cells in the blood and spleen of these animals were found to be transiently susceptible to the anti-CD52 antibody alemtuzumab, but tumor cells in the bone marrow were resistant to such treatment. Subsequent studies suggested that the resistance was a consequence of a tumor-induced process that rendered macrophages unable to mediate ADCC (Figure). But when cyclophosphamide was given within one day before or after alemtuzumab treatment, efficient phagocytosis was observed along with a reduction in tumor burden (Figure). The combination of cyclophosphamide and alemtuzumab was synergistic, producing a reduction in tumor burden 160-fold greater than that predicted if the effects of the combination were additive, and durable complete responses were observed in the animals treated with the antibody/cyclophosphamide combination.

Further analysis revealed that production of the stress-related cytokines IL8, TNFα, VEGF, and CCL4 (together called the "acute secretory activating phenotype" or ASAP), was induced by cyclophosphamide treatment, and that phagocytic activity was dependent on these cytokines. ASAP was also observed to be inducible by treatment with cyclophosphamide and rituximab in murine models of Burkitt lymphoma and BCR-ABL+ ALL.

When used as single agents, antibodies are ineffective in eradicating leukemia and lymphoma, but the combination of chemotherapy and antibody can be highly efficacious. The findings of Dr. Pallasch and colleagues that the tumor microenvironment inhibits macrophage function and that this inhibition can be ameliorated by treatment with chemotherapy (Figure) provides a plausible explanation for the success of chemoimmunotherapy combinations such as fludarabine, cyclophosphamide, and rituximab (FCR); chlorambucil–alemtuzumab; and R-CHOP. Understanding how ASAP is induced may result in rational development of specific pharmacologic modalities with favorable therapeutic profiles such that serious adverse effects (e.g., secondary malignancies) of stimulating agents including cyclophosphamide and chlorambucil can be avoided.

**Figure**

Presence of tumor cells in the bone marrow microenvironment can block macrophage activation and inhibit the ability of macrophages to engulf tumor cells. However, treatment with chemotherapy (CTX) can induce changes that activate the macrophages and promote phagocytosis.


2. Meads MB, Gatenby RA and Dalton WS. Environment-mediated drug resistance: a major contributor to favorable therapeutic profiles with favorable therapeutic profiles such that serious adverse effects (e.g., secondary malignancies) of stimulating agents including cyclophosphamide and chlorambucil can be avoided.
Dr. Bob Lowenberg (Editor-in-Chief) and Dr. Nancy Berliner (Deputy Editor-in-Chief) have combined efforts to identify some of the most outstanding Blood articles that have appeared either in print or online during the two-month interval between issues of The Hematologist. The citations are annotated to provide readers both with a concise description of the thrust of the article and an explanation of why the paper is particularly important. The goal is to underscore the remarkable research that is published in Blood and to highlight the exciting progress that is being made in the field.

**MAY 15, 2014**


Dr. Joaquin Martinez-Lopez and colleagues report the results of the first clinical study using deep sequencing of bone marrow for analysis of minimal residual disease (MRD) in multiple myeloma in patients with at least a very good partial response. The study showed that detection of MRD using this technology can be used to predict both risk of progression and length of survival. Further, the study identified a cohort of patients in whom residual disease was undetectable, suggesting that, in some cases, myeloma may be curable. These findings have the potential to inform clinical management once the technology becomes widely available.

**MAY 8, 2014**


Dr. Javed Ihapal and colleagues use gene-expression profiling to delineate clinically important subsets of peripheral T-cell lymphoma (PTCL). They report that GATA3 expression and an associated gene-expression signature define a poor-prognosis subset of PTCL, NOS (not otherwise specified). A linked paper by Dr. Tianjun Wang and colleagues confirms the prognostic importance of aberrant GATA3 expression and links it to GATA3’s influence on T-helper cytokine expression and the resulting macrophage polarization.

**MAY 1, 2014**


A growing body of evidence indicates that neutrophil extracellular traps (NETs) are generated during thrombus formation in humans and that NET biomarkers in plasma reflect disease activity. This review article from Dr. Kimberly Martinod and Dr. Denisa Wagner covers the important and novel role of NETs in thrombosis.

**APRIL 24, 2014**


A unique set of papers from the FANTOMS (functional annotation of the mammalian genome) consortium that describes deep sequencing of the global transcriptome of a range of hematopoietic subtypes and examines epigenetic regulators of hematopoiesis was highlighted in an Inside BLOOD commentary. In addition to representing incredibly rich resources for future study, the referenced manuscripts that were published in e-BLOOD provide new insights into hematopoietic ontogeny.

**APRIL 17, 2014**

Workentin TE and Sheppard JL. Serological investigation of patients with a previous history of heparin-induced thrombocytopenia who are re-exposed to heparin. Blood. 2014;123:2485-2493.

Heparin re-exposure, despite a history of previous heparin-induced thrombocytopenia (HIT), can be appropriate if platelet-activating antibodies are no longer detectable. In a relatively large study consisting of a group of patients with a previous history of HIT who were re-exposed to intraoperative (but not postoperative) heparin, Dr. Theodore Workentin and Dr. Jo-Jun Sheppard show that the risk of recurrent HIT is low, but still possible, if antibodies with heparin-independent platelet activating properties are formed.

**APRIL 10, 2014**


Dr. Neil Shah and colleagues present long-term follow-up of a Phase III study of dasatinib in 670 patients with imatinib-resistant or imatinib-intolerant chronic myeloid leukemia (CML). This manuscript presents novel and important data about the outcomes of CML patients in a second-line treatment with dasatinib. They observed that these patients can experience long-term benefit and that early molecular and cytogenetic responses are associated with improved progression-free and overall survival.

**APRIL 3, 2014**


Dr. Feng-Yen Li and colleagues describe the clinical features of a new primary immunodeficiency syndrome, X-linked immunodeficiency with magnesium defect, EBV infection, and neutropenia (XMen). The disease is linked to defects in a magnesium transporter (MAGT1), providing surprising insight into a critical role for intracellular magnesium in the normal immune response.

**MARCH 27, 2014**


The humanized monoclonal anti-C5 antibody eculizumab blocks the complement-mediated intravascular hemolysis of paroxysmal nocturnal hemoglobinuria (PNH) by inhibiting formation of the cytolysic membrane attack complex. However, due to deficiency of C5SS, a membrane protein that inhibits activation of the alternative pathway of complement, PNH erythrocytes in patients treated with eculizumab become opsonized with C3, resulting in clinically significant extravascular hemolysis in some cases. In this study, Dr. Antonio Risitano and colleagues report on small molecule inhibitors that prevent C3 activation and block complement-mediated hemolysis in vitro and in non-human primates. Clinical development of C3 inhibitors may lead to new therapies for PNH and other complement-mediated diseases.

**MARCH 20, 2014**


Long non-coding RNAs represent a recently identified class of RNAs whose biologic functions are just beginning to be explored. To date, their expression and activity in hematopoietic systems has not been investigated in great depth. This study by Dr. Vikram Paralkar and colleagues characterizes a new class of long non-coding RNAs with a functional role in erythroid terminal maturation.


Immunosuppressive therapy (IST) is standard of care for patients with aplastic anemia who are not candidates for allogeneic hematopoietic stem cell transplantation, but treatment failure and relapse are relatively common. Therefore, additional therapeutic options are urgently needed. Investigators at the National Institutes of Health, including Dr. Cynthia Dunbar and Dr. Neal Young, reported on the efficacy of the thrombopoietin mimetic eltrombopag in the treatment of patients with refractory aplastic anemia. Now, these investigators and their colleagues present data on a larger patient cohort with longer follow-up. The overall response rate was 40 percent, and, in some cases, response persisted after discontinuation of eltrombopag. Clonal evolution, including monosomy 7 and partial deletion of chromosome 7, was observed in a subset of patients that included both responders and non-responders. Ertrombopag has efficacy in the treatment of aplastic anemia, but further studies are needed to better define both the risk/benefit ratio and the optimal timing and duration of therapy.

**MARCH 13, 2014**

In late May, the new and improved Blood website launched. Check out the newly redesigned site at bloodjournal.org. As you browse the site, you will notice a similar look and feel to the ASH website, which underwent a design change that launched in early April.
Looking Back – Looking Forward: 40 Years in Hemophilia Research

GILBERT C. WHITE, II, MD
Richard H. and Sara E. Astor Chair for Medical Research, Executive Vice President for Research; Director, Blood Research Institute at BloodCenter of Wisconsin

The following is excerpted from and based on an article published by the author in the Transactions of the American Clinical and Climatological Association (Trans Am Clin Climat Assoc. 2010;121:61-73) with permission.

Author's Note: This paper is dedicated to those hemophiliacs who died of AIDS, to Harold R. Roberts, MD, and Campbell McMillen, MD, mentors who taught me commitment and compassion in the treatment of all human conditions, and to Martha Warren Turner, Aime Beebee, and Brenda Nielsen, nurses at the Harold R. Roberts Comprehensive Hemophilia Diagnosis and Treatment Center at the University of North Carolina, Chapel Hill, truly remarkable individuals who provided the very highest possible care in the very worst possible times.

Prior to the 1960s, the average life expectancy for an individual diagnosed with hemophilia was 11 years.1 When I came into clinical practice during the mid-1970s, treatment of hemophilia had been greatly improved by advent of the availability of glycoprotein-precipitated plasma concentrates and, consequently, the average lifespan of an individual diagnosed with hemophilia had increased to about 42 years. But life for patients with hemophilia remained challenging as treatment often meant a trip to the emergency room followed by an inpatient admission; hepatitis B and a non-A, non-B form of blood-borne hepatitis (that we now know was hepatitis C) were starting to appear at a high frequency among patients; hemophiliacs who developed inhibitory antibodies had no treatment options; and joint dysfunction and deformity continued to plague the population.

What has happened in the 40 years since then is a story not only of terrible tragedy but also of remarkable accomplishment that has led us to the threshold of a cure. Ultimately, though, it is a compelling story of the power of medical research that is driven not only by curiosity but also by passion and by the resiliency of patients and their caregivers, who persevered in the face of overwhelming adversity and, in the end, triumphed.

The Tragedy – AIDS in Hemophilia

The availability of glycoprotein-precipitated factor VIII concentrates enabled hemophiliacs, for the first time, to initiate treatment at home. This treatment option opened up a whole new world in which hemophiliacs could treat themselves more rapidly, thereby limiting the severity of hemarthroses and other bleeding complications, reduce dependency on emergency room and inpatient treatment, treat prophylactically prior to activities that might cause bleeding, travel, and have safe surgery for joint disease and other disease- and non-disease-related problems. But all of that progress ended abruptly in 1982 with the first report of AIDS in hemophilia patients.1 Over the course of the next few years, almost 5,000 hemophiliacs in the United States were found to be infected as a result of transmission of HIV through plasma-derived treatment products, and more than 4,000 of the estimated 10,000 hemophiliacs in the United States would eventually die of AIDS. These were dark times for the hemophilia community. The freedom that patients had gained from the availability of factor concentrates disappeared. Patients stopped treatment, and there was confusion among physicians about how to manage patients. Patients that I and the other members of the hemophilia team in Chapel Hill had followed for years began to die of complications related to AIDS. Because the treatment of hemophilia is from birth to death, the staff of a hemophilia center becomes unusually attached to their patients and the staff to the stool. We knew their goals and aspirations; we had seen how their goals had to be modified by their hemophilia and now, with AIDS, we saw that their goals might never be achieved.

The advent of the AIDS epidemic, however, drove scientific discoveries that ironically have now improved the treatment of hemophilia. These discoveries were fueled by the tragedy that was happening in the hemophilia community and driven by a robust collaboration among academic medical centers, the then fledging biotechnology industry, and pharmaceutical companies. In retrospect, these discoveries came rapidly, but they did not come fast enough to save the lives of nearly half of the U.S. hemophilia population.

Cloning the F8 Gene and the Development of Recombinant Products

The first of the landmark discoveries that changed the management of hemophilia was the cloning of the F8 gene.2 In 1987, the genes for two of the most common forms of hemophilia, factor VIII (FVIII) and factor IX (FIX), were cloned and sequenced.3 This was a landmark discovery because the cloning of the F8 gene allowed scientists to begin to understand the molecular structure of FVIII and FIX, the way the genes worked, and how they were repaired when they were damaged. This understanding, in turn, led to the development of recombinant products for therapeutic use. Two groups, one at Genetics Institute in Boston and one at Genentech in San Francisco, cloned the F8 genes simultaneously.4 One of the key elements in the race was to use recombinant technology to develop a human-safe product.5 According to a report of an interview with a patient at Harvard University's General Clinical Research Center in 1982, the patient who was to receive the product was a 43-year-old man who was told the product had been generated in Chinese hamster ovary (CHO) cells and that he was one of the concerns was that, being produced in a non-human animal cell, the factor VIII might not fold properly and antibodies might develop. I explained that by all methods of comparison, the CHO-protein was identical to the protein isolated from human plasma, but antibody formation was still a potential risk. On the day of the initial infusion, there was a news crew in the General Clinical Research Center to record the historic occasion. The lights were bright, and I was nervous. As I was infusing the material, I noticed that the patient was sitting with his eyes closed with his chin resting on his chest. I asked if he was okay, but he did not answer. I asked again, but again no answer. Louder, I said, “speak to me.” He looked up at me and started making hamster noises, intending to suggest that the infusion of a product synthesized in hamster cells had transformed him into a hamster. After the product was licensed, the patient was invited to Genetics Institute Headquarters in Cambridge, Massachusetts, for a celebration. During the festivities, Gabe Schmergel, the CEO of Genetics Institute, invited the patient to make some comments. After slowly and painfully climbing to a balcony half way up the stairs, he delivered a powerful story about what it was like to grow up with hemophilia without adequate treatment, how as a child he had lost a beloved older brother as the result of a bleed, and how important the development of safe recombinant factor was to him and all people with hemophilia; for the employees at Genetics Institute, his comments made their work relevant in an unforgettable way. Humor, selflessness, and undaunted spirit in the face of adversity were the signature characteristics of the hemophilia population during the dark days of the AIDS era, but there was also anger.

Today, more than 88 percent of the factor administered in the United States is a recombinant product, free of all human proteins.

Genetic Engineering and Molecules of the Future

One of the great promises of recombinant DNA technology is its use to reduce the cost of treatment through improvements both in the efficiency of FVIII and FIX production and by engineering functionally enhanced molecules, especially those with a longer half-life in circulation. The cloning of F8 and F9 was a critically important step in developing genetically improved molecules as the availability of large quantities of FVIII and FIX made feasible crystallization of portions of these proteins that allow determination of their molecular structure.6 Cloning and expression of factors VIII and IX permitted the development of antibodies that could be used as tools to investigate the structure, function, production, and catalysis of FVIII and FIX, serving as the foundation for the current success in generating recombination and derivatized proteins with favorable therapeutic properties.

A significant limitation of the current recombinant products is their rate of clearance. The half-life of FVIII is approximately 12 hours and that of FIX is somewhat longer. The clearance of FVIII seems particularly complex. FVIII binds with favorable therapeutic properties (Table).

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<td>One of the great promises of recombinant DNA technology is its use to reduce the cost of treatment through improvements both in the efficiency of FVIII and FIX production and</td>
<td>by engineering functionally enhanced molecules, especially those with a longer half-life in circulation.</td>
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The Hematologist: ASCN NEWS AND REPORTS

Validating Activated CSF3R Signaling as a Therapeutic Target in CNL and Atypical CML

STUDY TITLE: Prospective Evaluation of Ruxolitinib Efficacy for CNL/aCML Patients with Mutation of Colony Stimulating Factor 3 Receptor (CSF3R)

CLINICALTRIALS.GOV IDENTIFIER: NCT02892324

COORDINATOR: Oregon Health and Sciences University Knight Cancer Institute

PARTICIPATING CENTERS: 9 sites in the United States

ACCRCAL GOAL: ~30 patients

STUDY DESIGN: This is an open-label, phase II, investigator-initiated study of ruxolitinib in patients 18 years or older with World Health Organization (WHO)-defined chronic neutrophilic leukemia (CNL) or atypical chronic myeloid leukemia (aCML). Disease-specific eligibility includes the following: 1) platelet count > 25,000/mm3; 2) discontinuation of alternative therapies such as hydroxyurea, or biologics such as interferon-α; and 3) adequate hepatic and renal function defined as ALT/SGPT and direct bilirubin < 4X ULN and creatinine clearance > 150 mL/min, respectively. Subjects with CNL or aCML who have already been taking ruxolitinib as part of their standard of care may enroll as long as they meet the following criteria: 1) have pre-treatment cells or DNA in storage to allow sequencing of CSF3R or other relevant genes; 2) allow the transcriptional permissiveness to implement primary or targeted treatments and to data relevant to study endpoints, and 3) meet other trial eligibility criteria.

Treatment with ruxolitinib is initiated at a total daily dose between 5 mg and 40 mg (range from 5 mg qd to 20 mg bid). The starting dose is based both on existing guidelines used for treating patients with intermediate to high-risk myelofibrosis and on whether a patient is taking, concomitantly, drugs categorized as moderate or potent CYP3A4 inhibitors. Duration of treatment is 96 weeks; for patients with therapeutic benefit, the drug may be continued either on a commercial basis or enrollment in an extension study sponsored by the manufacturer (Incyte). The primary objective is to determine hematologic response rate. Secondary objectives include evaluation of safety/tolerability, correlation of response with type of CSF3R mutation, degree of mutant allele burden reduction, and assessment of changes in the total symptom score as measured by a modified Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF).

RATIONALE: No standard of care exists for the rare entities CNL and aCML, and the estimated median overall survival for these disorders is ~2-30 months. In 2013, gain-of-function mutations in CSF3R (the gene that encodes the G-CSF receptor) were identified in the majority of patients with CNL and in some patients with aCML (Maxson JE et al. N Engl J Med. 2013;368:1781-1790). CSF3R mutations cluster into two distinct regions of the receptor, with the majority occurring just extracellular of the transmembrane domain (membrane proximal mutations) and a small number resulting in truncation of the cytoplasmic tail (truncation mutations). The most common membrane proximal mutations (e.g., Thr158 and Thr163A) result in ligand-independent activation of CSF3R, which initiates downstream signaling through JAK2, and cells harboring membrane proximal CSF3R mutations are sensitive to the Jak1/2 inhibitor ruxolitinib, with IC50s of ~100-200 nM. In mice transplanted with CSF3R-T618I-expressing hematopoietic cells, a fatal myeloproliferative disorder characterized by overproduction of granulocytes and granulocytic infiltration of the spleen and liver developed. In this animal model, treatment with ruxolitinib lowered the white blood count and reduced spleen size with a concomitant gain in body weight (Frieschman AG et al. Blood. 2013;122:3268-3263). In addition, an index patient with CSF3R T618I-driven CML treated with ruxolitinib exhibited an excellent clinical response with near normalization of white blood cell neutrophil counts and normalization of the platelet count (Maxson JE et al. N Engl J Med. 2013;368:1781-1790). Truncation mutations are less common, but data from primary bone marrow colony assays suggest that cells harboring truncation mutations of CSF3R may be more sensitive to ruxolitinib, though these cells are also sensitive to inhibitors of SRC-family kinases, such as dasatinib.

COMMENT: The preclinical findings cited above and anecdotal patient data underscore the need to investigate the therapeutic potential of Jak inhibition in CSF3R-mutated CNL and aCML. In addition to establishing the hematologic response rate and effects on other clinical endpoints, such as splenomegaly, this trial will evaluate whether there is potential for modification of the natural history of these poor-prognosis neoplasms. The trial will also correlate biomarkers of response, such as type of CSF3R mutation, quality of response, and whether meaningful effects on mutant allele burdens are achievable, to the molecular remissions obtained by tyrosine kinase inhibitors in BCR-ABL1-positive CML. Patients who meet diagnostic criteria for CNL and aCML without molecular abnormalities of CSF3R may alternatively carry mutations in proteins downstream of this signaling axis or in parallel pathways. The collection of DNA for exome sequencing will permit identification of alternative or cooperating oncogenic driver mutations that contribute to the pathogenesis of CNL and aCML and may guide use of therapeutic agents such as ruxolitinib or of other signaling pathway inhibitors that are in development.

~ Jason Gottlieb, MD, MS

Dr. Gottlieb receive funding from Incyte for the administration of clinical trials, travel and honoraria from the advisory board. He is also a principal investigator of one of the participating trial sites.
Interested in Getting More Involved in ASH?

**Apply for the 2014 ASH Advocacy Leadership Institute**

The fourth annual ASH Advocacy Leadership Institute will take place in Washington, DC, October 1-2, 2014. This two-day workshop provides an opportunity for ASH members to gain a better understanding of the Society and its activities and to learn about legislation and health policy affecting hematology research and practice.

The first day of the Institute will focus on learning about the legislative process and health policy and will include training in advocacy. Sessions will feature guest speakers from Congress, the Presidential Administration, and the National Institutes of Health, as well as other health agency officials. On the second day, participants will visit their respective Congressional delegation on Capitol Hill to apply what was learned on the first day.

The selection of participants is based on a nomination process, and participation will be by invitation only. Up to 20 participants will be invited to attend the two-day workshop.

Nominations are being accepted through July 31. The exemplary candidate is an ASH member who is a U.S. citizen, is interested in health policy and advocacy, and wants to become more involved in ASH activities. Self-nominations are welcome. For more information or to submit a nomination online, please visit [www.hematology.org/ALI](http://www.hematology.org/ALI).

You may also send nominations to ASH Legislative Advocacy Manager Tracy Roades at troades@hematology.org. Please include the following information: nominator’s name and phone number; nominee’s name and institution (and contact information, if available); and reason for nomination (short paragraph describing the nominee’s interest in this opportunity).

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**MARK YOUR CALENDAR**

### July

**21**  
ASH webinar on Featured Topic: Hot Targets in Hemostasis and Thrombosis  
Washington, DC  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**24**  
Members-only registration and housing opens for 2014 ASH Annual Meeting and Exposition  
San Francisco, CA  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**28**  
ASH webinar on Physician Quality Reporting System and Pay for Performance for Hematologists  
Washington, DC  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

### August

**1**  
ASH Active and International Membership application deadline  
Washington, DC  
[www.hematology.org/membership](http://www.hematology.org/membership)

**1**  
Application deadline for Translational Research Training in Hematology  
Washington, DC  
[www.hematology.org/awards](http://www.hematology.org/awards)

**1**  
Application deadline for Scholar Awards  
Washington, DC  
[www.hematology.org/awards](http://www.hematology.org/awards)

**2-8**  
Clinical Research Training Institute  
La Jolla, CA  
[www.hematology.org/awards](http://www.hematology.org/awards)

**5**  
Deadline to submit abstracts for the ASH annual meeting  
Washington, DC  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**6**  
Application and letter of recommendation deadline for the Minority Graduate Student Abstract Achievement Award  
Washington, DC  
[www.hematology.org/awards](http://www.hematology.org/awards)

**10-13**  
ASH Meeting on Lymphoma Biology  
Colorado Springs, CO  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**13**  
ASH annual meeting advance registration and housing open to members and non-members  
San Francisco, CA  
[www.hematology.org/annual-meeting](http://www.hematology.org/annual-meeting)

### September

**12**  
ASH Consultative Hematology Course (Malignant)  
Chicago, IL  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**12-13**  
ASH State-of-the-Art Symposium  
Chicago, IL  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**30**  
Application deadline for Physician–Scientist Career-Developments Award  
Washington, DC  
[www.hematology.org/awards](http://www.hematology.org/awards)

### October

**1-2**  
Advocacy Leadership Institute  
Washington, DC  
[www.hematology.org/Advocacy/ALI](http://www.hematology.org/Advocacy/ALI)

**17**  
ASH Consultative Hematology Course (Non-Malignant)  
Baltimore, MD  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**17-18**  
ASH State-of-the-Art Symposium  
Baltimore, MD  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**21**  
ASH annual meeting late-breaking abstracts submission site opens  
Washington, DC  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

**28**  
Deadline to submit late-breaking abstracts for the ASH annual meeting  
Washington, DC  
[www.hematology.org/meetings](http://www.hematology.org/meetings)

For additional meetings dates and award deadlines, go to [www.hematology.org/Meetings/Non-ASH.aspx](http://www.hematology.org/Meetings/Non-ASH.aspx).