Genes to Tailor Warfarin Dosage


How much would you pay to know your patient’s response to warfarin before you even gave the first dose? This question may be more than academic as accumulating data indicate that gene variants of enzymes mediating the pharmacokinetics and pharmacodynamics of warfarin are predictive of an individual patient’s response to warfarin.

Studies have focused primarily on two genes. VKORC1 encodes vitamin K epoxide reductase, which recycles vitamin K epoxide to the reduced form of vitamin K and is the major target of warfarin. Individuals with different VKORC1 haplotypes require different doses of warfarin for therapeutic anticoagulation. CYP2C9 encodes cytochrome P-450 2C9, the primary enzyme required for metabolic clearance of warfarin. Polymorphisms of CYP2C9 also contribute to variability in sensitivity to warfarin. In a recent prospective study, Schwarz and colleagues evaluated the association of variants of VKORC1 and CYP2C9 with changes in INR following initiation of warfarin therapy.

Bleeding risk associated with warfarin therapy is relatively greater in the first month of therapy. Among the primary outcomes evaluated by Schwarz, et al. was the association of gene variants of VKORC1 and CYP2C9 with the first INR within therapeutic range and time to first INR >4. The rate of achieving the first INR in therapeutic range was higher by nearly 2.4-fold in patients with the A/A haplotype of VKORC1 compared with non-A haplotypes. The rate to first INR >4 was increased by >2.5-fold in patients with the A/A haplotype. Rates for achieving these endpoints were even increased in patients with a single A allele. CYP2C9 polymorphisms had less influence on initial response to warfarin. No effect on time to first therapeutic INR was detected. CYP2C9*2 and CYP2C9*3 variants were associated with somewhat shorter times to first INR >4 than the CYP2C9*1 variant. Thus, genetic variability in VKORC1 is more closely associated with initial sensitivity to warfarin than genetic variability in CYP2C9.

Warfarin remains the most widely used oral anticoagulant for patients with venous thromboembolic disease. However, its narrow therapeutic window is a substantial liability because supratherapeutic anticoagulation can result in bleeding, subtherapeutic anticoagulation is a risk for thrombosis, and there is a wide variation in individual dose requirements. The ability to predict an individual patient’s response to warfarin therapy a priori could reduce the time to achieve a therapeutic INR, increase time within the therapeutic range, and potentially reduce bleeding and recurrent thrombotic episodes. This study demonstrates that genetic variants of VKORC1 constitute a significant determinant of initial response to warfarin therapy. Screening for VKORC1 haplotypes may improve warfarin dosing algorithms.

The FDA has recently updated the label of warfarin to encourage pharmacogenetic testing to help guide dosing of individuals initiating warfarin therapy. As information on the role of genetic profiling for warfarin therapy becomes available, the impact of such screening to improve efficacy and safety will be subject to increasing scrutiny. Is the screening cost-effective? Should all patients be screened? Should we test only subpopulations at substantial risk for bleeding or clothing? Can test results be obtained rapidly enough to influence initial dosing? Genetic screening to improve warfarin therapy may or may not find a role in the routine management of patients with thrombotic disease. However, it has already proven to be an important test case in the nascent field of pharmacogenetics.
Heparin has been in the spotlight recently. The U.S. Food and Drug Administration (FDA) began receiving reports of allergic reactions to heparin in January 2008. The extent and significance of the problem have become increasingly clear over the subsequent months, and it has been the topic of much news and debate.

The FDA has received reports of 131 deaths among individuals exposed to heparin between January 2007 and April 13, 2008. Of these, 81 deaths were thought to be linked to heparin use, given the reported clinical syndromes of allergic reactions/hypotension. Hundreds of cases of non-fatal allergic reactions have also been reported in the United States and in 10 other countries worldwide. Following the initial reports of adverse outcomes, Baxter Healthcare Corporation, the supplier of the majority of heparin in the United States, recalled all heparin formulations in February. Covidien and B. Braun, other companies that supply heparin in the United States, subsequently issued heparin recalls in March.

More recently, Medtronic (makers of heparin-coated medical devices) and Atrium Medical Corporation (makers of heparin-coated drainage catheters) have also issued product recalls.

Heparin is prepared from porcine intestinal mucous membranes. The active ingredient of heparin manufactured by many companies is produced in China, where the initial steps of procuring the active ingredient occurs at unregulated “workshops,” often run as family operations before being sold to larger manufacturing plants via “consolidators.” Contamination of heparin with over-sulfated chondroitin sulfate (OSCS) at some early point in this manufacturing “pipeline” has been identified as the cause of the allergic phenomena. While chondroitin sulfate is also a glycosaminoglycan, it does not occur naturally in the over-sulfated form that is structurally similar to heparin.

A group of researchers led by Dr. Ram Sassekharan at the Massachusetts Institute of Technology investigated the link between OSCS and the reported allergic reactions. Heparin lots were procured from the FDA (13 associated with adverse events, 16 without) and analyzed for the presence of OSCS in a blinded fashion. All 13 heparin lots associated with adverse events showed evidence of contamination with OSCS ranging from 2 percent to 27 percent of the total glycosaminoglycan content. The contaminated heparin samples also demonstrated the ability to activate the kinin contact system (kallikrein activation) while natural chondroitin sulfate A did not. Additionally, contaminated heparin induced the production of complement C5a, which was dependent on activation of the contact system via factor XII activation. These findings were confirmed in a pig model by infusion of both contaminated and control heparin, as well as synthetic OSCS. While the animal studies showed induction of kallikrein with contaminated heparins, it did not always result in clinical manifestations, suggesting a possible role for presence of additional risk factors and variations in regulatory mechanisms. These findings may also explain why certain subsets of patients, such as those receiving hemodialysis, may be more susceptible to adverse outcomes. The results of these studies were recently published in the New England Journal of Medicine, and the authors should be commended for their contributions.

THE HEPARIN WOES: CONTAMINATION, CONTACT ACTIVATION, REGULATION, AND LEGISLATION

RAJ S. KASTHURI, MD, MBBS, AND NIGEL S. KEY, MBChB, FRCP

Dr. Kasthuri is Assistant Professor of Medicine at the University of North Carolina School of Medicine.

Dr. Key is Harold R. Roberts Distinguished Professor and Director of the Hemophilia and Thrombosis Center at the University of North Carolina School of Medicine.
Election Ballots to Mail in August

Active members in good standing will receive election materials by mail in late August for this year’s ASH leadership election for Vice President, Secretary, and three Councillors. Ballots are due by September 30, and the results of the election will be announced in the November/December issue of The Hematologist.

Trainee Research Award Recipients Announced

ASH is proud to announce the 2008 recipients of its Trainee Research Awards. Forty medical students, undergraduates, and residents will each receive $4,000 to conduct research on blood and blood-related diseases through this program, which is designed to encourage the pursuit of research and spark an interest in hematology. To see the list of names, go to www.hematology.org/media/05062008.cfm.

ASH Member Elected to the National Academy of Sciences

Ronald Levy, MD
Professor of Medicine, Robert K. Summy and Helen K. Summy Professor, and Chief of the Division of Oncology at Stanford University School of Medicine

ASH Members Named Fellows of the American Academy of Arts and Sciences

Jerome Groopman, MD
Professor of Medicine at Harvard Medical School and Chief of Experimental Medicine at Beth Israel Deaconess Medical Center

Judy Lieberman, PhD, MD
Senior Investigator at the Immune Disease Institute, Professor of Pediatrics, and Director, Division of AIDS, at Harvard Medical School

Samuel I. Rapaport, MD
Eminent Professor of Medicine and Pathology and Former Director of the Hemostasis and Thrombosis Research Laboratory at University of California, San Diego School of Medicine

Leonard I. Zon, MD
Howard Hughes Medical Institute Investigator, and Grousbeck Professor of Pediatric Medicine at Children's Hospital Boston of Harvard Medical School

ASH Members Named Fellows of the American Academy of Arts and Sciences

Attention Trainees: This One’s for You

This year’s Trainee Day will take place on Friday, December 5, 2008, from 7:00 a.m. to 12:00 noon. This half-day workshop is designed to support and encourage trainees in the field of academic hematology and to enhance their career development. The program will be presented through didactic and interactive small-group breakout sessions that will provide attendees with time for discussion, questions, and answers.

Didactic sessions will include:

• Defining the Research Hypothesis/Question
• How to Identify and Pursue Appropriate Funding for Your Research

Small-group breakout sessions will include:

• Developing Collaborative Research Teams
• Identifying and Pursuing Post-Training Career Opportunities

Trainee Day is limited to 200 participants; pre-registration is required and will begin in August. E-mail training@hematology.org for more information.
In March 2008, Blood started accepting electronic copyright transfer agreements (CTAs) from authors of papers published in the journal. By implementing electronic CTAs, Blood has eliminated paper processing and fax transmission of forms and documents. The Blood submission and peer-review process is now entirely electronic.

How the Process Works

The process works as follows:

- The corresponding author is required to provide valid e-mail addresses for all authors upon submission. Authors are notified of submission.
- Each author is required to double-click [i.e., confirm] their acceptance of the transfer. An "I agree" response is equivalent to signature and copyright transfer to ASH.
- Staff can track the progress or upload a PDF of a hard copy of the form. Otherwise, the staff is not able to fill out or alter the form on behalf of an author.

The Advantages

Although this new process may slightly extend the time from acceptance to prepublication in First Edition for authors who are nonresponsive, electronic CTA submission offers these advantages:

- Preempts possible authorship disputes by informing all authors via an automatic e-mail message at submission and at acceptance that a submission to Blood with their name has been made. It notifies all authors of the authorship order.
- Eliminates sending the CTA and COI form via fax and the need to maintain the paper records in perpetuity.
- Keeps all records in electronic format. Paper forms can be scanned and added to the system.
- Potentially speeds up the process of receiving the signed forms.

For more information, see the Blood Author Guide at http://bloodjournal.hematologylibrary.org/authors/authorguide.dtl.

THE HEPARIN WOES

It appears that the contamination of heparin was intentional, revealed many irregularities in the manufacturing "pipeline." It seems that the contamination of heparin was intentional, as OSCS does not occur naturally. The lack of regulation of overseas manufacturing plants, as well as the FDA itself, has come under significant criticism. It is apparent that current budget restrictions significantly limit the capabilities of this organization to inspect foreign manufacturing plants (>500) every two years — the norm within the United States. This issue has received considerable attention within the House and the Senate in the last few months, and whether this will result in implementation of useful steps to prevent such tragedies is uncertain from occurring again remains to be seen.


Dr. Key is a member of an advisory committee at Baxter Healthcare.

THE RESPONSE

I agree with this approach in this patient in the context of mild hemophilia A (factor VIII level of 21 percent of normal); however, my recommendations are empirical and are derived from my own experience, since these clinical situations are too unusual to develop evidence-based guidelines. In addition, the approach to mild hemophilia A is going to be somewhat less challenging than what would be required with severe hemophilia A. In any case, a factor VIII level of 21 percent should be adequate to sustain the anti-platelet aggregation effects of ASA (81 mg daily).

The fundamental question here is whether aspirin is an effective single agent to prevent the cardioembolic complications of atrial fibrillation. Recent large clinical trials and meta-analyses provide some insights. Warfarin appears to be superior to anti-platelet agents in preventing embolic stroke in patients over the age of 75 and in those with hypertension, prosthetic heart valves, mitral stenosis, or congestive heart failure. Others with atrial fibrillation seem to do equally well with aspirin alone. The 2007 Cochrane Report on this topic analyzed data from eight trials including more than 9,500 non-coagulopathic individuals (mean age = 69 years) with non-valvular atrial fibrillation. There were mixed statistical messages delivered in this publication in that while warfarin reduced the risk of all strokes by 32 percent compared with aspirin (P=0.0007) and decreased the incidence of disabling or fatal stroke by 31 percent (P=0.06), the mortality rate in both groups was similar. Of note, aspirin increased the risk of intracranial hemorrhage by 90 percent compared with warfarin. This latter risk issue has particular relevance for an individual with hemophilia, since it is very likely that any intracranial bleed (warfarin- or aspirin-induced) would result in exaggerated morbidity and mortality.

For the patient at hand with mild hemophilia A, I would place him on aspirin (81 mg daily) and avoid warfarin until or unless an embolic event occurs or unless it is determined that there is a mitigating circumstance contributing to the onset of atrial fibrillation (e.g., valvular disease, cardiac disease, etc.). I would not expect any significant aspirin-induced hemorrhagic events to occur at this level of factor VIII activity. If the patient has severe or moderate-severity hemophilia A (FVIII <5 percent), I would recommend the same aspirin dose, initiate a secondary prophylaxis regimen of FVIII replacement therapy with a recombinant factor VIII concentrate (25-30 IU/kg three times weekly), maintain the trough FVIII level greater than 5 to 10 percent, and subsequently titrate the dose of FVIII concentrate to achieve a FVIII activity adequate to minimize the development of spontaneous bleeding complications. If warfarin is needed in the context of hemophilia, I would aim for a target INR of 2 to 2.5 and administer once or twice daily FVIII concentrate to maintain FVIII levels of >50 percent, titrated upward based on the propensity of the patient to experience bleeding complications.

As for cardioversion, I would replace this patient’s factor VIII activity level to at least 50 percent of normal with recombinant factor VIII concentrate prior to the procedure and then proceed with cardioversion without concurrent anticoagulation. Lastly, I think that this patient should be screened for the von Willebrand disease (VWD) variant 2 Normandy, since a number of patients with mild cases of hemophilia have been shown to be phenotypically similar to and genotypically confirmed to actually have this VWD variant.

any hematologic malignancies remain lethal despite intense research that has uncovered many of the underlying molecular lesions. Below we address the role of mouse models of cancer in developing and testing new therapies for treating these diseases.

Goals of mouse models

Model systems aim to provide robust platforms for investigating the basic genetic and biochemical components of malignant behavior. Furthermore, mice potentially can be used in preclinical evaluation of novel therapies.1,2 A common theme is the ability to perform controlled experiments that are difficult or impossible in humans. Unlike patients, mice can be designed to have both a defined genotype and congenic siblings that serve as controls.

Design strategies

Based on the extensive molecular understanding of human leukemias, many of these diseases have now been modeled in mice. Several distinct methods can be used to introduce oncogenic mutations into the murine hematopoietic system. In conventional transgenic models, an oncogene is integrated at a random site in the genome. While these systems have proven quite valuable, they suffer from poor control over oncogene copy number and expression pattern due to integration effects. Conditional gene targeting addresses these concerns by modifying the endogenous locus of a proto-oncogene or tumor suppressor gene and allows a mutation to be induced at a specific time and/or lineage. To date, this approach provides the most accurate genetic model of oncogenic mutations. Finally, retroviral transduction is a rapid method for generating series of genetically related leukemias. In some cases, proviral insertion can be deliberately exploited to generate leukemias by insertion mutagenesis.3

Useful aspects of hematopoietic cancer models

Genetic diversity may be regulated: Acute leukemia in humans or mice involves multiple cooperating mutations, but most mouse models are designed with only one mutation in the germline. Therefore, additional genetic events are taken during leukemic transformation. These secondary events occur at random, leading to some genetic diversity among the tumors. Retroviral transduction is a rapid method for generating series of genetically related leukemias. In some cases, proviral insertion can be deliberately exploited to generate leukemias by insertional mutagenesis.4

Clear disease endpoints: Besides survival, intermediate endpoints of clinical appearance, peripheral blood counts, and/or lymphadenopathy are robust measures of efficacy in preclinical therapeutic studies. Noninvasive imaging systems can also quantify tumor burden over time if cells are marked with appropriate reporter genes. These measures collectively establish a basis for comparing disease progression in treatment studies. The clarity of these endpoints lends considerable power to statistical analysis, allowing small trials to yield meaningful results.

Well-established cell biology assays: Decades of research into basic mechanisms of hematopoiesis have revealed extensive similarities between human and murine hematopoiesis and have yielded a large set of techniques to assess cell biology in normal and diseased states. As a result, the effects of an oncogenic mutation on cell fate can be determined with some clarity. For example, traditional colony-formation assays readily demonstrate the enhanced self-renewal imparted by some oncogenic transcription factors and the hypersensitivity of myeloid progenitors to GM-CSF in models of human myeloproliferative disease.5 In one example of applying a classic cell biology assay to leukemia therapeutics, the therapeutic index of imatinib mesylate was predicted by its differential effect on myeloid progenitor colonies grown from CML but not normal bone marrow.6

Primary tumor cells are accessible for analysis: Once established, murine malignancies can be harvested and subjected to biochemical or genomic analysis. The high proportion of malignant cells in target organs results in nearly pure populations for study, although subtraction fractionation can be performed if desired. Tumors may be quiescent for secondary genetic mutations to accumulate and, importantly, the inhibition of the molecular target of therapeutic agents in the target cell population.7,8

Transplantability: Hematopoietic malignancies are almost always transplantable into naive hosts. The reproducibility and growth of tumors facilitates performing controlled, replicated experiments to examine responses to therapeutic intervention in vivo. The system is easily scaled up, making complex comparisons of multiple strains and treatment regimens feasible.

Genetic manipulation of primary tumor cells: Retroviral transduction of primary leukemia cells can produce a series of tumors with related genetic alterations. By varying both transduced genes and the genetic background of transduced cells, genetic contributions to disease and response to therapy can be analyzed efficiently. The ability to simultaneously produce large numbers of recipients with defined mutations in the hematopoietic compartment allows such hypotheses to be tested more quickly at a fraction of the cost and complexity involved in mating mouse strains.9,10 Recent advances in RNA interference technology have extended this approach to inhibiting target gene expression.

Tumor transplantaibility: Besides survival, intermediate endpoints of clinical appearance, peripheral blood counts, and/or lymphadenopathy are robust measures of efficacy in preclinical therapeutic studies. Noninvasive imaging systems can also quantify tumor burden over time if cells are marked with appropriate reporter genes. These measures collectively establish a basis for comparing disease progression in treatment studies. The clarity of these endpoints lends considerable power to statistical analysis, allowing small trials to yield meaningful results.

The ease of quickly generating large cohorts of disease-animal models, combined with the statistical power of small but uniform samples, is especially valuable when evaluating a large number of potential therapies. Therefore, mice may prove particularly helpful when novel agents are tested in combination.

Potential weaknesses

Inaccuracies: There are three fundamental ways in which mice can model human disease poorly. First, the engineered genetic lesions can only approximate those found in patient samples. Some conditional models come very close, but most are imperfect in some way. A second type of inaccuracy results from the inability to ensure that a single cell of the proper type undergoes the initiating oncogenic mutation. In most systems, the mutation occurs in a large “field” of genetically identical cells, failing to model the important process of clonal selection. Finally, whereas many mouse cancer models rely upon engineering a specific mutation into the germline, it is not always certain that these mutations represent bona fide initiating events in human hematologic cancers. Discordant results between mouse preclinical studies and human trials may reflect the fact that a specific mutation that initiates leukemogenesis in the mouse may not play the same role in human disease.

Pharmacology: A second concern relates to the mouse host, regardless of how accurately the tumor is modeled. Potential differences in pharmacology or toxicology can affect the interpretation of therapy trials, which depend on finding anti-tumor effect at a nontoxic dose. A notorious example involves investigations of camptothecins, which have vastly different pharmacology in mice and humans.11

Alternatives: Some translational researchers prefer xenograft models, in which human tumors are engrafted into mice. These have one potential advantage—they use actual human cancer cells. However, these systems lack the power of genetic systems applicable to mice and suffer from the same potential pharmacologic problems. Lastly, immortalized cell lines have revealed important facets of biochemistry and molecular biology, but are less useful for discovering more subtle and complex effects that are restricted to primary cells. Studies of drug sensitivity in cell lines are relatively fast, cheap, and easy, but experience has shown that they do not necessarily provide lasting value.

Will they work?

Will the increasing sophistication of mouse cancer models yield systems with enough predictive value and throughput to identify effective treatments for human diseases? Experience with models of acute promyelocytic leukemia (APL) is particularly encouraging. First, leukemias in PML-RARA transgenic mice were found to remain sensitive to retinoic acid (ATRA) and arsenic trioxide (As2O3) with differentiation and apoptosis, as had already been seen in pioneering human clinical trials.12,13 In this sense, the patients correctly predicted outcomes in the mice. Later, the model systems predicted a synergistic interaction between ATRA and As2O3, an effect only recently validated in the clinic.14 New predictions that CAMP agonists also cooperate with ATRA will soon be tested in patients.15 Perhaps the mouse models will soon replace traditional Chinese medicine as the inspiration for novel therapeutic approaches in APL and other leukemias.
Biomedical research and health-care delivery has been transformed over the past 36 years. The costs of health-care delivery have increased exponentially. Health-care disparities remain, and in some areas have increased. Most people born with SCD now live well into middle age and older, with substantial burden of disease experienced by them and their families, who often have serious problems accessing quality health care. The findings of a recent NIH Consensus Development Conference on Hydroxyurea Treatment for Sickle Cell Disease underscores the problems of ensuring that all who may benefit have access to research advances.

In this setting, NHLBI felt that it was appropriate to analyze its portfolio in SCD to assess the effectiveness of its investment in achieving its goals and identify areas in which adjustments should be made to funding mechanisms. We examined the history of investments and advances, solicited comments and suggestions from multiple constituencies, including ASH, and asked a subcommittee of our Advisory Council to provide an objective summary with recommendations. The report of the committee was adopted by the full Advisory Council at its February 13, 2008, meeting.

In response to the findings and the wisdom of the community, we have implemented changes in our approach to SCD research. We have reconfigured the Centers to form a Basic and Translational Research Program in SCD, which will undergo further modifications as the full research agenda evolves. We expect to address an aggressive basic science agenda, designed to bring investigators in disciplines who have not previously been involved in SCD research into the field, to ensure that scientific approaches not yet brought to bear can enhance the research agenda, and to create the “Manhattan project” for SCD research recommended by the ASH workshop. We plan to create new mechanisms for translational clinical research, which will require that the investigator community rigorously establish priorities, and we will invite all capable investigators and patients who wish to participate in research to do so. We will enhance our support of training and career development, including broadening the specialties involved in such activities. We will collaborate with the Clinical and Translational Science Award (CTSA) Consortium to both conduct clinical research and support training of investigators. We will involve multiple constituencies in the development of practice guidelines and educational materials for professionals, advocacy groups, patients, and families.

We are now actively engaged in discussions across HHS to support the missions of our sister agencies, including surveillance and assessment of public health needs by Centers for Disease Control and Prevention and Health Resources and Services Administration’s goal of ensuring access to care for the medically vulnerable and those affected by health disparities. The NIH missions are research and education. The Institute is not positioned to implement changes in medical practice, and is thus heavily dependent upon partnerships with payors and providers to address these issues. We will both repurpose existing funds and partner extensively to achieve our ultimate goals, which are to realize the promise of intervention in the prototypical molecular disease and to prolong and enhance the quality of life for patients with SCD. ASH members and leadership are an integral part of implementation; we all have a lot of work to do!

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President Bush Signs Genetic Nondiscrimination Legislation into Law

On May 22, President Bush signed into law the Genetic Information Nondiscrimination Act (GINA), which prohibits discrimination based on the results of genetic tests. Under the bill, employers cannot make decisions about whether to hire potential employees or fire or promote employees based on the results of genetic tests. In addition, health insurers cannot deny coverage or charge higher premiums to members because of genetic test results. The House voted 414-1 to approve the bill, while the Senate approved the legislation 95-0. ASH has supported this legislation for several years and thanks all members who joined the Society’s advocacy campaigns.

ASH Committee on Practice Meets with Congress to Urge Support of Physician Payment Fix

The ASH Committee on Practice met with nearly 30 Congressional offices during its spring meeting to urge Congress to prevent the scheduled 10.6 percent cut in Medicare physician payments scheduled to go into effect July 1, 2008, and replace the cut with a positive update for 18 months. In addition, the ASH members asked for support of a permanent replacement of the formula currently used to calculate physician payment rates. As this issue of The Hematologist was going to press, Congress had not yet taken action on this important legislation. To receive the most up-to-date information about this issue, please visit the ASH Web site at www.hematology.org.

NIDDK Announces September Workshop on Erythropoietin Expression and Function in Non-Hematopoietic Tissues

The Hematology Program at NIDDK is planning a two-day workshop on erythropoietin receptor (EpoR) expression and function in non-hematopoietic tissues. This workshop will take place on September 8-9, 2008, at the Doubletree Bethesda Hotel Meeting Center, Bethesda, MD, and will address evolving insights into the distribution and function of EpoR in non-hematopoietic tissues. The workshop will summarize clinical observations of non-hematopoietic erythropoietin (Epo) effects in patients with renal failure and solid tumors, and it will review experimental and clinical findings of Epo effects on the development, growth, and function of vascular endothelial cells, neuroblasts, and cardiovascular and neural tissues. This workshop is designed to promote interactions and discussion among workshop participants and to define key unanswered questions and highlight priorities and directions for future research. The program will include presentations by both invited speakers and speakers selected from submitted abstracts, together with poster presentations.

A limited number of travel grants will be made available to registrants whose abstracts are selected for oral presentations at the workshop. Registrants who submit abstracts for presentation at the workshop will be given preference if the number of registrants exceeds available space. Registration information is available at www3.niddk.nih.gov/fund/other/ sponseuro2008.htm. Individuals who wish to attend this workshop are also invited to contact Amy Amerson of The Scientific Consulting Group at 301-670-4990, or e-mail her at aamerson@sccorp.com.

CDC Appoints New Director for the Division of Blood Disorders

Dr. Hani Atrash, an obstetrician/gynecologist with training in epidemiology and preventive medicine, has been appointed Director of the Division of Blood Disorders (DBD) in the National Center on Birth Defects and Developmental Disabilities (NCBDDD). Dr. Atrash has worked at the Centers for Disease Control & Prevention (CDC) since 1979. In 2001, he joined NCBDDD as an Associate Director for Program Development, where he managed activities related to global health, workforce development, women’s health, relationships with national organizations and state and local health departments, and minority health. Dr. Atrash is also an accomplished scientist and prolific writer. He serves on a variety of key national advisory committees. Dr. Atrash has received numerous honors and awards within CDC and at the national level for his work in improving the health of mothers and children. He replaces Dr. Rosni Kulkarni, who is returning to Michigan State University.
ASH: A GLOBAL SOCIETY

As the world’s premier professional association representing hematologists, ASH has grown substantially in its international influence and impact around the globe. International membership continues to expand, international professional attendance at the ASH annual meeting hovers around 50 percent, and ASH continually seeks means of increasing the scope and breadth of programs targeted toward international hematologists.

Hematologists outside of North America may be elected to International membership upon the recommendation of the Executive Committee. These members represent roughly a quarter of the Society’s total membership and have the same privileges as Active members, except the rights to vote and hold office. To begin membership in January 2009, applications must be received by August 1, 2008.

Benefits of International membership include:

- A subscription to Blood: Journal of the American Society of Hematology
- All annual meeting mailings in advance
- Reduced registration rates and priority hotel reservations at the annual meeting
- Eligibility to sponsor abstracts
- Access to Consult a Colleague, an online service to submit clinical questions to respected colleagues in specific areas of hematology
- Society publications and the membership directory

Below are some misconceptions about international membership and the opportunities available to international members.

**MYTH:** I am not eligible to serve on an ASH committee.

**FACT:** International members may become members of ASH standing and scientific committees. Currently, international members serve on four standing and 12 scientific committees.

They are represented in the leadership of the Society by the International Members Committee, which advises the Executive Committee about issues relevant to international hematologists and makes recommendations to the Nominating Committee about international representation in the Society’s leadership and potential committee members. IMC membership is restricted to members from outside of North America. As a volunteer-run association, self-nomination for committee service is encouraged and welcomed as a good indication of interest in leading the Society.

**MYTH:** As an international member, I cannot submit an abstract to the annual meeting.

**FACT:** Abstracts are accepted from every corner of the globe, although abstracts submitted by non-members must be sponsored by an ASH member.

**MYTH:** I cannot participate in Blood’s publication process.

**FACT:** International hematologists may participate in every stage of the publication process for Blood, including reviewing manuscripts, serving on the editorial board, and authoring articles; in fact, more than half of submitted papers come from outside North America. Read the article, “International Blood,” written by Blood Editor-in-Chief Cynthia Dunbar.

**FACT:** International hematologists are eligible to receive several career-achievement awards, including the Ham-Wasserman Lecture, which is traditionally awarded to an individual from outside of the United States; the William Dameshek Prize; the Henry M. Stratton Medal; the E. Donnall Thomas Lecture and Prize; the Mentor Award; and the Wallace H. Coulter Award for Lifetime Achievement in Hematology. International hematologists are also eligible for career development awards; in particular, European hematologists are eligible for the EHA-ASH International Fellowship Award. The ASH Web site contains descriptions of each of these awards, including eligibility criteria and application/nomination deadlines.

**MYTH:** ASH never recognizes International members for career-achievement awards.

**FACT:** ASH recently partnered with Health Volunteers Overseas (HVO) to recruit ASH members to teach basic hematology in the most impoverished areas of the world. Recruitment has begun for sites in Kampala, Uganda, and Siem Reap, Cambodia; more sites in Asia and Latin America are anticipated.

Further information on ASH’s outreach programs in developing countries is available online.

**INTERNATIONAL OUTREACH PROGRAMS**

To meet the unique needs in research, practice, and training of hematologists in developing countries, ASH offers a spectrum of programs.

- The Visiting Trainee Program provides short-term funding to individual hematologists who seek specific clinical laboratory or technological training at a host institution under the mentorship of an ASH member. During this period, the participants will gain new knowledge and skills that can be applied upon returning to their home institutions.
- Through the International Outreach Initiative, hematology-related institutions can receive ASH educational materials free of charge, including a subscription to Blood, annual meeting materials, and materials from small meetings.
- The International Consortium on Acute Promyelocytic Leukemia (IC-APL) fosters interactions between clinicians and researchers with the goals of improving clinical care and creating the infrastructure for ongoing clinical trials and translational research.

ASH recently partnered with Health Volunteers Overseas (HVO) to recruit ASH members to teach basic hematology in the most impoverished areas of the world. Recruitment has begun for sites in Kampala, Uganda, and Siem Reap, Cambodia; more sites in Asia and Latin America are anticipated.

Further information on ASH’s outreach programs in developing countries is available online.

**IMPORTANT DATES**

August 1, 2008: To begin membership in January 2009, applications must be received by this date. Annual dues for International members are $245.

August 21, 2008: Applications must be submitted by this date to be considered for the annual meeting.

August 21, 2008: Application deadline for trainees who seek need- and merit-based travel awards.
Optimism and Caution on the Use of MSCs in Patients Undergoing HSCT for Hematologic Malignancies

Mesenchymal stem cells (MSCs) are culture-derived, non-hematopoietic, adherent progenitors that are defined by specific immunophenotypic features and their ability to differentiate into adipocytes, chondrocytes, or osteoblasts. In vivo, MSCs can migrate to sites of tissue injury and inflammation where they produce trophic and growth factors that facilitate repair and regeneration. MSCs also support hematopoiesis; they are relatively non-immunogenic and can down-modulate T-cell-mediated alloreactivity. Pilot and phase II studies in allogeneic hematopoietic stem cell transplantation (HSCT) suggest that donor or mismatched, “third party,” narrow-derived MSCs are safe and can enhance engraftment in certain patients or treat corticosteroid-refractory graft-versus-host disease (GVHD). Importantly, MSCs are also recruited to tumor microenvironments, and studies in murine or human xenograft tumor models show that systemic delivery or co-implantation of MSCs can promote malignant cell survival, proliferation, and/or metastasis. Thus, the safety of MSCs in patients undergoing HSCT for malignancies remains a major concern.

The report by LeBlanc, et al. describes a multicenter, non-randomized phase II trial of donor or third-party marrow MSCs for severe, corticosteroid-refractory acute GVHD after myeloablative or non-myeloablative HSCT for a hematologic malignancy (78 percent), solid tumor (4 percent), or non-malignant disease (12 percent). One intravenous infusion of MSCs induced a complete response (CR) in 27 of 55 patients (49 percent), and CR occurred in 30 patients overall (55 percent). Compared with patients without CR, those with CR had significantly lower one-year transplant-related mortality (37 percent vs. 72 percent) and higher two-year survival (53 percent vs. 16 percent). Response was not related to GVHD grade, MSC source, or total MSC dose. No acute or late side effects were reported; relapse occurred in three of 43 patients (7 percent) with hematologic malignancy. In the randomized controlled trial by Ning, et al., HLA-matched donor MSCs were co-transplanted with marrow and/or peripheral blood stem cells on day zero after myeloablative conditioning for hematologic malignancies. Patients were randomized by age, disease type, stage, and prognosis. Only 10 of 15 patients allocated to the treatment arm received MSCs; their engraftment was not enhanced, but only one developed GVHD, compared with eight of 15 non-MSC control patients. The study was closed early because six of the 10 patients who received MSCs relapsed (including five recurrences by day 150), compared with three relapses in the 15 non-MSC patients. The three-year disease-free survival rates for the MSC and non-MSC groups were 30 percent and 66.7 percent, respectively (log rank p=0.035).

These observations add to the growing experience of using culture-expanded MSCs in HSCT. The results of LeBlanc, et al. are highly encouraging. If confirmed in current randomized clinical trials, MSCs could offer the safest and most effective salvage therapy option for corticosteroid-resistant acute GVHD. This enthusiasm must be tempered, however, by the observations of Ning, et al. that remind us that MSCs can promote malignant cell survival and growth. Reassuringly, high relapse rates were not observed in a similar, but non-randomized, study of 48 patients with hematologic malignancies undergoing myeloablative HSCT with MSC co-transplantation on day zero, nor have increased relapse rates been reported after administering MSCs for GVHD. Moreover, the results of Ning, et al. may not be broadly applicable because their study groups were small and the technical limitations that prevented optimal donor MSC expansion could have introduced confounding variables. Additional clinical and pathobiological studies are needed to address whether MSCs enhance disease recurrence after HSCT, especially when co-transplanted on day zero, and if this might occur through direct cell-cell interactions, paracrine effects, and/or suppression of graft-versus-tumor alloreactivity.


Figure

Culture-derived MSCs, which are defined by specific immunophenotypic features and their ability to differentiate into adipocytes, chondrocytes, or osteoblasts in vitro, exhibit pleiotropic functions in vivo:

- Migrate to sites of tissue injury and inflammation; facilitate repair and regeneration
- Down-modulate T-cell-mediated alloreactivity
- Suppress clinical graft-versus-host disease
- Support hematopoiesis; enhance engraftment after hematopoietic stem cell transplantation
- Recruited to tumor micro-environments; promote malignant cell survival, proliferation, and metastasis
- Support hematopoiesis; enhance engraftment after hematopoietic stem cell transplantation

Dr. Linenberger indicated no relevant conflicts of interest.
Mutations in tyrosine kinases are a common theme in myeloid leukemia. The hallmark example is the inappropriate activation of Abl through the Bcr-Abl translocation in CML. Mutations in the FLT3 tyrosine kinase are quite common in AML. Recently, point mutations resulting in a valine to valine substitution (at amino acid 617) of the FLT3 kinase (FLT3-V617F) have been found in >90 percent of cases of polycythemia vera (PV), and approximately 50 percent of primary myelofibrosis (PMF) and essential thrombocytosis (ET). How can one mutation be associated with three different diseases?

A recent study by Tiedt, et al. paints a fascinating picture of how the mutant gene level can actually influence the malignant phenotype. The authors used elegant genetic engineering to create three mouse models: one with a balanced expression of the wild-type JAK2 and mutant JAK2-V617F, one with relatively high JAK2-V617F, and one with very high JAK2-V617F. The mice developed a hematologic disease influenced by the relative amount of wild-type to mutant allele. Thus, mice expressing balanced expression of wild-type and mutant Jak2 developed an ET-like disease, with increases predominately in platelet counts, splenomegaly, and fibrosis in the bone marrow. Mice that expressed higher levels of mutant JAK2-V617F showed increasing levels of erythroid expansion, with a phenotype that appeared PV-like. A study of 82 patients with myeloproliferative disease and 11 healthy people showed a similar pattern as the mouse model. Quantitative RT-PCR showed the highest mutant: wild-type ratio in cases with PV, followed by PMF, then ET. Expression of the mutant and wild-type JAK2 correlated with the gene copy numbers found in the samples. Thus, cases with PV tended to have samples where the chromosomal number of mutant JAK2 was greater than wild-type.

A variation of this theme has been found in AML cases with the FLT3 mutation. Approximately 15 percent to 30 percent of AML cases with normal cytogenetics harbor FLT3 mutations characterized by a head-to-tail duplication in gene coding for the juxtamembrane region of the protein. The occurrence of this FLT3 internal tandem duplication (FLT3-ITD) alone has had a variable prognostic import across different studies and treatments. However, several studies have now shown that the allelic ratio (the ratio of mutant FLT3-ITD to wild-type allele) drives prognosis. Cases with predominately mutant FLT3-ITD have a very poor prognosis; cases with predominately wild-type allele tend not to have a poor prognosis.

These findings run counter to the conventional (and, perhaps, wrong) wisdom of leukemia being a single clonal event. If AML really is only derived from a single clone, there could only be three possible allelic ratios in respect to the FLT3 mutation in an AML sample: all wild-type; heterozygous wild-type and mutant; or all mutant. The fact that one can have a variety of allelic ratios in AML cases suggests that there must be multiple clones in most leukemic cases, each clone having a different state of the three conditions outlined above.

Of interest, the allelic data suggest not only the case of a loss of the normal FLT3 [resulting in one mutant gene, no wild-type], but in some cases, a duplication of the mutant gene. How does a patient develop two copies of a mutant gene? Bad luck twice? In some cases of malignancy, wild-type alleles are dropped through chromosomal loss (for example, deletion of an arm of chromosome 17 eliminates a copy of the p53 tumor suppressor gene). This loss of heterozygosity, however, does not appear to be the case in the JAK2-and FLT3 story. Here, rather, the process of chromosomal repair causes a duplication of the mutant allele (see Figure). In this situation, a double-stranded DNA break takes place, and, in order to facilitate repair, a second copy of one of the genes is made. In the process of repair, one of the copies of the genes is lost. If the recombination process selects the wild-type gene to duplicate, then the cell has two copies of the wild-type gene, and order is restored. If, in this process, the wild-type gene is selected, then the cell has two copies of the mutated gene.

Thus, the mere presence of a mutated tyrosine kinase does not tell the whole story in myeloid malignancies. The dosage level of the mutated gene can affect the disease phenotype and the biology of response. Once again in science and medicine, the more we know, the more we need to know.

Reworking Our Textbooks


Who’s your daddy? This seems like a simple question, but in hematopoiesis the answer may not be so simple. We all know that blood is made up of red cells, white cells, and platelets. They all are derived from a hematopoietic stem cell. The red cells and platelets are derived from a common precursor. White blood cells, on the other hand, come in many different flavors with a very specialized function. They also seem to segregate broadly into differentiated myeloid and lymphoid cells through a series of less well-understood intermediates. The bifurcation or restricted commitment of these two lineages is thought to occur early on in differentiation. The myeloid lineages are derived from a common myeloid progenitor (CMP), and the lymphocytes are derived from a common lymphoid progenitor (CLP). This picture came from the description of the CLP, which was able to give rise to T, B, and NK cells but not to myeloid cells. The cartoon is well entrenched in our thinking of hematopoietic development and in every slide set of hematopoiesis.

But, is it correct? Two recent publications have challenged this dogma and provide definitive data that the picture is not so cut-and-dried. Before delving into the data, a very brief review of thymopoiesis is needed. While all the other blood elements mature in the marrow spaces, T-cell development is thought to proceed from a CLP that migrates from the bone marrow to the thymus where a tightly orchestrated set of events results in the release of a naïve T cell that has been positively selected (to recognize the appropriate MHC molecules) and negatively selected (to remove auto-reactive cells). It seems clear that T-cell precursors could give rise to myeloid cells.

However, using clonal analysis with single-cell assays, these two groups of investigators demonstrated that a substantial number of T-cell precursors in the DN1 and DN2 stage (prior to T-cell receptor rearrangement) demonstrate myeloid potential, which is lost at the DN3 stage. These myeloid cells were predominantly macrophages, but granulocytes and dendritic cells were also observed. Transfer of DN1 cells into T-cell-deficient mice demonstrated that up to one-third of the macrophages were derived from the T-cell precursors. Even more surprising was that these myeloid cells demonstrated rearrangement of the T-cell receptor and even expressed RAG recombinase, the enzyme necessary to create T-cell receptor diversity. Taken together, these data demonstrate that the early T-cell precursor is not yet fully committed to becoming only T cells.

What are the ramifications? These early T-cell precursors could explain the origin of some leukemias, which are biphenotypic. But more importantly, it is clear that a precursor’s potential can be different from what actually occurs in vivo. Simple characterization of such precursors may not fully describe their potential. We do not know what the molecular signals are in this case or whether it is similar to the need for PAX5 expression for a B-cell lineage commitment, where a single switch may determine the fate of the cell. However, these data go a long way in getting our lineages right.

Epidermal Sensing of Oxygen Regulates Systemic Hypoxic Response


Amphibians respond to changes in environmental oxygen at least in part through their skin, and frogs can use their permeable skin to derive oxygen directly from the atmosphere. Mammalian skin, however, has generally been thought of as an impermeable barrier, with no direct communication between outside environment and inner respiratory physiology. Mammals are known to sense changes in oxygen pressure by carotid bodies that regulate cardiovascular and respiratory response and by the kidneys and liver that regulate erythropoiesis by erythropoietin production. Boutin and colleagues, however, have created a series of experiments that demonstrate unanticipated regulation of erythropoiesis by novel regulation of renal erythropoietin production via epidermal O2 sensing.

The erythropoietin (EPO) gene is one of many “hypoxia-regulated” genes whose expression is controlled by the master transcription factors, hypoxia-inducible factors-1 and -2 (HIF-1 and HIF-2), each composed of dimers of α and β subunits. Even the HIF-α subunits are regulated by hypoxia, and their expression is controlled post-translationally. Under normoxic conditions, the prolly residues of HIFs are hydroxylated by the enzyme prolyl hydroxylases, which allows the von Hippel-Lindau protein (pVHL) to bind to HIF α, leading to rapid degradation by the ubiquitin-proteosome pathway. During hypoxic conditions, HIF α is stabilized (by not being targeted for proteasome degradation) and forms a transcriptional complex with HIF β that leads to increased expression of multiple target genes involved in diverse processes, including cell proliferation and survival, metabolism, angiogenesis, and erythropoiesis. HIF-1α and HIF-2α exhibit high sequence homology but have different mRNA expression patterns. HIF-1α is expressed ubiquitously, whereas HIF-2α expression is restricted to certain tissues. Both HIF-1α and HIF-2α are regulated by identical mechanisms during hypoxia and form a heterodimer with the same HIF-β subunit. HIF-1 is the principal regulator of EPO gene transcription in the kidney. In other tissues, such as brain and liver (that generates ~20 percent of circulating erythropoietin), EPO gene transcription is HIF-2-dependent.

Boutin and colleagues created a mouse with conditional deletion of Vhl in epidermal keratinocytes, which caused cutaneous vasodilation and increased expression of Hif-1α and Hif-2α. Although keratinocytes do not make erythropoietin, the erythropoietin level was nonetheless found to be increased, and the mouse became polycythemic. Further studies of this epidermal Vhl knockout mouse revealed that the elevated levels of hif-1 caused upregulation of inducible nitric oxide synthase, which in turn led to increased constitutive nitric oxide (NO), a potent vasodilator. This NO-induced skin vasodilation resulted in decreased perfusion of other organs, most notably the liver, with subsequent hypoxia-induced, increased expression of hepatic Hif-2α, which in turn caused increased expression of the Epo gene. In follow-up experiments using mice with wild-type Vhl, the authors deleted the cutaneous genes for either Hif-1α or Hif-2α, but not both. Under conditions of normoxia, the loss of Hif-1α and Hif-2α had no effect on erythropoietin levels. Under hypoxic conditions, however, the Hif-1α epidermal knockout mice did not display an appropriate increase in renal Epo gene transcription and were unable to mount an appropriate renal erythropoietin response. Two experiments showed the importance of epidermal Hif in sensing environmental oxygen levels and regulating systemic hypoxic responses in mice, with physiologic regulation mediated primarily by hif-1α while the pathologic loss of Vhl is mediated primarily by hif-2α.

Although these experiments were carried out using mice, the question of whether these results suggest a broader role for mammalian skin in general is compelling. While mice are not people, the partial inhibition of VHL in humans causes Chuvash polycythemia, a condition with a complex phenotype, the pathophysiologic and molecular basis of which is not yet fully defined, but includes elevated erythropoietin levels and an increased risk of thrombosis that remains unexplained. This human phenotype underlines the essential importance of HIF sensing in controlling multiple physiologic pathways, and future studies looking at whether human skin, in particular, responds directly to decreases in atmospheric oxygen via HIF mediation may provide a basis for the development of new strategies for the treatment of anemia, hypoxia, and oxygen delivery in humans.

Artificial Blood – Not Too Sweet

Soon after William Harvey described the circulation of blood in the early 17th century, Christopher Wren unsuccessfully experimented with replacing wine for blood. During a cholera outbreak in the late 18th century, Gaillard Thomas also failed in his attempts to substitute milk for blood. It was not until 1933 that the first successful blood substitution experiments were performed. William Ruthrauff Amberson of the University of Tennessee Medical School used hemolyzed red blood cells for an exchange transfusion in a cat and demonstrated that he could keep the animal alive for 36 hours. Unfortunately, infusion of a similar product into humans produced oliguria and bradycardia. These toxicities were initially thought to be due to the lipids within erythrocyte membranes. Yet, when Dr. Amberson infused hemoglobin free of red-cell membranes into a hemorrhaging post-partum woman who had depleted her hospital's inventory of cross-matched blood, she developed bradycardia and hypertension, and ultimately died from renal failure. These pioneering studies that spanned three centuries have demonstrated that the ideal blood substitute is an elusive goal.

Today, the blood supply in the United States is safe and usually sufficient to meet the needs of our patients. However, the current system does have two large fundamental problems. First, our reliance on altruistic blood donation creates seasonal shortages. During the summer and winter holidays, there is a significantly lower blood donation rate, and emergency transfusions of human products, and for emergency infusions at the scenes of trauma (civilian and military).

Most modern blood substitutes are either perfluorocarbons or hemoglobin-based oxygen carriers. Perfluorocarbons are non-water soluble, biologically inert, artificial, organic fluids with a high solubility for oxygen. Gas molecules are not chemically bound to perfluorocarbons, but instead are absorbed and released by simple diffusion. A large phase III trial using the perfluorocarbon, Oxygen, was halted early because of an increase in stroke rates in patients who were undergoing cardiopulmonary bypass. Hemoglobin-based oxygen carriers have held more promise. Four different methods have been used to avoid the toxicities induced by free hemoglobin: 1) cross-linking of the alpha chains, 2) polymerization of the hemoglobin chain tetramers, 3) conjugation of the hemoglobin to a larger molecule such as polyethylene glycol, and 4) encapsulating hemoglobin within liposomes. Since 1996, at least a dozen trials have been performed that analyzed the utility of these agents in a variety of clinical settings.

In a systematic review of the available literature on purified hemoglobin-based blood substitutes published since 1980, Natanson, et al. identified 70 trials, focusing only on the 16 randomized controlled trials involving 3,711 patients who received one of five cell-free hemoglobin products. One product, HemAssist, was cross-linked hemoglobin; three products, Hemopure, Hemolink, and PolyHeme, were polymerized hemoglobin; and one product, Hemspan, contained hemoglobin conjugated to polyethylene glycol. Disappointingly, but not surprisingly, the use of any of these products was associated with an almost three-fold increased risk of myocardial infarctions. Overall mortality was only mildly worse (relative risk 1.30) in subjects exposed to the blood substitutes. Further analysis did not indicate that any one hemoglobin product or any one indication for therapy was particularly worse than any of the others. These results demonstrate that the use of the available cell-free hemoglobin products is associated with too much morbidity, and an unacceptable rate of mortality, to be of any clinical benefit.

Selecting Patients Most Likely to Respond to Therapy

Multiple myeloma (MM) is a heterogeneous disease with a broad range of biological and clinical features. More studies have therefore attempted to identify genetic and biologic markers, as well as clinical characteristics to define subgroups of patients. For example, prognostic factors such as beta 2 microglobulin and albumin form the basis of the International Staging System, which is predictive of disease course. Conventional cytogenetics can identify patients with adverse outcome to conventional low- and high-dose therapies (i.e., 4;14 translocation or deletion of chromosome 13). More recent studies have used microarray profiling and array comparative genomic hybridization to form the basis for defining mRNA-based and DNA-based prognostic subgroups of myeloma. Importantly, prognostic factors must be defined in a particular clinical context. For example, in patients treated with novel therapies such as bortezomib, chromosome 13 deletion and 4;14 translocation are no longer of adverse prognostic import. Moreover, recent studies have utilized microarray profiling to define patient populations with gene signatures predictive of response to specific therapies (i.e., bortezomib).

Mateo and colleagues have recently carried out a prospective study of 685 newly diagnosed patients with myeloma treated uniformly with six alternating cycles of vincristine, BCNU, melphalan, cytoxan, and prednisone (VBMC); alternating with vincristine, BCNU, adriamycin, and prednisone (VBAPA) therapy, followed by melphalan 200mg/m² and autologous stem cell transplantation. The median progression-free survival (PFS) and overall survival (OS) were 37 and 67 months respectively. In order to delineate patient subgroups with differential outcome, CD138 positive bone marrow plasma cells were purified and immunophenotyped prospectively as well. Importantly, CD19+CD28+CD117+phenotype on CD138+BM plasma cells predicted poor outcomes significantly different PFS and OS. An immunophenotype-based staging system, defined on the basis of CD28 and CD117 expression on tumor cells, identified poor-, intermediate-, and good-risk patient subgroups with significantly different PFS and OS. In addition to its clinical utility, understanding the biologic significance of the observed antigen-expression profiles conferring this differential outcome may both delineate mechanisms of sensitivity versus resistance to therapy and also yield insights into MM pathogenesis. Moreover, correlation of these phenotype profiles with microarray profiling and mutational analyses may identify, and yield, new insights into MM pathogenesis to be exploited in novel, single agent or combination targeted therapeutics.

Several novel therapies are now available for myeloma, and the challenge is to use them most effectively. These investigators are to be congratulated on their major effort of prospectively providing patient tumor cells for phenotypic analysis and correlation with clinical outcome. Although VBMCP/VBAP is no longer utilized and incorporation of novel therapies such as bortezomib into the initial therapy pretransplant improves response post high-dose melphalan and autologous stem cell transplantation, this study by Mateo and coworkers is an example for future efforts of how correlative science can inform the selection of patients most likely to benefit from novel therapeutics.

Erythrocyte Indigestion: A Surprising Role for the Mitochondrial Pathway of Apoptosis in Reticulocyte Autophagy


Many dramatic changes of terminal mammalian erythroid cell differentiation occur during reticulocyte maturation, including completion of hemoglobin synthesis, degradation of nuclear organelles, conversion from aerobic to anaerobic metabolism, and acquisition of a uniform biconcave discoid shape. Recent publications have shed light on the role of autophagy, an intracellular process by which organelles are degraded, in mitochondrial loss during reticulocyte maturation. Two of the studies have demonstrated that knockout mice deficient in Nix, a BH3 domain-only member of the Bcl2 family, show retarded degradation of mitochondria in reticulocytes. This inability to degrade mitochondria leads to a shortened erythrocyte survival and anemia (i.e., partially compensated hemolytic anemia). Because Bcl2 and its family of proteins are key regulators of the mitochondrial pathway of apoptosis, the role of Nix in reticulocyte mitochondrial degradation suggests that it can mediate either apoptosis or survival, depending upon circumstances of the individual cell.

In nutrient-deprived cells, autophagy may be an alternative to apoptosis in that essential metabolites required for survival of the nutrient deprivation are salvaged by degrading organelles such as the mitochondria and recycling the crucial metabolic products. Similar to nutrient deprivation, maturing reticulocytes reach a crucial stage when they can no longer maintain mitochondrial function. Mitochondria appear to be both degraded and extruded from the maturing reticulocyte by autophagy.1 (See figure.) The failure of mitochondria to undergo autophagy in reticulocytes appears to be detrimental because a similar hemolytic anemia with mitochondria-retaining erythrocytes as found in Nix knockout mice was found in knockout mice with deficiency of Ulk1, the mammalian homologue of atg 1p, a mitochondrial autophagy regulatory protein in yeast.

In the sequence of events in mitochondrial autophagy in reticulocytes, Nix acts at the stage of mitochondrial depolarization and targeting for inclusion in autophagosomes. Nix interacts with the outer mitochondrial membrane, leading to loss of inner membrane polarization.2 Reticulocytes from Nix-deficient mice retain polarized mitochondria, but they are localized to areas adjacent to autophagosomes in reticulocytes. This result suggests that targeting of the mitochondria to the autophagosomes may be intact, but Nix's induction of mitochondrial depolarization is required for normal mitochondrial incorporation into autophagosomes.

Further evidence for this role of Nix in mitochondrial depolarization is that chemical depolarization of mitochondria in reticulocytes from Nix-deficient mice leads to their autophagic clearance. Unlike reticulocytes and erythrocytes from Nix-deficient mice, those from Ulk1-deficient mice have retention of ribosomes and mitochondria. Furthermore, the retained mitochondria in the Ulk1-deficient mice are not localized to areas adjacent to autophagosomes, suggesting that Ulk1-deficient mice have a defect at a different point than do Nix-deficient mice in the reticulocyte autophagy pathway.

Further studies of maturing reticulocytes have the potential to determine the specific range, targeting, and fate of the organelles that are removed by autophagy. This information will not only provide insights into the pathophysiology, but also interest those researchers and physicians interested in other cellular processes that involve autophagy, such as differentiation, aging, and survival following chemical or physical stress.


TARGETING NF-κB IN CLL


The NF-κB family of transcription factors has been implicated in diverse cellular processes, including cell proliferation, differentiation, survival, and inflammatory responses, among numerous others. At least three NF-κB cascades have been characterized: the classical or canonical pathway, which is induced by cytokines such as TNFα; the non-classical, or alternative pathway, which is triggered by BAFF and CD40 ligation; and the atypical pathway, which is engaged by DNA damage. Activation of NF-κB leads to transcription of numerous genes, many of which, for example, XIAP and Bcl-xL, serve survival functions. Not unexpectedly, NF-κB activation occurs in many tumor types, particularly hematologic malignancies. For example, multiple myeloma cells have long been thought to depend heavily upon NF-κB activation for their survival, and several recent studies have documented a high incidence of abnormalities involving genes associated with NF-κB activity in patient-derived, primary myeloma cells. In addition, NF-κB activation is characteristic of AML cells in general, as well as in AML stem cells. A corollary of these observations is that NF-κB represents a logical candidate for therapeutic intervention.

CLL is an accumulative disease of mature, differentiat ed lymphocytes. Although activation of the classical and alternative pathways may not only be an important prognostic determinant in CLL cell survival, the clinical relevance of these observations has not been clearly defined. However, a recent study by Hewamana, et al. may shed significant light on this issue. In this study, the authors examined NF-κB DNA binding, reflected by EMSA assays, in cells from a series of patients with CLL and sought correlations with more established prognostic indicators. They also tested whether the extent of NF-κB activation predicted resistance of cells to conventional and novel agents. While the authors observed considerable inter-sample variability in basal NF-κB activation status, clear correlations were observed between activation and certain known negative prognostic indicators (e.g., high white count, short doubling time), although not between others (e.g., ZAP-70 expression).

Interestingly, cells exhibiting high basal NF-κB activity were less sensitive to the established agent fludara bine, but more sensitive to the novel agent, LC-1, a panphosphoinositol that inhibits IKK and has recently been shown to be active against AML stem cells.

One implication of this study is that, as recently suggested in the case of other hematologic malignancies such as multiple myeloma and AML, the NF-κB pathway may not only be an important prognostic determinant in CLL, but could also represent a logical target for pharmacologic intervention in this disorder. In this context, the results of a recent preclinical study suggested that synergistic interactions between the proteasome inhibitor bortezomib and histone deacetylase inhibitors in primary CLL cells involved, at least in part, NF-κB inactivation.1 The broader implication of the present study is that, as more sophisticated gene and protein profiling classification systems are developed in CLL and other hematologic malignancies, their ultimate benefit may lie in guiding the development of more rational, mechanism-based, targeted forms of therapy.

The Minority Medical Student Award Program (MMSAP) encourages minority medical students to pursue an interest in hematology research. For an eight- to 12-week period, MMSAP participants will work closely with their mentors on a hematology-related research project. The subjects investigated by this year's students include lymphoma, leukemia, sickle cell anemia, thalassemia, and stem cells. The awardees will also have the opportunity to present the results of their research at ASH’s annual meeting in December.

This year’s program has the largest number of participants yet, including three returning students. Each award recipient will receive the support of a research mentor and a career-development mentor, travel stipends to attend medical meetings, and a subscription to The Hematologist and Blood.

ASH Hosts Thrombosis Surveillance Workshop

ROY SILVERSTEIN, MD, AND GARY EDWARD RASKOB, PhD

Dr. Silverstein is Professor of Molecular Medicine and Chairman of the Department of Cell Biology at the Lerner Research Institute. He is also Chair of the ASH Committee on Government Affairs.

Dr. Raskob is Dean of the College of Public Health, Professor of Epidemiology, and Professor of Medicine at The University of Oklahoma Health Sciences Center.

Venous thrombosis affects up to 1 million Americans each year and has been called by some the “silent killer.” Despite the large impact thrombosis has on the population, the United States has not yet developed/implemented a surveillance system for thrombosis. To improve understanding of the scope and scale of the problem and begin to identify key elements in a surveillance system, ASH convened a successful workshop in Washington, DC, on June 12.

ASH worked closely with the Centers for Disease Control and Prevention (CDC) and National Heart, Lung, and Blood Institute (NHLBI) in planning the workshop with the ultimate goal of identifying key questions that need to be answered through a national surveillance system and scientific approaches that can answer them. ASH scheduled the workshop in the first half of the year because interest about thrombosis within the U.S. Department of Health and Human Services is growing. The Surgeon General’s Office is expected to issue a “Call to Action” this year stating that the United States is facing a major public health crisis regarding venous thrombosis, and leadership at the CDC National Center on Birth Defects and Developmental Disabilities (NCBDDD) identified thrombosis as one of its top four priorities in 2008.

We had the privilege of jointly chairing the one-day symposium that assembled a group of 30 participants including representatives from relevant federal agencies, patient groups, and the medical and public health communities. Experts from various subspecialties discussed their “front-line” perspective of dealing with venous thromboembolic disorders (VTE). The subspecialty experts included representatives from the following communities: pediatric and adult hematology, obstetrics and gynecology, geriatrics, emergency medicine and trauma, radiology, and surgery. A hospitalist and intensivist were also present. In addition, experts in epidemiology and health-care policy leaders lent their expertise to the discussion.

A workshop writing committee is developing a summary of the meeting’s deliberations and will produce a report identifying recommendations and next steps. Once finalized, the document will be shared with the ASH membership, relevant federal agencies, patient groups, and the medical and public health communities.

Dr. Raskob consults for and receives honoraria from Bayer, BMS, Daiichi-Sankyo, GSK, Johnson & Johnson/Scios, Pfizer, Sanofi-Aventis, and ThromboGenics.
Do election politics leave you cold? Have political pundit's got you down? Have you grown tired of the political bickering? One possible solution has been offered by Vice President for Public Policy Glenn Mones at the National Hemophilia Foundation (NHF). Mr. Mones is developing something refreshing and relevant. He is creating a patient-focused guide about the upcoming presidential election and its potential impact on health care in general and on individuals with bleeding or clotting disorders in particular. Dr. Craig Kessler, of Georgetown University, and chair of NHF’s Medical and Scientific Advisory Committee (MASAC), asked Mr. Mones to give a short presentation on the subject at their recent meeting in Chicago. Dr. Kessler mentioned that he had received a lot of positive feedback on the presentation; thus, the idea of the patient guide came about. Mr. Mones’ analysis is not intended to be an endorsement of any candidate, but rather a look at where the candidates stand on key health-care issues and how their proposals might affect individuals with bleeding and clotting disorders.

As Mr. Mones describes, “This is a campaign: We are hearing what the candidates are saying but not necessarily whether they will carry through on their promises, nor whether their policies will work. None of the plans are detailed enough to determine how well they would meet the particular needs of the bleeding and clotting disorders community.”

While Mr. Mones’ guide does not attempt to solve your patients’ health-care issues, his effort highlights how one can make this presidential campaign more relevant to the hematology community. Interested readers can obtain the guide by contacting the National Hemophilia Foundation at 800-42-HANDI, or by visiting www.hemophilia.org. The chart to the right displays a few examples from the guide. ASH has also prepared a side-by-side comparison of the presidential candidates’ positions on health care that is available on the ASH Web site at www.hematology.org/policy/news/02142008.cfm.

Dr. Ragni is a member of the medical/scientific advisory committee at the National Hemophilia Foundation.

O B I T U A R Y

Oscar D. Ratnoff, MD
(1916-2008)

Dr. Oscar D. Ratnoff was the consummate triple threat. He was an excellent clinician, basic and clinical researcher, and teacher and mentor. His 302 publications are filled with seminal observations that moved his field forward in the 1950s to the 1980s. His observations populated 29 Journal of Clinical Investigation and 63 Journal of Laboratory Clinical Medicine publications, many with trainees as first authors.

Dr. Ratnoff started his investigative career in liver disease, which introduced him to plasma proteins. At Johns Hopkins with Dr. Calvin Menzie, Dr. Ratnoff described a simple method to measure fibrinogen. In 1950, he moved to Cleveland and Case Western Reserve University (CWRU). In 1955, Dr. Ratnoff and Dr. Jane Colopy reported on a patient, John Hageman, who had reduced surface-activated blood coagulation times without bleeding. He and Dr. Earl Davie later identified, in 1961, the missing protein in the Hageman trait as factor XII and showed that it activated factor XI. This collaboration led directly to publication of the coagulation cascade hypothesis in 1964. They observed that isolated factor XII autoactivates on negatively charged surfaces leading to factor XI activation and then a waterfall of proteolytic reactions. At the time, the model synthesized the biochemical basis of blood coagulation and led to 44 years of refinements.

Dr. Ratnoff had a long-standing collaboration with Dr. Irwin Lepow, the discoverer of factor VII, and together they described the C1 inhibitor of the first components of the complement system. In 1969, Drs. Ratnoff and George Naff showed that the C1 inhibitor inhibits plasma kallikrein and plasmin, and, with Dr. Charles D. Forbes, described in 1970 that the C1 inhibitor inhibits factor Xa and factor Xa. In the 1970s, as part of an evaluation of a patient who had a long APTT without bleeding, but had normal factor XII, Drs. Ratnoff and Hidehiko Saito simultaneously, with the laboratories of Weupper, Colman, and Kaplan, described high-molecular-weight kininogen deficiency in humans.

Dr. Ratnoff’s contributions to medicine were recognized by his election to the American Society for Clinical Investigation, the Association of American Physicians, and the National Academy of Sciences. He served as President of ASH and was selected to present the Henry M. Stratton Lecture in 1972. At CWRU and University Hospitals, he served as Division Chief of Hematology and Oncology and interim Chairman of the Department of Medicine. To his trainees, he was the skillful and talented editor, tireless reviewer, and constant scientific protagonist, but his greatest legacy is his influence on countless physicians, internists, and hematologists as the accomplished physician-scientist.

Dr. Ratnoff is survived by his wife, Marian Foreman Ratnoff, his daughter, Martha Ratnoff, and son, William Davis Ratnoff, MD.

For those who wish to donate, contributions may be made to the Dr. Oscar D. Ratnoff Research and Education Fund at the Case Western Reserve University School of Medicine.

~ Alvin H. Schmaier, MD, and Stanton L. Gerson, MD

Dr. Ratnoff was profiled in the January/February 2008 issue of The Hematologist as part of ASH’s ongoing series for the 50th anniversary. The article can be accessed online at www.hematology.org/publications/hematologist/50th/profiles.cfm.
William Dameshek (1900-1969) was the preeminent American clinical hematologist of his time. A polymath, his interests ranged from diseases of the blood to pre-Columbian statuettes. He was a lead investigator in the first-known multi-institutional trial of chemotherapy (nitrogen mustard for Hodgkin lymphoma). Dr. Dameshek pioneered in the treatment of immune thrombocytopenia with corticosteroids, introduced antimitabolite therapy for autoimmune diseases, developed the concepts of the myeloproliferative and lymphoproliferative disorders, and proposed that CLL is the result of a gradual accumulation of lymphocytes. He was the founder of Blood, an architect of the American Society of Hematology, and an organizer of the International Society of Hematology (ISH).

“Above all, he was deeply empathetic with patients for whom there were few effective treatments.”

Dr. Dameshek was named Ze’ev at his birth in Voronezh, Russia, and, at the age of three, was brought to the United States by his parents, who settled in Medford, MA, and renamed him William. An exceptional student at Boston’s English High School (the oldest public high school in America), he went to Harvard College, and in 1923 he graduated from Harvard Medical School and married Rose (Ruddy) Thurman. During his internship at Boston City Hospital, he worked with Dr. Ralph Larrabee, a Tufts professor who had established a “Blood Laboratory” in the basement of the hospital. Dr. Dameshek’s first research paper was titled “The reticulated blood cells — their clinical significance.” Dr. Dameshek was also drawn to hematology by George Minot, director of the Thorndike Memorial Laboratory of the hospital, who in 1925 was treating pernicious anemia — then a fatal disease — with raw liver. In 1939, Dr. Dameshek established the Blood Research Laboratory at what was to become the New England Medical Center (now Tufts Medical Center). There he did the work that brought him international recognition.

Dr. Dameshek was a gifted teacher. He ran the most popular course (hematology) for medical students at Tufts University School of Medicine; his Saturday morning hematology Grand Rounds at the New England Medical Center were legendary. He trained more than 100 hematologists from 20 countries. Former trainees still remember walking rounds with Dr. Dameshek — 20 fellows and students trailing the master through the hospital. Dr. Dameshek knew better than any of us how to take a history, perform a physical examination, and interpret a blood smear. In the hallway, after leaving the patient, he would quiz, cajole, and tease the fellows but never embarrass, and never parade his knowledge. He could effortlessly admit, “I don’t know,” (“We’ll vote,” he’d say about a difficult problem or decision.) Above all, he was deeply empathetic with patients for whom there were few effective treatments. He never denied them hope, never seemed rushed, never failed to touch them.

Dr. Dameshek was not a laboratory investigator. He was a busy clinician who took the time to think deeply about his patients and their diseases. He was tireless. He wrote five books and more than 350 articles and editorials. He would arrive in his office on Monday morning with a bundle of lined white paper on which he had, over the weekend, written in characteristic green ink his latest thoughts on PNH, CLL, or myelodysplasia. On some Mondays, he would arrive bursting with a wonderful new idea about the origin of lymphomas, or the relation between PNH and aplastic anemia, or why immune thrombocytopenia develops in systemic lupus erythematosus. Soon after we (puppies) had yelped our objections to the latest idea, it would appear as an editorial in Blood.

Dr. Dameshek demanded from us fellows his own level of enthusiasm, commitment, and effort. It wasn’t easy to emulate the boss. On Friday, October 4, 1957, the day the Sputnik satellite went into orbit, we received a memorable lecture on our deficiencies. Later, he apologized even though we knew that he meant every word.

Patients came from everywhere to seek Dr. Dameshek’s counsel. Luminaries arrived regularly. He collected art, lived in a beautiful house in Brookline, loved music (Serge Koussevitzky was his patient), and entertained grandly thanks to Ruddy. Dressed by Louis, Boston’s high-end haberdasher, Dr. Dameshek cut a stylish figure. He was, from outward appearances, the antithesis of the staid, underpaid Boston academic. He would brush off petty jealousies with a shrug. His favorite response to the critics was, “Every knock is a boost.”

Dr. Dameshek had an extraordinary influence on the development of hematology in America. He used his intelligence, independence, and influence in ways that benefited not only hematologists but also patients. His legacy is unique.
The ASH Web site offers a convenient way for ASH members to find information relating to upcoming Society events and provides easy access to the many valuable products and services offered by ASH.

Participate in ASH’S 50TH ANNIVERSARY and learn more about the rich history of ASH and hematology by visiting www.hematology.org/about/50thanniversary. On this Web page you can meet legends in hematology and read their oral histories, in which they reflect upon their careers. Check out the latest additions for Drs. Ernest Beutler and Joseph F. Ross in the Legends of Hematology section at www.hematology.org/education/legends.

Add your name to the “FIND A HEMATOLOGIST” resource. This is an online service to connect patients with hematologists in their area. Help us make this resource even more valuable by agreeing to be listed in our public directory. Go to www.findahematologist.org for more information.

Go to www.hematology.org/education/awards/ifa.cfm to download your letter of intent for the EHA-ASH INTERNATIONAL FELLOWSHIP AWARD. Letters are due by September 4. The EHA-ASH International Fellowship Award is offered in partnership with the European Hematology Association (EHA) to provide hematologists in training or early in their careers the opportunity to conduct research in another country.

Get up-to-date ANNUAL MEETING information at www.hematology.org/meetings/2008/index.cfm. Join ASH in San Francisco for its 50th anniversary celebration.

Read THE HEMATOLOGIST ONLINE (www.hematology/publications/hematologist) and catch up on the latest news in the field of hematology right on your desktop.

MARK YOUR CALENDAR

**JULY**

3 – 6
The World Congress on Controversies in Cardiovascular Diseases
Berlin, Germany www.comtecmed.com/ccaere

9 – 12
The 37th Annual Scientific Meeting of the Society for Hematology and Stem Cells
Boston, MA www.iseh.org

21 – 26
Fourth International Barth Syndrome Conference
Clearwater, FL www.barthsyndrome.org

22
Annual meeting early-bird registration opens for ASH members
Washington, DC www.hematology.org

**AUGUST**

5
Annual meeting advance registration opens to members and non-members
Washington, DC www.hematology.org

21
Annual meeting abstract submission deadline
Washington, DC www.hematology.org

21 – 29
Hematology and Medical Oncology Board Review
Washington, DC www.hemoncboardreview.com

27 – 28
Workshop on Vasculopathy in Sickle Cell Disease
Bethesda, MD www.sicklecellmeeting.net

**SEPTEMBER**

4
EHA-ASH International Fellowship Award letter-of-intent deadline
Washington, DC www.hematology.org

5
Institutional Insights in Multiple Myeloma
Atlanta, GA www.multiplemyeloma.org

5 – 7
10th International Conference on Chronic Myeloid Leukemia
Boston, MA www.esh.org/agenda08.htm

12 – 14
Annual Meeting of the Society for the Advancement of Blood Management
Baltimore, MD www.sabm.org

16 – 17
NIDDK: Heme Regulation During Erythropoiesis
Bethesda, MD www3.niddk.nih.gov/fund/other/hemeregulation/index.htm