Oncometabolites Mediate Alterations in Covalent Cytosine Modifications in AML

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Cytogenetically normal acute myeloid leukemia (CyN-AML) makes up 40 to 50 percent of cases of adult AML. The heterogeneous nature of CyN-AML makes prognosis uncertain, and despite the clinical heterogeneity, induction therapy remains largely uniform. Technological advances in DNA sequencing, however, are providing new insights into the molecular basis of CyN-AML, suggesting new diagnostic and prognostic categories and novel approaches to therapy.

In the case of CyN-AML, leukemogenesis is not driven by the consequences of a specific chromosomal rearrangement. Hence, investigators have focused on delineating molecular abnormalities that define disease subgroups. Mutations within the FLT3, NPM1, and CEBPA genes are now evaluated routinely at diagnosis, and the identification of one or more mutations in these genes gives prognostic information and aids in the selection of a consolidation strategy. In the case of FLT3 mutations, targeted therapies have been developed and are currently undergoing clinical trials.

In addition to the three genes cited above, mutations in several genes encoding components of epigenetic pathways are common in CyN-AML. ASXL1 and MLL both encode modifiers of the histone proteins that compact DNA into chromatin, and DNMT3A, TET2, and IDH1/2 encode proteins that alter the balance of covalent cytosine modifications (Figure, page 5). Cytosines that are part of a 5′-CpG-3′ dinucleotide are potential substrates for DNA methyltransferase (DNMT) enzymes, and a decreased translocation (TET) proteins convert 5-methylcytosine to 5-hydroxymethylcytosine in an α-ketoglutarate (α-KG)-dependent reaction. Although the function of S-adenosylmethionine as a trans-acting repressor is well established, the precise role of 5-hydroxymethylcytosine in facilitating transcription and/or as an intermediate in DNA demethylation is incompletely understood.

The TET gene family was originally identified when TET1 was discovered as a chromosomal fusion partner of MLL in acute lymphoblastic leukemia (ALL) but was subsequently found to be a target of diverse transcriptional fusions in hematopoietic malignancies, including chronic myeloid leukemia (CML) (driven by BCR-ABL1) and select cases of non-small-cell lung cancer (NSCLC) (driven by activating mutations of the epidermal growth factor receptor [EGFR]). It is a testament to the imaginative, creative power of biomedical research. Nonetheless, room for continued progress in the field remains. For example, in the IRS1 trial of matribin-treated patients with newly diagnosed CML, primary hematologic resistance occurred in 2 percent of patients, and 8 to 13 percent of patients failed to achieve either a major or a complete cytogenetic response. After a median of five years of treatment, secondary resistance (hematologic or cytogenetic relapse, or progression to accelerated/blast crisis CML) developed in 15 to 20 percent of patients but was uncommon after four years of treatment. Secondary resistance is primarily due to the acquisition of point mutations within the tyrosine kinase domain of BCR-ABL1 and much less frequently due to BCR-ABL1 overexpression. BCR-ABL1-independent mechanisms of resistance include 1) clonal evolution; 2) suboptimal plasma levels of imatinib as a consequence of more rapid drug metabolism mediated by cytochrome P450 enzymes; and 3) reduction of intracellular bioavailability of imatinib due to decreased influx (mediated by the human organic cation transporter [OCTN1]), and/or increased efflux mediated by the same adenosine triphosphate-binding cassette (ABC) membrane transporter ABCB1 that is responsible for multidrug resistance. The basis for primary resistance is less clear; however, it is observed more frequently in patients from East Asia (e.g., Singapore, Malaysia, and Japan) than in patients from North America and Europe, suggesting that a genetic predisposition may account for suboptimal TKI responses.

Prior studies have shown that the BCL2 family member BIM (BCL2L11) is needed for TKIs to induce apoptosis in malignancies driven by kinases, and the BH3 domain of BIM is required for this function. In an elegant study, investigators from the National University of Singapore and Genome Institute in Singapore identified a BIM isoform lacking the pro-apoptotic BH3 domain. The BH3 domain-deficient isoform is the result of a relatively common germline deletion polymorphism. This 2,903 base-pair genomic deletion in intron 2 alters the splicing pattern of the gene such that transcripts lacking exon 4 (that encodes the BH3 domain) predominate. The deletion polymorphism was found in 12.3 percent of healthy East Asian individuals, but it was absent in African and European populations.

The deletion-containing CML cell line KCL22 (derived from a Japanese patient) exhibited resistance to imatinib-induced apoptosis, with the resistance being reversed either by transfection of the cells with the exon-4-containing, BCR-ABL1 domain transcript or by treatment with the BIM3-mimetic drug ABT-737. When the BIM deletion polymorphism was introduced into imatinib-sensitive K562 CML cells, susceptibility to imatinib-mediated apoptosis decreased, and this effect was reversed either by treatment with ABT-737 or by forced expression of BIMEL, a BH3-containing BIM isoform. In support of the findings in the CML-based studies, the NSCLC cell line HCC2299 that contains the BIM deletion polymorphism was also found to be resistant to EGFR inhibitors, and treatment with ABT-737 sensitized these cells to apoptosis induced by the TKI gefitinib.

The authors retrospectively analyzed East Asian patients with CML and found a statistically significant increase in prevalence of the deletion polymorphism in resistant subjects compared with optimal responders. In addition, the polymorphism was more prevalent in patients with resistance without BCR-ABL1 mutations compared with other patients resistant to TKIs with BCR-ABL1 mutations or patients sensitive to TKIs. In patients from Singapore and Japan with NSCLC and activating EGFR mutations, the BIM deletion polymorphism was associated with a shorter progression-free survival (6.6 months vs. 11.9 months), and the BIM deletion polymorphism and the TKI-resistant exon 20 mutation of EGFR were the only independent prognostic factors identified in a multivariate analysis.

The discovery of an inherited BIM polymorphism that confers resistance to TKI therapy in CML and NSCLC likely has applicability in the pathobiology of other leukemias and solid tumors. This seminal finding imparts prognostic utility by identifying patients who may exhibit relatively less responsiveness to TKIs. Such individuals may benefit from incorporation into their treatment regimens of BH3 mimetics or similar drugs that act in concert with TKIs to promote the apoptotic death of cancer cells. Therefore, knowing the “BIM you’re born with” is a dividend of the germline age that permits a more individualized approach to cancer care.

Dr. Goliath indicated no relevant conflicts of interest.
A Post-Modern View of Women in Hematology: A Focus on Work/Life Balance

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The concept of work-life balance is everywhere these days. In blogs, newspapers, and throughout professional societies, the topic is being discussed and debated. Several months ago, a friend gave me a book titled, “Good Enough Is The New Perfect.” The authors are working professionals with children who have honed a philosophy to simultaneously bring satisfaction to the workplace and the home. Skeptical, I began to read it in the moments before nodding off. Ultimately, I found the advice compelling and the anecdotes relatable.

Naturally, I began to examine my own situation as a part-time hematologist/oncologist and mother to Oliver, age 5, and Julian, age 3. Reflecting on the start of my career, I looked to an article I wrote for The Hematologist in May 2005 titled “Women in Hematology — Where Are We Today?” (www.hematology.org/Publications/Hematologist/2005/2100.aspx). At the time, I was a hematology/oncology fellow and first-time mother of a three-month-old. Aspects of hematopoiesis have changed in this interval, from new drugs and new guidelines, to concerns over drug shortages and cost constraints. How has the field changed for women? Are there new ways to approach the “work” component of the work-life balance conundrum to better accommodate one’s personal and professional goals? What strides have we made, and what new challenges do we face? Is there a successful formula for being a physician, mother, and so much more all at the same time? Let’s start by going back seven years.

When I interviewed Barbara M. Alying MD, then acting director of the National Center for Research Resources at the National Institutes of Health, her perspective and advice, based on more than two decades of experience, were particularly insightful. She spoke of the value of women’s communication and socializing skills in developing collaborations that move projects forward. She warned against giving up in the face of rejection and urged women in the field to “pick yourself up, keep showing up, and keep publishing papers.” She stressed the importance of bringing a family, and she urged me, as a fellow female hematologist, to think strategically about time commitments, to get help with tasks not essential to career or family life, and to always provide time for myself.

Many female physicians have taken her advice, as well as that of other thoughtful male and female mentors. In doing so, we have created successful hematology careers in academic medicine, private practice, and industry. And yet, the story does not end there. Research journals and the lay press continue to inform us that, across many professional contexts, women do not negotiate, self-promote, or earn wages concordant with their male colleagues. Because of the time and resources invested in education and training, most female physicians choose to work. However, many limit or opt out of career development and leadership roles through childrearing years. Because many women have delayed starting families during the rigors of training, their optimal childbearing years coincide with the optimal period for career development. The negative result of this convergence appears to be fewer female physicians, which is not responsible for any errors or omissions in the materials. The Hematologist welcomes Op-Ed pieces or letters to the editor on any subject.
ASH Invites You to Atlanta, GA, for Our 54th Annual Meeting Taking Place December 8-11, 2012

Important Dates

- Early-bird registration and housing: July 18
- Deadline to submit abstracts: August 14

ASH Offers Additional Programs Geared Toward Clinicians

ASH will be extending its reach to clinical hematologists and community-based practitioners by expanding its State-of-the-Art Symposium (SAS) to a second location and offering a Consultative Hematology Course in conjunction with the SAS in Chicago.

2012 State-of-the-Art Symposia

**Chicago, IL • September 28-29**

**Los Angeles, CA • October 12-13**

Now offered in two locations, SAS is a CME-accredited activity designed to offer the same high caliber of clinically focused educational content for which the ASH annual meeting is known, while the small-meeting format provides attendees with exclusive access to leading hematology experts to discuss individual cases. The distinguished faculty includes Nancy L. Bartlett, MD; Thomas L. Ortel, MD, PhD; Terry B. Gernsheimer, MD; James Douketis, MD; Joachim Deeg, MD; Gary J. Schiller, MD; John P. Leonard, MD; Tracy Balchelor, MD; Nathan Fowler, MD; Steven H. Bernstein, MD; Barbara Pro, MD; and Jacqueline C. Barrientos, MD, who will present the most current developments in key areas of hematology and discuss the impact of new data on current practice. The focus of this year’s SAS meetings is recent advances in hematologic malignancies with a special focus on thrombosis. Don’t miss this valuable meeting. To register, go to www.hematology.org/SAS12TH.

2012 Consultative Hematology Course

**Chicago, IL • September 28**

*Held the morning prior to the 2012 SAS*

Are you a board-certified hematologist(-oncologist) who sees patients with non-malignant hematologic disorders infrequently? Would you like to update your core knowledge of hematology? Then this refresher course is for you. The one-day program, geared toward North American community practitioners, will cover commonly encountered clinical problems that arise in everyday practice and require the expertise of a hematologist. It will focus on non-malignant hematology and will use case-based presentations and interactive discussions on topics such as thrombosis, thrombocytopenia, bleeding, and white blood cell abnormalities. The hands-on course will be led by faculty – Terry B. Gernsheimer, MD; Lawrence A. Solberg Jr., MD, PhD; Richard Lottenberg, MD; Marc J. Kahn, MD; Barbara A. Konkle, MD; and Thomas L. Ortel, MD, PhD – familiar with consultative practice issues. To register, go to www.hematology.org/CHC12TH.
The Question
Is there a standard of care for treating older patients with chronic lymphocytic leukemia (CLL)?

Our Response
Despite recent progress in the understanding and management of CLL, the disease remains essentially incurable. Incidence of CLL is significantly higher in the elderly (those > 65 years old), as the median age at diagnosis is 72 and an estimated 70 percent of newly diagnosed patients are over 65. Despite the predominance of elderly CLL patients, this group has been under-represented in clinical trials because many have major medical co-morbidities or are perceived likely to tolerate therapy poorly. This omission leaves clinicians who treat CLL without definitive data on which therapy to choose for the large population of older adults.

At initial presentation, the clinical spectrum of CLL ranges from an asymptomatic patient, identified by routine blood work, to a symptomatic patient who experiences rapid progression to death from complications of CLL. Therefore, the first decision to make is whether or not treatment is required. Indications for consideration of treatment include: clinical symptoms (fevers, night sweats, weight loss, or painful lymphadenopathy or splenomegaly), non-immune-mediated cytophenias (hemoglobin < 11 g/dL or platelets < 100 x 10^12/L), autoimmune hemolytic anemia, or thrombocytopenia (ITP) that is poorly responsive to standard immunosuppressive therapy and rapidly progressive disease (lymphocyte count rising to > 300 x 10^12/L or rapidly enlarging lymph nodes, spleen, or liver). Isolated mild thrombocytopenia (platelets 70-100 x 10^12/L) often represents chronic ITP and can be followed closely without treatment if symptoms are absent.

Treatment decisions can be informed by identifying factors that influence response rates, duration of response, and prognosis, including specific interphase cytogenetic abnormalities such as del(17p13.1) and del(11q22.3), complex cytogenetics (> 3 abnormalities) determined by stimulated metaphase karyotype, unmutated immunoglobulin heavy chain gene status, Rai stage III/IV, ZAP-70 expression in 20 percent or more of peripheral blood lymphocytes, and elevated serum β2-microglobulin concentration. Trials by the German CLL Study Group have factored in co-morbid features assessed by the CRBS-G score for choosing therapy in elderly patients; however, this score is subjective, and, to date, no study has documented the benefit of this scale over simpler methods. Only performance status and the presence of del(17p13.1) or del(11q22.3) influence our treatment choice (Figure).

If an elderly CLL patient requires therapy and is eligible for and willing to participate, we recommend enrollment in a clinical trial. Outside of a trial, if the patient’s performance status will allow therapy, he or she should be assigned to one of three groups according to the presence of FISH/cytogenetic abnormalities: del(17p13.1), del(11q22.3), and all others.

Standard frontline therapy for younger patients with cytogenetic abnormalities other than del(17p13.1) or del(11q22.3) is the combination of fludarabine, cyclophosphamide, and rituximab (FCR) or fludarabine and rituximab. However, a subset of CLL patients ≥ 60 years in a large study of frontline therapy with FCR were more likely to require early treatment discontinuation due to myelosuppression and other non-hematologic toxicities. Although fludarabine improved the overall survival rate (ORR), improved the percentage of complete remissions (CR), and increased the time to treatment failure, there was no difference in progression-free survival (PFS) or overall survival (OS) between the two groups. Notably, the fludarabine group demonstrated a shorter median survival time and higher rate of toxicity, indicating that there is no major clinical benefit of using fludarabine over chlorambucil in the older population. These findings were confirmed by Woyach and colleagues who reviewed the experience of elderly CLL patients across all completed Cancer and Leukaemia Group B trials and demonstrated no benefit for fludarabine treatment in patients > 70 years. In contrast, patients of all age groups appeared to benefit from the addition of rituximab. Recently, Hillmen and colleagues published a phase II trial in which rituximab was combined with chlorambucil in 50 patients (median age ~ 70.5 years). ORR was 84 percent, with infection and neutropenia being the most common adverse effects. Therefore, for an elderly CLL patient without either del(17p13.1) or del(11q22.3), we recommend therapy with chlorambucil (10 mg/m² orally days 1-7 of a 28-day cycle) +/- rituximab (375 mg/m² day 1, cycle 1 and 500 mg/m² day 1, cycles 2-4).

Patients with del(11q22.3) do not have favorable outcomes in the absence of fractionated alkylator-based therapy. For older CLL patients with del(11q22.3), we recommend therapy with FCR, PCr (pentostatin, cyclophosphamide, and rituximab) or bendamustine (70 mg/m² days 1 and 2 of 28-day cycle) and rituximab (375 mg/m² first cycle, 500 mg/m² subsequent 5 cycles). Using the latter regimen, Fischer et al. reported an ORR of 90.9 percent in 117 patients (median age ≥ 64 years) with minimal major toxicities. A subgroup analysis of patients with del(11q22.3) showed a 90.5 percent ORR.

Treatment options are limited for older patients with del(17p13.1). Many propose the use of alemtuzumab, but we don’t recommend this therapy due to significant risk of palliative care.
infected complications and limited response duration. Instead, we recommend rituximab (375 mg/m² weekly for 12 weeks) in combination with high-dose methylprednisolone (1 g/m² days 1-3 of each 4-week cycle). Using this regimen, James and colleagues reported that in a relatively small study of 28 patients (29% > age 70) the ORR was 96 percent (100% in the > 70-year-old subset) with CR observed in 32 percent (38% in > 70 years). Adverse effects were found to be minimal. In general, for patients being treated with this regimen, we recommend hospitalization during the first three days of cycle 1 as tumor lysis syndrome, metabolic aberrations, and fluid retention can occur.

For elderly, unfit patients without del(17p13.1), for whom we have concerns that therapy will be poorly tolerated, we typically recommend chlorambucil or rituximab alone or palliative supportive care. Elderly, unfit patients with del(17p13.1) are unlikely to respond to either of these therapies and should be supported to care expectancy.

In summary, due to paucity of definitive data on management of the elderly CLL population, we encourage enrollment of these patients in clinical trials. However, when enrollment in a clinical study is not feasible, we use a treatment approach based on FISH/cytogenetic analysis together with our assessment of the individual patient’s capacity to tolerate these agents. The CLL treatment landscape is evolving rapidly. Promising new therapies that are currently undergoing clinical trials include lenalidomide, ibrutinib, GS1101, ofatumumab, GA101, ABT-199, and TBI-0316. While we await the outcome of these and other studies, a carefully considered approach to the management of elderly patients with CLL is essential.

Oncometabolites Mediate Alterations

AML, but the enzymatic function of the TET proteins was identified more recently. Although TET1 was discovered first, mutations in the TET2 gene are found more commonly in AML, ranging from 8 to 23 percent, and are thought to confer an unfavorable prognosis.8 The activity of TET2, an α-KG-dependent enzyme, is inhibited by 2-hydroxylutarate (2-HG), a metabolite produced in excess by mutated isocitrate dehydrogenase 1 and 2 (IDH1/2) enzymes. The IDH family of enzymes includes IDH1 and its mitochondrial isoforms, IDH2 and IDH3. The IDH enzymes catalyze the conversion of isocitrate to α-KG in the citric acid cycle, but mutant IDHs can confer a unique gain-of-function to the enzymes that results in excess production of 2-HG, a molecule usually found in trace amounts in cells. When levels are high, 2-HG diffuses throughout the cell (including the nucleus) and inhibits TET2 (Figure).

IDH1 and its mitochondrial variant, IDH2, first came to the attention of oncologists in 2006, when IDH1 mutations were found in colorectal adenocarcinoma. Most of the early attention, however, focused on gliomas and secondary glioblastomas as IDH2 mutations are found in > 70 percent of these tumors. IDH mutations have since been implicated in AML, particularly CyN-AmL, with varying frequencies across case series—from 5.5 percent (based on 493 patients who had only IDH1 mutations) to 33 percent (based on 358 patients who had both IDH1 and IDH2 mutations).6

In the earlier glioma studies, oncogenic mutations were identified at the arginine 132 residue (R132) in IDH1, and its analog, arginine 172 (R172), in IDH2. Notably, these mutations were found to be mutually exclusive in gliomas. A third IDH2 mutation, arginine 140 (R140), was found to be IDH1/MDS-specific. Concurrent IDH1 and IDH2 mutations in AMLs have been reported to be either mutually exclusive9 or very rare,10 and concurrent TET2 and IDH mutations have not been identified.4 These observations lend credence to a model in which IDH mutations in younger adult patients with acute myeloid leukemia: a Cancer and Leukemia Gene expression and clinical outcomes in mDS patients (e.g., serum/urine levels) may be used in the future for monitoring of AML disease progression and/or relapse. Therapeutically, drugs that target IDH1/2 mutated AMLs are under development. Disappointingly, the first small trials using hypomethylating agents in TET2-mutated disease showed either no impact11 or worse survival.12 Further investigations of these and other mutations are ongoing and will likely affect clinical decisions in this dynamic field of investigation, we can look forward to the discovery of other novel, unanticipated pathologic profiles that will ultimately affect the diagnosis, prognosis, and management of hematologic malignancies.

NIH Faces Budget Crisis as Sequester Looms

As Congress begins to work on fiscal year (FY) 2013 appropriations, lawmakers warn that, because of federal budget constraints, the funding line for the National Institutes of Health (NIH) will be flat at best. After nearly a decade of modest budget increases that were consistently below the annual rate of biomedical industry-related inflation, NIH’s inflation-adjusted funding is close to 20 percent lower today than in FY 2003. Further complicating the appropriations process is the fact that FY 2013 spending is subject to a $97 billion across-the-board cut as a result of “sequestration.” Sequestration is looming because of the failure of last year’s Joint Select Committee on Deficit Reduction (commonly referred to as the “Super Committee”) to reach agreement on a workable approach to the deficit reduction. Sequestration goes into effect in January of 2013 unless Congress can agree on an alternative plan to control deficit spending. Barring a compensatory increase in FY 2013 appropriations for NIH, the effects of sequestration on biomedical research could be devastating. The Bipartisan Policy Center has estimated that compensatory increase in FY 2013 appropriations for NIH, the effects of sequestration on biomedical research could be devastating. The Bipartisan Policy Center has estimated that unless Congress can agree on an alternative plan that would obviate sequestration, most non-defense discretionary programs, such as the NIH, would be cut by as much as 9.3 percent for the year.

ASH is urging Congress to provide NIH with at least $32 billion in funding in FY 2013. This funding level represents the minimum investment necessary for NIH’s budget to keep pace with biomedical inflation. A smaller NIH budget would necessitate a reduction in the number of grants awarded, further lowering the percentage of funded applications that is already at a critically low level. More information about NIH funding is available at www.hematology.org.

House and Senate Debate Bills on User Fee Programs & Drug Shortages

Vote on Final Legislation Expected Near July 4

On May 30, the U.S. House of Representative passed its version of the Prescription Drug User Fee Act (PDUFA) legislation including important provisions to mitigate drug shortages, most notably mandating an early warning system for manufacturers. The U.S. Senate passed its version of PDUFA on May 24, which includes similar provisions to combat drug shortages. In addition to addressing drug shortages, both the House and Senate bills would create user fee programs for generic drugs and generic biologic drugs, or biosimilars, and include provisions that focus on the safety of the drug supply chain and other issues.

The House and Senate are now expected to begin the conference committee process to reconcile differences in their respective legislation and set up quick votes in both chambers for final passage. House and Senate leaders have set a goal of delivering a final bill to President Obama by July 4.

While ASH believes the bills help address drug shortages, the Society is concerned that the Senate bill exempts biologics from all provisions and both bills specifically exempt products derived from human plasma proteins and recombinant products replacing human tissue — products used for the treatment of hemophilia and other bleeding disorders — from the early reporting mandate in the legislation. As the legislation is finalized, ASH will continue to work with Congressional leaders to ensure that biologics, human plasma protein derivatives, and recombinant products are included.

Physicians to Face Medicare Payment Cut Unless Congress Passes a Legislative Fix; ASH Continues to Pursue a Permanent Solution

Once again, physicians face a significant reduction in the rate of Medicare reimbursement for services beginning January 1 unless Congress passes a legislative fix to correct the flawed physician payment formula.

For almost a decade, Congress has overridden the reductions in payment rates mandated by the Sustainable Growth Rate (SGR) act of 1997 that included a formula intended to keep Medicare costs under control. Although the draconian nature of the cuts dictated by SGR have been recognized by Congress, a permanent solution to the problem has been elusive.

Representatives Allyson Schwartz (D-PA) and Joe Heck (R-NV) have introduced bipartisan legislation that would eliminate the SGR-based formula and begin a series of pilot programs designed to test new payment models that could serve as a replacement. The Schwartz-Heck legislation (H.R. 5707) would establish a five-year transition period during which physicians would get small rate increases in reimbursement and the Centers for Medicare and Medicaid Services would develop and test various payment models. The $300 billion cost of fixing the SGR program would be covered by savings on military expenditures expected to accrue as the wars in Iraq and Afghanistan wind down.

Ironically, the flawed formula created by the SGR program has exacerbated the problem it was intended to address. Each time the SGR-mandated cuts are overridden, no Medicare savings are realized and a funding source for the higher reimbursement must be identified while the cost of developing and implementing an alternative strategy continues to rise.

ASH strongly opposes the nearly 30 percent physician payment cut scheduled to begin in 2013 and continues to advocate for a permanent fix to the Medicare physician reimbursement problem. The Society supports the Schwartz-Heck bill.

ASH Advocacy in Action

The Society continues to vigorously represent the interests of its members to the Congress and federal agencies. Below is a summary of recent activities:

- ASH shared its Agenda for Hematology Research with congressional offices, federal agencies, professional societies, and patient advocacy groups. ASH will use the Research Agenda as a tool both to educate the Congress on the value of NIH and to highlight the most promising areas of research that require continued government support.

- ASH submitted testimony to the House and Senate Appropriations Committees in support of a budget of at least $32 billion for NIH.

- In conjunction with the Committees on Government Affairs, Scientific Affairs, and Practice, ASH conducted three Capitol Hill Days. Committee members visited approximately 100 congressional offices to discuss the Society’s concerns about funding for NIH, persistent drug shortages, and the proposed reduction in physician reimbursement through Medicare.

- ASH joined with more than 160 organizations to urge Congress to restore the salary limit imposed on extramural NIH researchers to Level I of the Executive pay scale. Last year, Congress included a provision in the Consolidated Appropriations Act to reduce the salary limit to Executive Level II and extended this limit to all HHS funding agencies. This change represents a reduction of $20,000 (10 percent) in the salary cap and is particularly onerous as it comes at a time when the pool of discretionary funds generated from clinical revenues and other sources is smaller and more constrained, limiting the capacity of investigators’ home institutions to support research. Having to make up the difference resulting from NIH’s reduction in salary support further diminishes institutional research coffers.

- In the report accompanying the FY 2013 Agriculture, Rural Development, Food and Drug Administration, and Related Agencies Appropriations bill, ASH successfully persuaded the Senate Appropriations Committee to include language aimed at improving communication by the FDA with physicians on problems related to ongoing and impending drug shortages.

- ASH launched a grassroots advocacy campaign to strengthen legislation concerning drug shortages by ensuring that it included biologics, such as recombinant proteins, in all provisions.
A Post-Modern View of Women in Hematology

(Cont. from page 2)

professionals can enrich the workplace with their unique attitudes and skill sets. From these women, my own experience, and my friends and colleagues, I offer up a framework for the ongoing effort to strike a balance that fits and sticks.

Build your framework by RE-FRAMING

Hollee Temple is an attorney, professor, and co-author of Good Enough is the New Perfect. The book draws on the authors’ survey of more than 900 working mothers. The authors postulate that re-framing the working mother’s mindset from “never enough” to “good enough” allows women to be more satisfied with their choices, and ultimately achieve success without sacrificing in the home or the workplace.

Do what makes you HAPPY

In an article appearing on the American College of Physicians (ACP) website titled “Women in Medicine: Work Life Balance,” Dr. Janis Blair, an internist and infectious disease specialist at the Mayo Clinic in Phoenix, AZ, calls her life demanding, but exactly the life she wants to lead. Her disease specialist at the mayo Clinic in Phoenix, AZ, calls her “working toward common goals becomes easy when you make adversaries into friends.”

Take care of YOURSELF

One of my mentors during fellowship, Dr. Irene Ghobrial, another active member of ASH, has fashioned a successful translational career at a highly competitive institution. In her career, she has pushed forward, seemingly without fear, always with a smile and a palpable hum of enthusiasm. She passed on to me this advice from her mentor: “develop rhino skin.” She elaborated that removing emotion and personalization from criticism turns it into advice.

Be ORGANIZED and GET HELP

Dr. Kathleen Beekman is a part-time oncologist with three children that range in age from two to seven years old. She manages her life with “a small cadre of helpers from the children that range in age from two to seven years old. She manages her life with “a small cadre of helpers from the

interested in getting more involved in ASH?

The second annual ASH Advocacy Leadership Institute will take place in Washington, DC, September 12-13, 2012. This two-day workshop is a unique opportunity for ASH members to gain a better understanding of the Society and its activities and to learn about legislation and health policy affecting hematolgy research and practice.

The first day of the Institute will focus on learning about the legislative process and health policy; it 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 will be by invitation only. Up to 20 participants will be invited.

Nominations are now being accepted through July 5, 2012. The ideal candidate is an ASH member residing in the United States who 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.

Interested in getting more involved in ASH?

Please send your nominations to ASH Senior Manager for Scientific Affairs Ulyana V. Desiderio, PhD (udesiderio@hematology.org), and include the following: 1) nominator’s name/phone number; 2) nominee’s name/institution; 3) reason for nomination (short paragraph describing the nominee’s interest in this opportunity).
Genetic Horizon Scanning in Large B-Cell Lymphoma


Broad genomic analysis is providing fascinating insights into the pathobiology of diffuse large B-cell lymphoma (DLBCL), identifying both key driver mutations and potential therapeutic targets. This paper from Boston, that includes investigators from the Broad Institute, Massachusetts Institute of Technology, and the Dana-Farber Cancer Institute, is one of three recent studies that used whole-exome sequence analysis to map the mutational landscape in DLBCL. The other two analyses were conducted independently by the British Columbia Cancer Agency group in Vancouver and the Institute for Cancer Genetics, Columbia University, in New York.1 There are notable areas of overlap among the studies, pointing to mutational events that are fundamental to this type of lymphoma.

In the current study, tissue from 49 biopsy samples of DLBCL was subjected to whole-exome sequencing. To eliminate the dilutional effect of contamination by non-lymphoma cells contained in the biopsy specimens, the sequencing protocol mandated an average depth of exome coverage of 150-fold. A relatively high mutation rate (mean of 1.6 mutations per megabase with a range of 0.6–8.7) was observed. This rate was notably higher than that seen in chronic lymphocytic leukemia where the figure is usually below one mutation per megabase.

Among the 58 genes with rates of mutation significantly higher than would be expected by chance, is usually below one mutation per megabase.

Interestingly, this study also revealed frequent mutations in other epigenetic modifiers, such as MYC, MLL2, and E2F2. That these genes have been shown to be mutated in other malignancies, including medulloblastoma and multiple myeloma, suggest that their protein products function as a tumor suppressors. Further, observed mutations in members of the Histone 1 family of genes lend additional weight to the hypothesis that epigenetic dysregulation is an important component of the pathobiology of DLBCL. Other genes found to be frequently mutated included the TNFRSF14, and a cell-cycle regulator, BTG1.

Mutations in the apoptosis suppressor BCL-2 are a well-described finding in lymphoma, and this study confirmed a relatively high mutational frequency, but with some distinctive features. Many of the mutations involved the WRCY motif, known to be an AID (activation-induced cytidine deaminase) target, and they largely occurred in the presence of a t(14;18) translocation that effects a balanced translocation between BCL-2 and the IgH locus. This observation supports the idea that mutations in BCL-2 arise through the same process as somatic hypermutation in immunoglobulin genes when t(14;18) is present. Notably, most of the mutations observed were synonymous, thereby not altering the normal function of the BCL-2 protein. The majority of mutations that were non-synonymous were clustered outside the BH domain of BCL-2, the region of the protein that determines key binding interactions with prosapoptotic proteins. The observations suggest a selection pressure against non-functional mutations in the BH domain and are in keeping with the idea that suppression of apoptosis by BCL-2 is an important step in malignant transformation.

Mutant KRAS, NOTCH1, BRAF, SYK, and SGK1 are well recognized as drivers in some malignancies, but they have not been thought to contribute to the pathobiology of DLBCL. In the studies of Lohr and colleagues, mutations in these genes were identified but at a frequency that was insufficient to reach statistical significance. Nonetheless, identifying such mutations may be clinically relevant for an individual patient as targeted therapy aimed specifically at these driver mutations has already reached the clinic (e.g., the BRAF inhibitor vemurafenib) or is under development.

Although DLBCL is frequently cured with combination chemo/immunotherapy, the rapid increase in incidence during the latter part of the last century, particularly among older adults, has resulted in a large number of DLBCLs occurring among those who have difficulty tolerating intensive cytotoxic treatment. This group of patients is less likely to be cured, making identification of new approaches to therapy an imperative. Large-scale sequencing is one means of approaching the problem. The current study has confirmed some of the core pathalogical events in lymphoma development, highlighted new concepts in disease pathobiology of DLBCL such as epigenetic dysregulation, and shown that broad sequence analysis is a powerful tool for identifying new therapeutic objectives. There is little doubt that this current study will be an important part of our diagnostic and therapeutic armamentarium as we transition toward our twin goals of targeted therapy and personalized medicine.

A great mystery in hemostasis and thrombosis has been why patients with hemophilia have such severe spontaneous bleeding despite having a fully functional extrinsic pathway of blood coagulation. The answer lies in the activity of a protein called tissue factor pathway inhibitor (TFPI). Following vascular injury, coagulation is mediated by tissue factor (TF), a central component of the extrinsic pathway, which binds factor VIIa. This complex (TF VIIa) activates factor X to Xa, which, in concert with factor Va, generates a thrombin burst. TFPI almost immediately blocks the extrinsic pathway, but the combination of the thrombin burst along with activation of factor XI to Xla by TF-VIIa is sufficient to activate the intrinsic pathway. In this way, activation of factor X to Xa by the factor VIIIa/factor IXa complex counteracts the activity of TFPI, allowing coagulation to proceed. If factor VIIIa and factor IXa are deficient, as in hemophilia A and B, the activity of TFPI is unopposed and the combination of complete blockade of the extrinsic pathway and a normal functional intrinsic pathway results in uncontrolled bleeding. These observations raise the possibility that direct inhibition of TFPI in the setting of hemophilia might mitigate bleeding complications and has prompted investigators to develop TFPI inhibitors. Blocking antibodies or aptamers (oligonucleotide or small peptide inhibitors) directed at TFPI improve hemostasis in animal models of hemophilia, but inhibition of total intravascular TFPI poses a thrombotic risk. Platelets express an isoform of TFPI that is different from that expressed in vascular beds. Therefore, finer control of hemostasis could potentially be obtained by selectively inhibiting TFPI from one source and not another. Yet, whether the roles in hemostasis of TFPI from different cellular sources are distinct is incompletely understood.

Investigators working in the laboratory of Alan E. Mast at the Blood Research Institute of Wisconsin have created a series of genetically modified mice, and studies using these animals suggest an unexpected role for platelet TFPI in hemostasis. Initially, Maroney and colleagues attempted to breed FVIII/TFPI-double-null mice. However, like single TFPI-null mice, these double-null mice were not viable. Next, the investigators generated hemophilic mice (FVIII-null mice) that were also heterozygous for TFPI, but these mice were not protected from excess bleeding. However, when TFPI was neutralized using a blocking antibody in the hemophilic mice, an antibody concentration-dependent increase in protection against bleeding was observed. Notably, protection against bleeding continued to increase even after all plasma TFPI was neutralized, suggesting the existence of an extra-plasmic pool of the inhibitor. To identify this putative alternative source, the investigators generated a hemophilic mouse with hematopoietic cells that lacked TFPI and observed protection against bleeding with demonstration of fibrin generation at injury sites.

These studies provide further evidence that inhibition of TFPI can decrease bleeding in a mouse model of hemophilia. Unexpectedly, they also demonstrate that TFPI derived from a hematopoietic cell, presumably platelets, serves an essential function in inhibiting TFPI following vascular injury.

Hemophilia is the most common cause of inherited coagulopathy. Current treatment includes recombinant or purified factor therapy. However, these treatments are expensive and require intravenous infusion; and formation of inhibitors is common. Small molecule-based therapies that modulate natural anticoagulant pathways hold potential advantages of lower cost, oral administration, and absence of inhibitor formation. Yet the optimal targets for this strategy are incomplete and the risk of thrombosis remains. Inhibition of TFPI represents a potential new strategy for mitigating the bleeding risk associated with hemophilia, and the animal models developed by Maroney and colleagues provide valuable insights into how the TFPI system functions in vivo.

**The Enemy of My Enemy: Inhibiting TFPI in Hemophilia**


**Engineering AML-Targeted T Cells**


Case reports of an inverse relationship between incidence of disease recurrence and extent of graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (SCT) along with subsequent confirmatory retrospective studies in larger patient cohorts suggested that T lymphocytes have potent anti-tumor activity. This concept was strongly supported by the demonstration that donor lymphocyte infusions could produce disease remission in patients who had relapsed after allogeneic SCT. Frustratingly, the inability to segregate anti-tumor effects from anti-host reactivity (GVHD) in the allogeneic setting and the formidable technical challenges of in vitro manipulation of T cells in the autologous situation have hampered clinical development of effective cancer immunotherapy. Efforts to direct anti-tumor activity by cloning and transferring antigen-specific T-cell receptors (TCRs) revealed two additional problems: inadequate expression of the tumor-specific TCR and mispairing of components of the tumor-specific TCR complex with those of the endogenuous TCR. The compromised target specificity resulted in both low avidity and auto-reactivity.

Chiara Bonini and colleagues from Vita Salute San Raffaele University in Milan appear to have overcome these obstacles, and their report marks another milestone in the quest to implement effective immunotherapy through adoptive T-cell transfer. The authors describe an elegant two-stage approach in creating tumor-specific T cells. In the first stage, the endogenuous TCR α and β chains were disrupted using specifically designed zinc-finger nucleases (ZFNs). The idea behind ZFNs is to target a specific DNA site for disruption. The zinc-finger portion of the molecule is the recognition component that binds to the targeted sequence, positioning the endonuclease to cleave the adjacent DNA. In the current experiments, the zinc-finger DNA-binding domain targeted the catalytic portion of the endonuclease to a specified sequence in the endogenuous TCR coding region. Nuclease-induced, genomic double-strand breaks are typically repaired by an error-prone process (called non-homologous end joining) that almost inevitably disrupts the reading frame. Thus, T cells treated with the specific ZFNs fail to express their endogenuous TCR. Absence of expression of the CD3-TCR complex allowed enrichment of the TCR-negative population through immunoselection, and because central-memory T cells were targeted in these experiments, in vitro expansion did not require TCR engagement but could be accomplished through cytokine supplementation with interleukin (IL)-7 and IL-15. Part two of the process of creating tumor-specific T cells involved the transfer of a TCR specific for the target antigen into the edited T cells. In this case, a lentiviral vector was used to transfer a TCR specific for Wilms tumor 1 (WT1) antigen, an inherently leukemicogenic transcription factor commonly expressed in acute myeloid leukemia (AML) and already known to be a T-lymphocyte target in some patients. Complete editing with genomic elimination of endogenuous TCR chain expression and transgenic replacement by the WT1-specific TCR required sequential rounds of culture, transduction, and sorting, but avoided TCR mispairing while providing functional T cells. In the end, the edited T cells showed strong antigen-specific avidity without in vitro off-target reactivity, a normal immunophenotype and unaltered in vitro expansion characteristics. Finally, the authors demonstrated appropriate WT1-specific lytic activity against AML patient samples in vitro, and xenogeneic transplantation studies provided evidence that fully TCR-edited cells can eliminate WT1+ AML grafts in vivo in the absence of broad host-directed reactivity.

Success in murine xenotransplantation does not predict how edited T cells will perform in patient trials, but the studies of Provasi and colleagues demonstrate the way forward for clinical use. Several aspects of their protocol lend themselves to broad application. As recently demonstrated, the same ZFN-based TCR-editing strategy can be paired with the introduction of a chimeric antigen receptor. Similarly, cytome-driven rather than TCR-driven expansion of central-memory T cells may prove to be a generally effective method for ex vivo enrichment, and WT1 targeting serves as a paradigm for tailoring the technique to the desired malignancy-specific antigen. The strategy described by Provasi et al. produces functional T cells with redirected specificity and constitutes an important proof of concept on the way to generating generic, off-the-shelf T-cell therapy. The improved avidity, reduced off-target reactivity, and TCR-independent in vitro expansion represent key accomplishments. While the strategy is technically feasible and potentially effective, the considerable complexity of the human immune system requires careful translation of this exciting new technique into use in the clinic.

Adoptive, cancer-directed T-cell immunotherapy using genome engineering and gene transfer represents perhaps the ultimate progression of cellular therapy in the quest to eradicate tumors.


**PETER KURRE, MD**

Dr. Kurre indicated no relevant conflicts of interest.

**ROBERT FLAUMENHAFT, MD, PhD**

Dr. Flaumenhaft indicated no relevant conflicts of interest.

**NOTES**

The Hematologist • ASH News and Reports
Blood From a Stone; Erythropoietin From the Bone


New healthy people, erythropoietin produced in renal interstitial cells maintains the normal hematocrit. Aside from the kidney, only the liver and possibly brain glial cells are known to have the capacity to produce erythropoietin. This study from the laboratory of Amato Giaccia at Stanford University reveals that osteoblasts in the bone marrow also express erythropoietin. This remarkable observation expands the list of diverse functional properties of subendosteal osteoblasts, known essential components of the hematopoietic stem cell niche.

Erythropoietin production in the kidney is regulated by hypoxia inducible factor (HIF) signaling. In renal cells, hypoxia leads to increased levels of HIF proteins, which are transcription factors that promote expression of many genes including erythropoietin. HIF protein levels are tightly regulated post-transcriptionally by a rheostat system based on protein stabilization versus protein degradation. The proteins that promote HIF degradation are Von Hippel-Landau (VHL) protein and prolyl hydroxylase domain (PHD) enzymes. Although VHL mutant mouse models are known primarily for causing renal cell carcinoma, neuroblastomas, and pancreatic cysts, 15 to 20 percent of patients with congenital erythrocytosis, including those with Chuvash polycythemia mutations in the VHL gene, have a normal number of erythrocytes. VHL protein is functionally abnormal in its role in the HIF degradation pathway, leading to increased erythropoietin production as a consequence of persistent expression of HIF proteins. The novel finding in this paper is that VHL-HIF-erythropoietin signaling also occurs in osteoblasts, localizing this process to the bone marrow and, intriguingly, to the hematopoietic stem cell niche. Using a mouse model in which VHL and HIF protein levels could be genetically manipulated uniquely in osteoblasts, Rankin et al. showed that mice with knockout of VHL only in the osteoblasts had a phenotype consistent with that observed in patients with congenital erythrocytosis including elevated circulating levels of erythropoietin, polycythemia, and splenomegaly with extensive extramedullary hematopoesis. By knocking out different HIF genes, they show that this effect is dependent upon stabilization of HIF2α (but not HIF1α) in osteoblasts. And by blocking erythropoietin signaling using a soluble erythropoietic receptor antagonist, the authors proved that the polycythemia in these mice is erythropoietin-dependent. Notably, the erythrocytosis caused by erythropoietin upregulation in osteoblasts completely suppressed erythropoietin production in renal cells.

Deletion of VHL in osteoblasts had additional effects on bone and bone marrow: Mice had both increased trabecular bone and a relative increase in hematopoietic stem cells. However, analysis of the committed progenitor subpopulations determined that osteoblast-derived erythropoietin only increased transferin receptor and glycoporphin-A-expressing erythroid progenitors responsible for the erythropoiesis. As HIF2α is the downstream mediator of the effects of VHL deletion in osteoblasts, Rankin et al. deleted HIF2α in osteoblasts to understand the physiologic role of VHL-HIF-erythropoietin signaling in osteoblasts in steady-state conditions. Interestingly, this manipulation led to a statistically significant decrease in erythroid precursors in the marrow, but it did not cause anemia. When anemia was induced with phenylhydrazine to “stress” the animals, recovery from the anemia did not require osteoblast-derived erythropoietin. While it is not clear why osteoblast-derived erythropoietin does not adequately compensate for the loss of renal erythropoietin in patients with kidney failure, the investigators next tested whether pharmacologic manipulation of osteoblast-derived erythropoietin production could potentially benefit patients with anemia caused by renal insufficiency. When the stabilized HIF proteins by injecting directly into the bone marrow small molecules (currently in clinical trials) that inhibit PHD, the mice developed the same erythrocytosis as that caused by deletion of VHL.

Thus, these data reveal that osteoblasts are a previously unidentified source of erythropoietin and suggest that manipulation of the osteoblast HIF/VHL pathway could be a therapeutic target to provide local erythropoietin in the BM with effects not only on red cell production, but possibly also on stem cell/early progenitor number and function.
D

The Hematologist: using the humoral response to an antigen, B cells differentiate into memory cells and antibody-secreting plasma cells. The response is triggered by the recognition of the antigen by the B-cell receptor (BCR). The BCR is an oligomer consisting of antigen-specific, membrane-bound immunoglobulin and a signaling component, heterodimer, Igα/Igβ (CD79). Cross-linking of the BCR by a multivalent Ag is the classical model of the activation of immunogenic signaling pathways. Antigen is presented to the BCR by follicular dendritic cells, dendritic cells, and macrophages. Subsequently, antigen is internalized into endosomal compartments containing major histocompatibility complex (MHC) class II molecules and undergoes proteolytic processing. Antigenic peptides bound to MHC class II are transported to the cell surface. As a consequence, B cells become antigen-presenting cells for helper T cells, which leads to further B-cell activation, affinity maturation of antibody, and immunoglobulin class switching.

Thaunat et al., in the laboratory of Facundo Batista at the London Research Institute, used sophisticated cellular imaging methods to follow the fate of internalized antigens in a murine model system. This system included fluorescently labeled transgenic B cells, designated MD4 cells, expressing BCR specific for hen egg lysozyme (HEL) and HEL-conjugated beads (bHEL) to simulate a multivalent antigen on a microorganism. Following adoptive transfer of MD4 cells into normal (C57Bl/6) mice and immunization of the mice with bHEL, the antigen-conjugated beads were shown to be localized in B-cell uropods (the protrusion at the rear of polarized motile cells). Additionally, MD4 cells cultured in vitro with bHEL and stimulated with both IL-4 and CD40 displayed polarized localization of bHEL. Importantly, B cells maintained a polarized distribution of antigen after cell division. The authors tested two possible models of antigen distribution on cell division. According to the first model, each daughter cell would receive an equal amount of internalized antigen. In this model, several cell divisions would be required for antigen to be diluted to undetectable levels in progeny cells. In the second model, one daughter cell would receive the entire antigen load. This model predicts the presence of daughter cells lacking antigen after only one cell division. Flow cytometric analysis performed on both in vitro and in vivo samples provided experimental support for the second model.

The authors examined the functional significance of the differential distribution of antigen during cell division. They sorted B cells into populations containing either high or low levels of bHEL and measured HEL peptide bound to MHC on the cell surface. HEL-MHC concentrations were higher for B cells that contained high levels of bHEL, indicating that they are more efficient at antigen presentation. Additionally, using bHEL-ovalbumin (bHEL-OVA) conjugate as the antigen and HEL-specific B cells as antigen-presenting cells, they observed that IL-2 secretion and proliferation of co-cultured OVA-specific T cells was greater in the presence of B cells containing higher amounts of bHEL-OVA.

This study raises the question of why it would be advantageous for the immune system to distribute antigen asymmetrically during B-cell expansion. The authors speculate that antigen-loaded daughter cells may compete more effectively for limiting amounts of T-cell help. Additionally, antigen-poor daughter cells are poised to mutate their BCR and will survive only if high-affinity surface immunoglobulin is generated. And in a Perspectives commentary accompanying the article, Dustin and Meyer-Hermann describe a mathematical model that predicts that asymmetric inheritance of antigen can lead to more efficient production of plasma cells.7

The study by Thaunat et al. produced the surprising observation that there is an asymmetric distribution of internalized antigen in polarized B cells that persists after cell division. This property may play an important regulatory role in the production of high-affinity antibodies, immunoglobulin class switching, and the generation of antibody-secreting plasma cells.


PETE LOLLAR, MD
Dr. Lollar indicated no relevant conflicts of interest.
cutaneous myeloid leukemia (AML) is the archetypal neoplasm for the characterization of cancer genomes. Dozens of recurrent, AML-associated chromosomal rearrangements and somatic point mutations are now recognized and these influence clinical risk stratification. However, such genetic information does not currently affect treatment decisions beyond identification of those patients with AML who have a reasonable chance of cure with high-dose chemotherapy alone versus those whose odds are poor enough to justify the risk of an allogeneic stem cell transplant. Most patients diagnosed with AML in 2012 will be treated with the same anthracycline–cytarabine combination regimens that were developed in the 1970s, or else (if the patient is especially old or infirm) with lower-intensity palliative strategies that improve median survival by only a couple of months.

In order to better understand how the full complement of AML-associated point mutations might influence outcomes in a uniformly treated cohort of patients, Ross Levine’s laboratory at Memorial Sloan-Kettering Cancer Center assessed the mutational status of 18 genes in samples from 502 patients between ages 18 and 60 (398 patients in the test cohort, 104 in the validation cohort) from among the 657 participants enrolled in the Eastern Cooperative Oncology Group (ECOG) E1900 randomized clinical trial. The ECOG E1900 study demonstrated superiority of daunorubicin 90 mg/m²/day compared with 45 mg/m²/day as part of the 3 and 7 induction regimen, at least for patients with favorable or intermediate-risk cytogenetics. According to the E1900 trial results, which were published in 2009, the higher dose of daunorubicin was associated with a superior rate of complete remission (71% vs. 57%) and improved overall survival (median, 23.7 vs. 15.7 months) compared with the lower dose, without increasing the rate of serious adverse events. Levine and his colleagues found that 97 percent of the samples from E1900 patients had at least one identifiable somatic mutation, the most common of which were FLT3 (37%), NPM1 (29%), DNMT3A (23%), and NRAS (10%). The major findings of the new analysis are that FLT3 internal tandem duplication (ITD) partial tandem duplication in AML, and mutations in ASXL1 and PHF6 were independently associated with reduced overall survival in the E1900 cohort, while CEBPA and IDH2 predicted better outcomes. NPM1 mutations were only favorable if present in conjunction with an IDH1/2 mutation. Of more immediate clinical relevance, higher-dose daunorubicin improved survival among patients with DNMT3A or NPM1 mutations or MLL translocations but did not influence survival among patients without such mutations. Complex interactions between the various mutations appear important but require further study.

Currently, the National Comprehensive Cancer Network (www.nccn.org) AML Clinical Practice Guideline recommends evaluation of the mutation status of only four genes at the time of diagnosis: NPM1, FLT3, CEBPA, and KIT. Few clinical pathology laboratories offer additional mutation testing beyond this short list, even for the subset of genes that are relatively commonly mutated in AML (e.g., DNMT3A and IDH1/2) and that were known to influence outcomes well before the new E1900 molecular profiling study. Additional mutational profiling seems likely to become more widely available in the near future, especially if other clinical groups are able to confirm that molecular analysis in AML can influence more than the single binary treatment decision about whether to proceed with transplantation. Next-generation sequencing approaches have dramatically decreased the cost of sequencing, enabling the profiling of a larger panel of mutations at a low cost. Nevertheless, obstacles remain, such as intellectual property claims for mutation testing of FLT3 and other genes and the logistics of returning such information in real-time to clinicians. Still, deep genetic characterization of patients enrolled in the numerous ongoing treatment trials of novel agents by the cooperative groups and other institutional networks promises to identify molecular subgroups with particular therapeutic sensitivities, hopefully making therapeutic calculations in AML more relevant than the current basic math of adding 3 to 7.

A Trial of EPIC Proportions

**STUDY TITLE:** A Phase IIIb Clinical Study to Assess Whether Regular Administration of F.VIII in the Absence of Immunological Danger Signals Reduces the Incidence Rate of Inhibitors in Previously Untreated Patients with Hemophilia A (The EPIC Trial)

**COORDINATOR:** Baxter Healthcare Corporation

**CLINICALTRIALS.GOV IDENTIFIER:** NCT01376700

**PARTICIPATING CENTERS:** 75 centers planned: 27 enlisted to date, including 20 in six European countries and seven in two Asian countries

**ACCRUAL GOAL:** 100 patients

**STUDY DESIGN:** This is a prospective, open-label, single-arm trial designed to test whether low-dose factor VIII (F.VIII), started at an early age, before the onset of severe bleeding and in the absence of other “danger” signals (e.g., infection or trauma), can reduce the incidence of inhibitors. Those who are eligible to participate in this trial include males, age < 1 year, with severe or moderately severe hemophilia A (F.VIII ≤ 2%), with three or fewer past exposures to F.VIII and adequate venous access without central line requirement. All subjects will receive 25 IU/kg of recombinant F.VIII once weekly by intravenous infusion for up to two years. For head injury, preventive low-dose F.VIII will be allowed. For a joint bleed, the dose may be escalated to twice weekly. The primary endpoint is the incidence of inhibitor formation within the first 50 F.VIII exposure days. Secondary endpoints include time-to-inhibitor formation, antibody titers, antibody isotype, bleeding, adverse event frequency, and documentation of risk factors for inhibitor formation including VIII genotype, HLA haplotype, F.VIII frequency, and documentation of risk factors for inhibitor formation. It will also collect immunologic and genetic marker data to better understand the basis of F.VIII tolerance (Mattus H. Blood. 2009;114:677-685).

**COMMENT:** Risk factors for hemophilia inhibitor formation have been well-established, but it is still difficult to identify prospectively the 25 percent of patients in whom inhibitors will develop. Most commonly, inhibitors develop during the first 50 F.VIII exposure days, hence the rationale for attempting to induce tolerance by exposing very young patients (< 1 year old) to low-dose recombinant F.VIII. If the innovative “pre-emptive” approach proposed by the EPIC trial is successful, such a preventive strategy will be potentially practice-changing and will set the standard for future randomized trials.

– Margaret Ragni, MD, MPH

**STUDY TITLE:** Induction of Donor Specific Tolerance in Recipients of Living Kidney Allografts by Donor Stem Cell Infusion

**CLINICALTRIALS.GOV IDENTIFIER:** NCT00497926

**STUDY SPONSOR:** University of Louisville

**COLLABORATORS:** Northwestern University, Department of Defense (OAR), and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

**PRINCIPAL INVESTIGATOR:** Joseph Leventhal, MD, Northwestern Memorial Hospital

**ACCRUAL GOAL:** 30

**STUDY DESIGN:** This is a phase II, non-randomized trial to assess safety and efficacy. The current trial utilizes a non-myeloablative preparative regimen that incorporates infusion of hematopoietic stem cells along with graft-tolerance-promoting-facilitating cells (FCRx). These tolerance-promoting cells are CD8+ but do not express the T-cell receptor. The kidney from the living donor is transplanted on Day 0 of the hematopoietic stem cell transplantation. Patients must be between the ages of 18 and 65 years old and meet the institution’s criteria for renal transplantation for end-organ failure. Primary outcome includes bone marrow engraftment and chimerism, while characterization of graft-versus-host disease (GVHD) is a secondary outcome.

**RATIONALE:** The aim of the study is to determine whether administration of donor-derived, G-CSF-mobilized peripheral blood cells, enriched for both hematopoietic stem cells and graft-facilitating cells and depleted of GVHD-producing T cells, will induce immune tolerance of mismatched renal allografts from living related or unrelated donors. In a recent report, five of eight kidney transplant recipients who underwent this procedure were taken off immunosuppressive agents by one year with no evidence of GVHD or engraftment syndrome (Leventhal J et al. Sci Transl Med. 2012;4:124ra28).

**COMMENT:** From the time of Medawar and Billingham and the first kidney transplants of Murray, the goal of developing a method that would allow donor organ acceptance in the absence of chronic immunosuppression has remained elusive. Despite identification of several successful approaches in mice, in adult humans, attempts to induce tolerance to solid organs transplanted across MHC barriers have produced inconsistent results. In the animal studies, demonstration of hematopoietic chimerism that included lymphocytes that neither rejected the transplanted organ nor attacked the recipient in a GVH-like reaction defined tolerance. In humans who received a bone marrow transplant and subsequently a kidney transplant from the same donor, this same definition of tolerance was observed even in the absence of immunosuppressive therapy. The prospective trial described herein weds basic immunology with transplantation science resulting in an exciting new approach to ameliorating the adverse consequences of mismatched renal allografts and minimizing (or eliminating) chronic immunosuppressive therapy, reducing the incidence of renal toxicity, drug-induced diabetes, post-transplant malignancy, hypertension, cardiovascular disease, and opportunistic infections. Conceivably, by inducing immune tolerance through use of non-myeloablative hematopoietic stem cell transplant, successful allografting of mismatched recipients may prove applicable in settings beyond renal transplant.

– Gregory M. Vercellotti, MD

Dr. Vercellotti indicated no relevant conflicts of interest.

**STUDY TITLE:** Of Immunological Danger Signals Reduces the Incidence of Inhibitors.

**ACCRUAL GOAL:** 100 patients

**STUDY DESIGN:** This is a phase II, non-randomized trial to assess safety and efficacy. The current trial utilizes a non-myeloablative preparative regimen that incorporates infusion of hematopoietic stem cells along with graft-tolerance-promoting-facilitating cells (FCRx). These tolerance-promoting cells are CD8+ but do not express the T-cell receptor. The kidney from the living donor is transplanted on Day 0 of the hematopoietic stem cell transplantation. Patients must be between the ages of 18 and 65 years old and meet the institution’s criteria for renal transplantation for end-organ failure. Primary outcome includes bone marrow engraftment and chimerism, while characterization of graft-versus-host disease (GVHD) is a secondary outcome.

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– Gregory M. Vercellotti, MD

Dr. Vercellotti indicated no relevant conflicts of interest.
This academic year stands as a landmark in residency training in the United States as the Accreditation Council for Graduate Medical Education (ACGME) announced new standards for resident duty hours and for the learning and working environment (Table). The effects of these new standards influence the day-to-day practice of subspecialty services. Hematology services in teaching hospitals rely on internal medicine (IM) residents for patient care and were required to assess these new standards, which necessitated modification of hematology training program rotations and inpatient services.

The new duty-hour standards aim to create a structure that allows residents to gradually develop their skills, knowledge, and attitudes as independent clinicians. Program directors are encouraged to design a graded and progressive training plan with proper supervision; this concept of graded and progressive responsibility is one of the core tenets of the ACGME. As a result, IM residency program directors were instructed to decrease the workload of the postgraduate year 1 (PGY1) residents and increase the clinical responsibilities of PGY2 and PGY3 residents (Table). The precise adjustments depend on the service and program. Some services hire moonlighters to cover work previously done by PGY1 residents while other programs create mixed resident teams with a variety of combinations of PGY1, PGY2, and PGY3 residents. On the medicine service, the classical structure of attending physician, resident (PGY2 or 3), and intern (PGY1) is usually maintained, but the structure in some of the subspecialty services has had to be changed.

An additional core concept is to ensure that the development of skills is achieved in a safe and effective environment for both the patients and residents. As a result, ACGME mandated a concern for the number of patients who are treated by the residents corresponding to the level of training (Table). In some cases, IM program directors made an independent decision to decrease the recommended cap based on the complexity of the patients on the teaching service and the availability of the residents. As a net result, the cap leads to an overall decrease in the number of patients who can be covered by the residents on the subspecialty services, forcing hematology programs to create a parallel, non-teaching service.

In the past, the co-existing teaching and non-teaching services contributed to the evolution of "hospital medicine" as a widely practiced specialty. Whether a similar process will occur routinely on inpatient hematology services is yet to be determined. In many centers, hospitalists and mid-level providers are being hired to cover non-teaching hematology services. While it is possible that this arrangement is one way to solve the challenges created by the new ACGME standards, it raises the question of what training and skills are needed for the care of hematology inpatients. This arrangement also brings into the conversation the possibility of creating a new niche — the "hematology hospitalist" that will be focused on inpatient care of hematology patients. Whether the approach to management of hematology inpatients needs to change significantly is debatable, however, awareness of the effects of the recent changes in ACGME standards encourages us to think broadly about this issue.

The role of the hematology fellow is no longer clearly defined in this time of change, but it is clear that hematology training programs will have to adjust to the new structures of the inpatient services. The ACGME core concept of gradual and progressive responsibility for residents will have to be applied to hematology fellow training as well. Program directors can no longer choose between defining the fellow as a "super-resident" or "junior attending"; instead, the training process should reflect a responsibility continuum throughout the fellowship. Defining the desired competencies for fellows in each year of training will most likely be the first step in this direction.

In many programs, the clinical experience of hematology fellows during inpatient rotations will be decreased as a result of creating parallel teaching and non-teaching services. The negative effect on experience of splitting up the inpatient service can be compensated for by giving more responsibility to the fellows during inpatient rotations to maximize the educational value of the rotation. Additionally, the academic curriculum will need to be adjusted to ensure that high-quality formal, didactic training will provide fellows with a reasonable replacement for the less intense inpatient experience. Fellows will need to learn how to communicate and work effectively with mid-level providers. This interaction will be a new experience for most fellows, as involvement with mid-level providers during residency is typically limited. However, the experience will be of value for trainees as mid-level providers work closely with staff physicians in the "real world."

The mandated changes create an opportunity to improve hematology fellowship programs and better prepare fellows to be independent and confident hematologists, but doing so calls for collaboration between ASH and ACGME to define the goals and competencies for every stage of the fellowship. It also creates an opportunity for fellows to take part in this process on a national scale, helping to design better programs for future generations of trainees.

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<th>Table: Resident duty hours comparison of 2003 and 2011 standards. From the Accreditation Council for Graduate Medical Education (ACGME).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2003 STANDARDS</strong></td>
</tr>
<tr>
<td>Duty hours are limited to 80 hours per week, averaged over a four-week period, including all in-house call activities.</td>
</tr>
<tr>
<td><strong>2011 STANDARDS</strong></td>
</tr>
<tr>
<td>Duty hours are limited to 80 hours per week, averaged over a four-week period, inclusive of all in-house call activities and all moonlighting.</td>
</tr>
</tbody>
</table>

| Maximum hours of work per week | Duty hours are limited to 80 hours per week, averaged over a four-week period, inclusive of all in-house call activities. | Duty hours are limited to 80 hours per week, averaged over a four-week period, inclusive of all in-house call activities and all moonlighting. |
| --- |
| **Mandatory time free of duty** |
| Residents must be provided with one day in seven free of duty every week. |
| **Maximum duty period length** |
| Continuous on-site duty, including in-house call, must not exceed 24-consecutive hours. Residents may remain on duty for up to six additional hours if needed. |
| **Minimum time off between scheduled duty periods** |
| Adequate time for rest and personal activities must be provided. This should consist of a 10-hour time period provided between all daily duty periods and after in-house call. |
| **Recommended maximum in-house on-call frequency** |
| In-house call must occur no more frequently than every third night, averaged over a four-week period. |
| **Recommended maximum number of patients** |
| PGY2 residents and higher must be scheduled for in-house call no more frequently than every third night. |
| **Recommended maximum number of patients** |
| PGY1 residents must not be responsible for the ongoing care of more than 10 patients. |
| **Recommended maximum number of patients** |
| When supervising PGY1 residents, the supervising resident must not be responsible for the ongoing care of more than 14 patients. |
| **Recommended maximum number of patients** |
| When supervising more than one first-year resident, the supervising resident must not be responsible for the ongoing care of more than 20 patients. |
**Med Students Pursue Interests in Hematology Research**

This summer, 14 medical students from various parts of the country will work closely with their mentors on a hematology-related research project. The subjects investigated by this year’s students include mantle cell lymphoma, hematopoietic stem cell transplantation, and acute chest syndrome. The Minority Medical Student Award Program (MMSSAP) participants will have the opportunity to present the results of their research during the Promoting Minorities in Hematology Presentations and Reception on Saturday, December 8 from 6:30 to 9:00 p.m.

Each award recipient will receive the support of a research mentor and a career-development mentor, a stipend of $5,000 during his/her research experience and a $2,000 allowance for travel to the annual meeting, and online subscriptions to *The Hematologist* and *Blood*.

### Returning Participants

**Ryanne Ashley Brown**  
Baylor College of Medicine  
**Research Project:** Identification of enzymes involved in the VWF-GPib binding-initiated signaling pathway that results in platelet activation and fibrinogen-ßIIßIII interaction using the R1450E VWF mutant under high shear stress  
**Research Mentor:** Miguel Cruz, PhD  
Baylor College of Medicine

**Michelle Long**  
Wake Forest University School of Medicine  
**Research Project:** Complex pediatric hematology consults: immune cytopenia following liver and multisystemic transplants  
**Research Mentor:** Ellis Neufeld, MD, PhD  
Harvard University

**Myntee T. Ngangana**  
The Ohio State University  
**Research Project:** Assessment of hematopoietic stem cell transplantation co-morbidities index in DNMT3A and Flt3-ITD AML patients receiving decitabine  
**Research Mentor:** Alison Walker, MD  
The Ohio State University

### First-Time Participants

**Rhe Battle**  
Meharry Medical School  
**Research Project:** Role of the Elf2 transcription factor in erythroid gene expression and terminal differentiation  
**Research Mentor:** Stephen Brandt, MD  
Vanderbilt University

**Hewan Belete**  
University of Minnesota  
**Research Project:** The role of omega-3 fatty acids in hematopoietic cell transplant patients with sirolimus-induced hyperlipidemia  
**Research Mentor:** Linda Burns, MD  
University of Minnesota

**Guensley Delva**  
UMDNJ-Robert Wood Johnson Medical School  
**Research Project:** Targeting MLL-AF9 fusion protein with PARP inhibitors  
**Research Mentor:**Jonathan S. Harrison, MD  
UMDNJ-Robert Wood Johnson Medical School

**Laurette Femnou**  
Johns Hopkins University  
**Research Project:** Evaluation of the effect of sodium salicylate on inflammation and iron homeostasis in aged mice  
**Research Mentor:** Cindy Roy, PhD  
Johns Hopkins University

**William Courtland Lewis**  
The Ohio State University College of Medicine  
**Research Project:** Analysis of Mll-PTD function in the absence of Mll-WT in adult murine bone marrow  
**Research Mentor:** Michael Caligiuri, MD  
The Ohio State University

**Ileanyi Nzewgu**  
University of Cincinnati College of Medicine  
**Research Project:** The role of cytokines and toll-like receptor 4 in acute chest syndrome  
**Research Mentor:** Solomon Ofori-Asafoe, PhD  
Emory University School of Medicine

**Shannalee Rene Martinez**  
Loma Linda University  
**Research Project:** Evaluation of juvenile myelomonocytic leukemia (JMML) cell sensitivity to natural killer (NK) cell-mediated lysis in the presence and absence of epigenetic sensitization  
**Research Mentor:** Dean Lee, MD  
University of Texas MD Anderson Cancer Center

**Elisa Quiroz**  
Ponce School of Medicine  
**Research Project:** Characterization of the expression patterns of NP-9 and Rec in HERV-K (HML2)-expressing cells  
**Research Mentor:** Scott Getlin, MD  
University of Michigan

**Jeneba Abass-Sherief**  
UMDNJ-Robert Wood Johnson Medical School  
**Research Project:** Targeting self-renewal pathways regulating mantle cell lymphoma-initiating cells  
**Research Mentor:** Roger Strair, MD, PhD  
UMDNJ-Robert Wood Johnson Medical School

**Lynda Villagomez**  
Albert Einstein College of Medicine of Yeshiva University  
**Research Project:** Evaluating a novel class of drug targeting PRMT5 enzyme dysregulation in mantle cell lymphoma  
**Research Mentor:** Robert Bao, MD, PhD  
The Ohio State University

**Tatiana Melissa Villatoro**  
San Juan Bautista School of Medicine  
**Research Project:** Progression of vasculopathy in children with sickle cell disease and abnormal transcranial doppler ultrasonography: rates and predictors  
**Research Mentor:** Janet Kwiatkowski, MD  
Children’s Hospital of Philadelphia
The ASH website offers a convenient way for members to find information about upcoming Society events and provides easy access to many valuable products and services.

ASH Quick Reference Guides
Mobile App – Now Featuring Guide for Immune Thrombocytopenia (ITP)

ASH Guides is a new mobile app that will house all of the Society's Clinical Quick Reference Guides. The initial release of the app included the 2009 Clinical Practice Guideline on the Evaluation and Management of Heparin-Induced Thrombocytopenia (HIT), and in May, ASH’s Clinical Quick Reference Guide for Immune Thrombocytopenia (ITP) was added. Throughout 2012, ASH will introduce additional mobile versions of the Society’s entire Clinical Quick Reference Guide collection, including von Willebrand disease, epoetin and darbepoetin, and its newest Quick Reference Guide on Anticoagulant Dosing and Management of Anticoagulant-Associated Bleeding Complications in Adults. The app is currently available for iOS, Android, and Blackberry devices. To install the app, simply search for "ASH Guides" in your device's app store. For more information about the guides and app, go to www.hematology.org/Practice/Guidelines/2934.aspx.

WHAT'S ON THE WEB

ASH Guide Conferences

July
18 Early-bird registration and housing opens to ASH members
Atlanta, GA www.hematology.org

August
1 Application deadline for active and international membership
Washington, DC www.hematology.org
8 Annual meeting advance registration and housing open to members and non-members
Atlanta, GA www.hematology.org
14 ASH annual meeting abstract submission deadline
Atlanta, GA www.hematology.org
23-26 Annual Scientific Meeting of the Society of Hematology and Stem Cells
Amsterdam, Netherlands www.iseh.org

September
12-13 ASH Advocacy Leadership Institute
Washington, DC www.hematology.org
28-29 ASH State-of-the-Art Symposium in Chicago
Chicago, IL www.hematology.org

October
12-13 2012 ASH State-of-the-Art Symposium in Los Angeles
Los Angeles, CA www.hematology.org
22 ASH annual meeting late-breaking abstracts submission site opens
Atlanta, GA www.hematology.org
25-27 Lymphoma & Myeloma 2012: An International Congress on Hematologic Malignancies
New York, NY www.lymphomaandmyeloma.com/2012
29 ASH annual meeting late-breaking submission deadline
Atlanta, GA www.hematology.org
31- Nov. 3 2012 American Society for Clinical Pathology Annual Meeting
Boston, MA www.ascp.org

MARK YOUR CALENDAR

Read The Hematologist online at www.hematology.org/hematologist, and catch up on the latest news in the field of hematology right at your desktop.