ASH Launches New Publication for Clinicians

This fall, ASH will begin publishing a monthly news magazine in hematology/oncology. ASH Clinical News will provide timely updates in the field by reporting on key medical meetings, clinical trials, innovative technology, and influential papers in the literature. Additionally, regular features will include clinical case studies, interviews with leaders in various hematology subspecialties, patient education information, news about individual ASH members, and updates on Society initiatives.

“With the new magazine, we hope to provide a different and engaging forum for the exchange of information while broadening our reach beyond the ASH community,” said Society President Linda Burns, MD. “We hope that this new magazine will provide an engaging forum for the exchange of information while broadening our reach beyond the ASH community.”

This important effort will be led by Mikkael Sekeres, MD, who was named editor-in-chief of the ASH Executive Committee after an extensive search led by Dr. Roy Silverstein. Dr. Sekeres is professor of medicine, vice-chair for Clinical Research, and director of the Leukemia Program at the Cleveland Clinic in Cleveland, Ohio.

“Mikkael’s participation in ASH’s committees on Quality and on Educational Affairs, his keen understanding of the drug development process through his service on the FDA’s Oncologic Drugs Advisory Committee, and his passion for writing make him the ideal person to help shape the direction of this new publication,” said Dr. Burns.

Dr. Sekeres gained an appreciation for journalism from his late father, Joel Sekeres, who was a newspaper writer and editor. He has been writing both scientifically and creatively his whole life, recently served as a clinical advisory editor of Oncology Times, and regularly contributes essays to the New York Times Well Blog.

(Cont. on page 7)

Gene Editing: A New Fix for Inherited Disorders of Blood Stem Cells


Much progress has been made in the quest to conquer, through gene therapy, monogenic diseases rooted in the hematopoietic stem cell (HSC), but challenges remain. Until recently, methods to “correct” the genetic defect involved introducing into the HSC genome a wild-type copy of the mutant gene, using a retroviral vector-based system. This technique has yielded considerable success, but concerns about insertional mutagenesis and difficulty procuring sufficient HSCs for transplantation, preserving transduced HSCs in an undifferentiated state ex vivo with intact engraftment capability have pushed investigators to identify techniques to enhance both safety and efficacy. One area where recent progress is notable is development of targeted DNA editing techniques that allow true correction of genetic defects in situ. Such editing functions use endonucleases, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases, or RNA-guided nucleases (e.g., the clustered, regularly interspaced, short palindromic repeat [CRISPR]-associated system (Cas9). Because only a small amount of DNA that encompasses the mutated site is excised by the endonuclease and repaired by naturally occurring mechanisms in situ, these techniques have the advantage over retroviral vector-based gene therapy in that native gene regulatory elements (e.g., promoters, enhancers) are preserved. The other major advantage of targeted editing over retroviral-based gene therapy is that the potential toxicity associated with random integration of the retrovirus (i.e., activation of oncogenes that can lead to development of acute leukemia, as reported in the early clinical trials) is likely eliminated.

Now, using a ZFN-based technique, Dr. Pietro Genovese and colleagues at the San Raffaele Telethon Institute for Gene Therapy in Milan, Italy, have successfully corrected, in long-term repopulating HSCs, the disease-causing mutation in the interleukin-2 receptor gamma (IL2RG) chain in a patient with X-linked severe combined immunodeficiency (SCID-X1). Two major innovations were critical for this accomplishment: 1) refinement of the technique to induce cycling of HSCs without differentiation so as to take advantage of high-fidelity homology-directed DNA repair (HDR); and 2) development of the method to efficiently introduce cells both the nucleas and the cDNA template needed to correct the mutant gene (Figure).

Umbilical cord blood stem/progenitor cells were incubated with the cytokines stem cell factor, Flt3 ligand, thrombopoietin, and interleukin-6, together with StemReginin 1 and 16,16-dimethyl-prostaglandin E2, such that cells would progress through the cell cycle (a requirement for HDR) but not undergo differentiation. For correction of the SCID-X1 mutation, the difference in the cell cycle phase was taken advantage of by introducing the ZFNs only in the G0/G1 phase of the cell cycle.

Correction of X-linked severe combined immunodeficiency (SCID-X1) by gene editing. The illustration of the stem/progenitor cell on the far left depicts the location of the gene mutation that causes the disorder. The affected cells are incubated in a cytokine cocktail that induces cell cycling without differentiation. Next, the targeted zinc-finger nuclease (ZFN) is introduced into the cell by electroporation, followed by transduction with integrase-deficient lentiviral vector (IDLV). The IDLV contains the cDNA for IL2RG across 5’ and 3’ green fluorescent protein (GFP) driven by the phosphoglycerate kinase (PGK) promoter. GFP functions as an easily identifiable marker expressed in parallel with IL2RG. This process results in high-fidelity homology-directed repair (HDR), and thereby, corrects the gene mutation that caused SCID-X1. The corrected stem cells can be used therapeutically as, following transplant, their hematopoietic progeny, including T cells and NK cells, will expand in vivo and thereby restore normal immune function (far right).
Envisioning ASH in 2025

It did you know that 22 percent of today’s ASH members live in nearly 100 countries outside of North America? Or that more than 50 percent of Blood manuscripts are submitted from other countries? Or that 46 percent of the 2013 annual meeting attendees and 52 percent of abstract submissions to that meeting were from outside North America? I didn’t either!

I’ve always thought of ASH as being a primarily North American society. Although it’s true that much of the Society’s focus is on domestic programs, if ASH is interested in continuing to attract the best science to Blood and the annual meeting, and to growing the field of hematology outside of North America, the issue of how best to meet the needs of international members while continuing to address those of our domestic members becomes ever more important to consider.

Over the years, ASH has actively engaged international hematologists and provided customized products and services to its members abroad through programs such as the Highlights of ASH in Latin America and Asia, the International Outreach Initiative, the International Consortium on Acute Leukemia, and the Visiting Training Program. If ASH expands its services, the context in which it develops, delivers, and funds its programs would likely need to change.

To begin to address these issues, the ASH Executive Committee engaged the members of the International Members Committee in active discussions to identify the most important programs and services that ASH should provide and to decide what opportunities ASH is uniquely positioned to address. In addition, the Executive Committee has established a task force charged with presenting two to three models representing different levels of benefit to the Society to better meet our mission of helping hematologists conquer blood diseases worldwide. All of the models will focus on maintaining the Society’s position as the preeminent source of hematologic research, education, and quality care. Each ASH standing committee will be invited to consider this matter and provide input to the task force.

The task force wants to receive input from all ASH members. Please consider the following questions: How should ASH evolve? What services and programs are most important? What is the most important issue that should address for hematologists and their patients worldwide? How do you envision ASH in 2025? Please send your responses to these questions to pbs@hematology.org by October 1, and look for an update from the Society’s international initiative task force in a future issue of The Hematologist.

Gene Editing: A New Fix for Inherited Disorders of Blood Stem Cells

(Cont. from page 1)

integrase-deficient lentiviral vector (IDLV) contained a cDNA construct composed of exons 5-8 of the IL2RG gene and the green fluorescent protein (GFP) marker (Figure). The ZFN was designed to excise exons 5-8 of IL2RG, and when the excised site was repaired by HDR using the cDNA construct contained in the IDLV as the template, the mutated DNA was repaired. Thus, the cDNA construct developed by Dr. Genovese et al. can be used to correct the genetic defect in any SCID-X1 case that arises from a mutation affecting any nucleotide residues downstream of exon 4. Analysis of the treated cells showed expression of the GFP marker gene by 100 percent of the primitive stem cells when transduction was induced on the third day of culture. The progeny of the corrected HSCs included myeloid and erythroid cells, as well as T and NK cells. The latter two cell lineages are strictly dependent on normal function of the IL2RG gene, and expression of the repaired gene was demonstrated by massive expansion of GFP marked T and NK cells that were shown to have the capacity to mediate rejection of an allogeneic tumor cell line.

Using this same technique, edited HSCs derived from normal adult bone marrow were shown to engraft in a mouse xenograft model, and cells from a four-month-old SCID-X1 patient with a missense mutation in exon 7 were corrected using this novel methodology.

SCID-X1 was among the first diseases in which gene therapy was successful using defective retroviruses to transfer a normal copy of the cDNA into mutant cells.12 Although this technique was effective in restoring T-cell immunity to the subjects, the procedure was associated with the risk of random insertion of the genetic material into the genome that resulted in development of acute leukemia through activation of oncogenes such as LMO2. Fortunately, four out of five of the children who developed acute leukemia due to insertional mutagenesis were successfully treated with chemotherapy. The elegant studies of Dr. Genovese and colleagues demonstrate proof of principle that gene editing can be incorporated into gene therapy strategies for disorders involving the HSC. Whether this new approach is clinically viable, however, remains to be proven, and the long-term consequences of this procedure including persistence of functional correction and degree of toxicity must be evaluated in clinical trials.


LETTERS TO THE EDITOR
SOLICITATION

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

Letters should be sent to: Juana Llorens, Managing Editor The Hematologist: ASH News and Reports 2021 L Street, NW, Suite 900 Washington, DC 20036 j{llorens@hematology.org

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Recap of 2014 Highlights of ASH® Meetings

North America
Highlights of ASH in North America took place in late January/early February 2014 in six cities: Dallas, New York, Atlanta, San Francisco, Miami, and Seattle. A total of 1,180 attendees registered for the 2014 meeting series.

Asia
The meeting was held March 29-30 in Singapore in partnership with the Cancer Science Institute, National University of Singapore. Representatives from the 13 partner organizations served as moderators for the meeting and worked directly with the speakers to provide regional and/or country-specific perspective (data/relevance) to each program topic. A total of 603 attendees from 22 countries participated in the meeting (174 from Singapore). Since 2011, when the program in Asia began, attendance from most of the partner organization countries has increased consistently.

To increase attendee participation, a poster session was included in the meeting format. The session was held at the end of the first day of programming. As a group, speakers and attendees walked through the poster viewing area and listened to short presentations prepared by the authors. Data from a total of 27 posters, each based on an accepted abstract from the 2013 ASH Annual Meeting, were presented.

For the first time, both international Highlights meetings (Asia and Latin America) were accredited for CME credit. A total of 10 AMA PRA Category 1 Credit(s)™ were available to physicians for the Highlights of ASH in Asia. In addition, the Singapore Medical Council accredited the meeting for up to 6 CME points for Singapore physicians.

Latin America
The meeting was held on April 25-26 in Florianopolis, Brazil, in partnership with the Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular (ABHH). A total of 689 attendees from 17 countries participated in the meeting (296 from Brazil). Moderators from all 10 Latin American partner organizations provided local context for the presentations.

Dates for Next Year’s Highlights of ASH Meetings
The 2015 Highlights of ASH meetings will take place on the following dates and in the following locations:

North America
January 16-17: Austin, TX, and New York, NY
January 23-24: Seattle, WA, and Washington, DC
January 30-31: Orlando, FL, and San Diego, CA

Asia
February 28 – March 1: Bangkok, Thailand

Latin America
April 23-24: Cartagena, Colombia

ASH Foundation 3K – 5K Run/Walk
On Sunday, December 7, come kick off your day in a fun and healthy way – run or walk in the ASH Foundation 3K – 5K Run/Walk! Participants can choose between the two distances and will have the option to run or walk. All race proceeds will fund programs supported by the ASH Foundation.

The route will take runners and walkers along San Francisco’s scenic Embarcadero. ASH will operate a continuous-loop shuttle service between the Moscone Center and the Embarcadero before and after the Run/Walk.

Registration is now open at www.hematology.org/runwalk.
Ask the Hematologist

JOSEPH MIKHAIL, MD, Med
Associate Dean, Mayo School of Graduate Medical Education; Deputy Director - Education, Mayo Clinic Cancer Center; Associate Professor, Mayo College of Medicine, Mayo Clinic in Arizona, Scottsdale, AZ

The Question
What is your approach to the evaluation of patients with an IgM monoclonal protein?

My Response
Benign monoclonal proteins were first described by Dr. Jan Waldenström in 1960 after he detected abnormal narrow hypergammaglobulinemia bands in serum protein electrophoresis (SPEP) samples from healthy individuals. The term monoclonal gammopathy of undetermined significance (MGUS) was coined by Dr. Robert Kyle in 1978 to describe an asymptomatic plasma cell dyscrasia characterized by a monoclonal protein of <1.5 g/dl, bone marrow plasma cells <10 percent, and the absence of end-organ damage commonly associated with multiple myeloma (MM). MGUS is a relatively common condition with a prevalence of three to five percent in the adult population over the age of 50 years. SPEP is a frequently performed laboratory test ordered by primary-care physicians evaluating patients with anemia, by nephrologists evaluating patients with renal insufficiency (possibly with associated proteinuria), and by neurologists evaluating patients with peripheral neuropathy. When the SPEP reveals a monoclonal protein, referral to a hematologist usually follows. Therefore, it is part of routine practice for hematologists to see patients with monoclonal proteins that are initially identified as the result of screening examinations. The hematologist must then decide which additional diagnostic studies are warranted, and based upon those results, develop management and follow-up plans.

Although it accounts for only 15 to 20 percent of MGUS cases (that also include IgG-MGUS, IgA-MGUS, and light chain-MGUS), IgM-MGUS poses a unique diagnostic challenge because of the association of monoclonal IgM proteins with B-cell lymphoproliferative disorders (particularly Waldenström macroglobulinemia [WM], amyloidosis, and peripheral neuropathy. This review is intended to provide the practicing hematologist with a focused diagnostic approach to patients with a monoclonal IgM protein that takes into account its associations with other disease processes.

MGUS
Three distinct classes of MGUS are recognized: Non-IgM-MGUS (essentially IgG-MGUS or IgA-MGUS as both IgM-MGUS and IgG-MGUS are fleetingly rare), light chain-MGUS, and IgM-MGUS. Distinguishing among these sub-groups is important, as doing so directs both the diagnostic plan and the follow-up recommendations, facilitates identification of diseases associated with MGUS, and impacts on management recommendations and prognosis. Although the majority of patients with IgM-MGUS will have a benign course, it is critical for the clinician to rule out a concurrent associated disease and to monitor for progression or transformation into a distinct entity that requires specific therapy.

The Risk of MGUS Progression
Unstratified patients with non-IgM MGUS have approximately a one percent per-year risk of their disease transforming into MM; however, the risk of transformation is double in patients with IgM MGUS. Risk for transformation can be more precisely stratified based on three parameters: IgG subtype versus non-IgG subtype, monoclonal protein concentration (<1.5 g/dl, versus >1.5 g/dl), and normal versus abnormal serum free light chain ratio. Stratification of risk can help guide the diagnostic evaluation and follow-up recommendations.

IgM not only has a higher risk of transformation than non-IgM MGUS, but also the states of diseases associated with IgM MGUS transformation is broader than that of non-IgM MGUS. Whereas non-IgM MGUS can progress into smoldering and active MM and AL amyloidosis, IgM MGUS can transform into WM, AL amyloidosis, and less commonly, IgM smoldering myeloma or IgM MM. For this reason, patients with IgM MGUS require closer follow-up than patients with non-IgM MGUS, and from a conceptual perspective, IgM-MGUS can be thought of as a “lymphoproliferative” MGUS while non-IgM MGUS behaves as a “plasma cell proliferative” MGUS.

Special Concerns for IgM MGUS
a. Association with neuropathy – Neurologists routinely screen patients with peripheral neuropathy for the presence of a monoclonal protein, and approximately five to 10 percent of such patients will be found to have a monoclonal protein by SPEP. A causal relationship between MGUS and peripheral neuropathy is supported by association of peripheral neuropathy with other plasma cell dyscrasias including WM, MM, AL amyloidosis, and POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes). The peripheral neuropathy of MGUS is classically bilateral, peripheral, and sensory with electrophysiologic studies showing a demyelinating pattern; and in patients with progressive, debilitating disease, biopsy may reveal axonal loss. Although peripheral neuropathy can occur with all forms of MGUS, it is most commonly associated with IgM-MGUS. Demonstration of IgM anti-myelin-associated glycoprotein (MAG) antibodies supports a causal relationship between IgM-MGUS and polyneuropathy but is not essential for the diagnosis. Because peripheral neuropathy can be caused by other processes that may co-exist with IgM-MGUS, the clinician is often faced with the dilemma of whether to assign the neuropathy to MGUS, and if so, what to do about it. The decision can be guided by the observations that the neuropathy associated with IgM-MGUS is characteristically a relatively benign, slowly progressive sensory process (although some cases can be severe and debilitating). Management is challenging as effective therapy is lacking. A minority of patients respond to rituximab, and the only effective treatments are generally ineffective. As is the case with other plasma cell dyscrasia-associated neuropathies, the monoclonal protein concentration in IgM-MGUS-associated peripheral neuropathy does not correlate with disease severity, arguing against the use of myeloma-directed therapy to reduce the plasma cell burden as a treatment strategy for the neuropathy of IgM-MGUS.

b. Association with AL amyloid – This complex disease can be associated with any form of myeloma, although usually one of the two processes dominates the clinical picture. That is to say that patients typically have either a myeloma-phenotype manifested by some combination of hypercalcemia, renal insufficiency, anemia, and skeletal involvement or an amyloid phenotype characterized by organ (liver, heart, kidney) infiltration by the pathologic immunoglobulin light chain. AL amyloid is more commonly associated with IgM-MGUS than other forms of MGUS. Thus, it is imperative that AL amyloid be carefully considered when evaluating patients with an IgM monoclonal protein.
c. Association with WM – The hallmark of this disease is an IgM monoclonal protein, lymphoadenopathy, hepatosplenomegaly, and bone marrow involvement by plasmacytoid lymphocytes. Frank disease may be preceded by a relatively asymptomatic smoldering phase in which IgM MGUS is the only apparent clinical manifestation.
d. Association with other B-cell lymphoproliferative disorders – Although IgM-MGUS is more commonly associated with WM, it can be observed in association with another B-cell lymphoproliferative disease such as CLL or non-Hodgkin lymphoma. As indicated in the algorithm (Figure 2), determining if organ damage is attributable to the monoclonal protein is critical, as in most cases the monoclonal protein simply “co-exists” with the lymphoproliferative disorder. Although the monoclonal protein may be discovered first as part of a laboratory evaluation, it is not usually the presenting clinical manifestation of lymphoproliferative diseases other than WM.
e. Association with IgM MM – This is a rare form of myeloma that is genuinely distinct from WM. IgM MM is distinguished from WM primarily by skeletal involvement (lytic bone lesions) in the former, but genetic studies may also be informative (e.g., t(4;14) in IgM MM and somatic mutation of MYD88 in WM).11, 12

Suggested Approach to the Evaluation of Patients with an IgM Monoclonal Protein
The strategy that I use in the evaluation of patients with an IgM monoclonal protein is described below and illustrated in Figure 2.

1. History – This remains a critical aspect of the evaluation, as symptoms elicited from a careful history focus attention on specific issues that require further investigation. Indeed, even subtle symptoms become important when the differential diagnosis includes such a wide spectrum of disorders as amyloidosis, POEMS, MM, and lymphoma. Key symptoms to address include the following:
   a. Constitutional (weight loss, extreme fatigue) – Such symptoms suggest AL amyloid or lymphoma.
   b. Gastrointestinal – Upper GI bleeding, early satiety, and chronic diarrhea raise the possibility of GI amyloid.
   c. Cardiac – Progressive shortness of breath, presyncpe/syncpe, and chest pain are consistent with cardiac amyloid.
   d. Neurologic – Bilateral, sensory neuropathy is consistent with the neuropathy associated with plasma cell dyscrasias; and vision changes, headache, vertigo, or dizziness raise the possibility of hyperviscosity associated with WM.
   e. Skeletal pain – This symptom suggests IgM myeloma.
   f. Skin – Urticarial rash raises the possibility of Schnitzler syndrome.12

Figure 1
Natural History of MGUS

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ASH does not recommend or endorse any specific tests, physicians, products, procedures, or opinions, and disclaims any representation, warranty, or guaranty as to the same. Reliance on any information provided in this article is solely at your own risk.
3. Venous Blood Sample
- CBC, platelet count, clotting time, prothrombin time, partial thromboplastin time
- Serum protein electrophoresis
- Light chain electrophoresis
- Immuneelectrophoresis
- Immunofixation
- Serum immunoglobulins
- Serum free light chains
- Urinalysis
- Urine protein electrophoresis
- 24-hour urine protein collection
- Urine electrophoresis
- Urine immunofixation
- Serum and urine Bence Jones protein
- Serum and urine free light chains
- Fasting lipid panel
- Calcium, phosphorous, uric acid, creatinine
- HbA1c
- LFTs
- Lipid profile
- TSH
- T4
- Free T4
- Thyroid ultrasound
- Chest X-ray
- CT scan of chest, abdomen, and pelvis
- MRI of spine
- Skeletal survey
- Bone marrow biopsy
- Bone scan
- PET/CT
- Flow cytometry
- Inmunostaining
- Genomic testing
- Cytogenetic analysis

4. Clinical Considerations
- Bone marrow evaluation
- Amyloidosis
- Myeloma
- Multiple myeloma
- Smoldering multiple myeloma
- Waldenstrom's macroglobulinemia
- Non-Hodgkin lymphoma
- Hodgkin lymphoma
- Paraproteinemia
- Monoclonal gammopathy of undetermined significance
- Malignant lymphoproliferative disorders
- Secondary amyloidosis
- Primary amyloidosis
- Plasma cell dyscrasias
- Systemic vasculitis
- Hypergammaglobulinemia
- Systemic lupus erythematosus
- Systemic sclerosis
- Rheumatoid arthritis
- Scleroderma
- Polymyalgia rheumatica
- Giant cell arteritis
- Polyarteritis nodosa
- Wegener's granulomatosis
- Churg-Strauss syndrome
- Primary biliary cirrhosis
- Primary sclerosing cholangitis
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5. Conclusion
Monoclonal IgM is associated with a diverse set of diseases that range from a generally benign process requiring no specific therapy (IgM MGUS) to overt disease requiring a specific management plan. Approaching the initial evaluation systematically and comprehensively will enable the clinician to accurately characterize the disease process.

Technologic advances in DNA analysis have revolutionized our understanding of the genetic basis of myelodysplastic syndrome (MDS). As this technology is incorporated into the routine evaluation of patients, the clinical benefits of genetic analysis will continue to expand. Currently available are two widely used and complementary strategies for querying genetic abnormalities in patients: single-nucleotide polymorphism-based genomic and array-based single-nucleotide polymorphism-based genetic arrays (SNPs) for detection of large-scale genomic gains and losses and next-generation sequencing (NGS) studies for detection of single-gene abnormalities, including small insertions/deletions and substitution mutations (Table). This technology is allowing for more accurate and timely diagnosis by the laboratory, particularly in those cases where the morphologic findings and the results of classical karyotyping are inconclusive. And the results of these tests are being used to personalize the management of patients with MDS. For example, the spectrum of genetic abnormalities often varies among patients with the same WHO category. In such cases, morphologic analyses can be used to subclassify patients into categories that have prognostic and therapeutic implications.

Conventional cytogenetic testing, including metaphase cytogenetics (MC) and fluorescence in situ hybridization (FISH) studies, is considered the gold standard for the genetic evaluation of patients with MDS (Table). However, these strategies have technical constraints that limit their clinical usefulness. MC requires actively dividing cells and results that are not always characteristic. FISH can overcome some of the limitations of MC, but because FISH is a targeted assay, only those abnormalities under analysis (e.g., 5q) will be detected. In addition, neither MC nor FISH can detect copy neutral loss of heterozygosity (CN-LOH), a frequent finding in MDS that can serve as a clonal disease marker (Table). These technical limitations impede the discovery of cryptic or low-frequency chromosomal lesions that may have diagnostic and/or prognostic significance. Conventional cytogenetic testing identifies chromosomal abnormalities in approximately 50 percent of patients with MDS. For the remainder with normal-karyotype MDS, genetic abnormalities will go undetected unless samples are analyzed further. The natural history of normal-karyotype MDS is variable, and in this setting, higher-resolution genetic analysis may provide clinically relevant information.

**SNP s**

SNPs have emerged as a valuable tool for identifying both copy number alterations detected by MC, or FISH and CN-LOH (Table). Because SNPs do not depend on the availability of actively dividing cells, it is especially useful when MC has failed to detect a clonal abnormality or when studying bone marrow cells in culture (Table). SNPs can detect chromosomal deletions in patients with MDS at a significantly higher rate compared with conventional cytogenetics (75% vs. 50%) due to its capacity to identify cryptic chromosomal deletions and gains and acquired CN-LOH (aCN-LOH). For example, recurrent small deletions beyond the resolution of MC and FISH involving genes such as TET2 on 4q24, DMRT3A on 2p23.3, and RUNX1 on 21q22 detected by SNPs have been reported in patients with MDS. In addition, SNPs detect aCN-LOH in 20 percent of MDS patients. aCN-LOH represents a major mechanism by which tumor suppressor genes are inactivated or by which oncogenes become hypermethylated. In MDS, abnormal DNA methylation changes (MLCs) are often observed, and they can be modified such that only the protein coding components of each gene are sequenced. This process is called whole-genome sequencing and reduces the amount of sequence data generated by 99 percent compared to whole genome sequences. NGS can also be used to sequence a specific set of genes (called mutation panels) that are usually related in some way. For example, one sequencing panel might consist of genes known to be frequently mutated in MDS, or another panel of genes might be associated with the signaling pathway involved in leukemia pathogenesis (Table). Initially, NGS was used primarily as a research tool, but the combination of lower cost and improvements in both sequencing technology and bioinformatics has made feasible the implementation of NGS protocols in clinical laboratories. At a number of larger medical centers throughout the US, NGS is a validated analysis of large samples from patients with MDS and other myeloid malignancies, with some results (depending upon the complexity of the analysis) being reported within a week (Table). A recent study involving DNA samples from 944 patients that used a panel consisting of 104 genes demonstrated that 90 percent of MDS patients have at least one of these genes mutated, with three being the median number of mutated genes observed in this study.1 Similar to the results of NGS testing, the information derived from NGS can be valuable in establishing a diagnostic hypothesis, identifying patients without sufficient morphologic evidence of dysplasia or when there is no evidence of cytogenetic abnormalities using traditional methods. Data from NGS can also be used to determine if a patient fits into a prognostically significant disease subclassification.

MDS-associated mutations recurrently affect discrete cellular pathways, including regulators of transcription (RUNX1, TET2), cell-cycle regulators (TP53), components of the RNA splicing machinery (SF3B1, SRSF2, U2AF1), regulators of epigenetic functions such as methyltransferases (DNMT3A, TET2), and components of the core histones (H2B, H3). In terms of frequency, mutations in one of the components of the spliceosome complex are present in almost half of cases, with SF3B1 and SRSF2 being most commonly affected.6 Mutations in TET2, DNMT3A, RUNX1, and ASXL1 also appear commonly in MDS patients with frequency of more than 5%. Many other genes are recurrently mutated in MDS, albeit at a lower frequency. These same abnormalities may be found across the spectrum of myeloid malignancies, including in acute myeloid leukemia, in myelodysplastic neoplasms (MPNs), and in MPN/MPD neoplasms such as chronic myelomonocytic leukemia. In general, a higher overall number of driver mutations correlates with worse outcomes in MDS.2 Specific mutations also have independent functional significance. For example, SF3B2 mutations are associated with a higher frequency of progression to AML,2 while mutations in SBF2 are associated with longer event-free survival.3 Specific pairwise associations between mutations and clinical outcomes have also been observed, suggesting co-dependence or co-evolution during disease progression. Mutations may also demonstrate mutual exclusivity. Although the revised international prognostic scoring system (IPSS-R) does not incorporate mutation status of individual genes, in many cases, IPSS-R-independent prognostic information is gained through this type of analysis. With the goal of developing more informative scoring systems, comprehensive prognostic models have been proposed that incorporate the entire spectrum of clinical, laboratory, and genetic information.

SNP testing and NGS-based mutation studies are revolutionizing the clinical and laboratory evaluation of patients with hematologic malignancies including MDS. The analyses can usually be performed on either peripheral blood or bone marrow, but are also possible in other tissues.2,11 As use of the technology becomes more widespread, pathologists and bone marrow aspirates will be relied upon to ensure the appropriate integration of newly available genetic information with traditional clinical, laboratory, morphologic, and histopathologic findings.


**Comparison of Common Clinical Strategies for Assessing Genetic Abnormalities in Patients with Myelodysplastic Syndromes**

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This table was adapted from a table published in the Journal of Molecular Diagnostics, 16, Nybakken GE, Bagg A. The genetic basis and expanding role of molecular analysis in the diagnosis, prognosis, and therapeutic design for myelodysplastic syndromes. 147; Copyright Elsevier 2014.

*Depending on the informatics pipelines in place, it is possible to detect copy number changes and balanced rearrangements by NGS, although it is still uncommon in clinical testing.
ASH Contributes to New Report Highlighting Devastating Impact of Deep Budget Cuts on Biomedical Research

A new report released in mid-July, and supported by ASH, details the dire consequences of recent deep budget cuts to federal health programs, including the National Institutes of Health (NIH), that continue to retard progress in biomedical research and threaten the health and safety of Americans.

The Coalition for Health Funding (CHF), an alliance of more than 90 public health advocacy organizations including ASH, invited scientists, public health advocates, and others to share stories of how their programs have been hurt by deep budget cuts enacted by Congress in recent years in an effort to control federal spending. The resulting report, titled "Faces of Austerity, How Budget Cuts Hurt America’s Health," details the ways in which reductions in funding are stifling scientific discovery and innovation, dissuading a generation of scientists and health practitioners from pursuing research and academic careers, hindering access to essential health and social services, and undermining government programs designed to respond to health hazards and natural disasters. Among the stories included in the report are two from ASH members who detail how a decade of flat NIH funding and a 5 percent budget cut in 2013 have shuttered labs and jeopardized translational research projects aimed at discovering new and more effect therapies.

"Faces of Austerity" is available online at www.cathcart.org.

Appropriations Process Stalled in Congress; Outlook for 2015 NIH Funding Uncertain as New Fiscal Year Approaches

October marks the beginning of the fiscal year and the date by which congressional spending bills are to be completed. But despite bipartisan support for the National Institutes of Health (NIH) and a bipartisan commitment earlier this year to restore "regular order" to the annual appropriations process, the House and Senate have reached a virtual stalemate in this year’s quest to move the federal spending bills through both chambers.

At this time, whether the bill that includes funding for NIH will be brought to the House and Senate floor for a final vote by that date is unclear.

As this issue of The Hematologist went to press, action on spending bills had stalled in both the House and Senate. Though in early June the Senate Labor, Health and Human Services, and Education (Labor-HHS) Appropriations Subcommittee approved its fiscal year (FY) 2015 spending bill, which seeks to restore the NIH budget to its pre-sequestration level, action by the full Senate Appropriations Committee was postponed indefinitely, and the fate of the bill in the Senate remains uncertain. Of further concern, the House scheduled no significant action on its version of the Labor-HHS bill prior to the month-long August congressional recess.

Given the lack of momentum to complete the bills and a limited number of legislative workdays in September, completion of the spending bills in either chamber by the end of the current fiscal year will be challenging. If Congress cannot reach a decision about spending bills by October 1, it has two options: pass a temporary continuing resolution that extends federal agency and program funding unchanged from the current year or shut down the government until it can come to an agreement on spending (an unlikely scenario in an election year).

Take Action

ASH is continuing its advocacy, urging Congress to provide NIH with at least $32 billion in funding in FY 2014 – the minimum investment necessary for NIH’s budget to keep pace with biomedical inflation. Senators and representatives need to hear from their constituents about the negative impact that cuts in funding have had (and will continue to have) on hematology research. ASH members, along with ASH staff, have met with numerous congressional offices to discuss the importance of biomedical research and the need to protect NIH from further funding cuts.

You can also have your voice heard in the halls of Congress and back home in your congressional district by participating in the Society’s advocacy efforts. As Congress continues to formulate the FY 2015 budget, the Society encourages you to visit the ASH Advocacy Center (www.hematology.org/takeaction) and take action in support of funding for NIH in FY 2015. You are also encouraged to let the ASH Government Relations, Practice, and Scientific Affairs Department know when you are in Washington, DC, and available to meet with your congressional delegation. To find the latest information about the FY 2015 budget and its potential impact on NIH, please visit www.hematology.org.

ASH Comments on Proposed Federal Rules Impacting Medicare Physician Payment

ASH is in the midst of commenting on changes to payment and quality rules for Medicare scheduled for the upcoming year. Early in July, ASH addressed the proposed 2015 Inpatient Hospital Rule, supporting funding for a factor prothrombin complex concentrate, expressing concern about potential changes to the rules that determine inpatient status, and commenting on the removal of performance measures aimed at preventing venous thrombois.

In August, ASH offered comments on the outpatient hospital rule and the physician fee schedule rule. While many hematologists offer services in the outpatient hospital setting, there were relatively few items of note within that rule. The physician fee schedule rule was estimated by Medicare to have a 1 percent positive impact on hematology/ondcology physicians, but that impact would vary considerably depending on the mix of services and ownership status of the practice. While the Centers for Medicare and Medicaid Service did not propose changes for next year, they indicated that they are considering reviewing payments for chemotherapy and therapeutic injections and potentially recommending changes that would be implemented in 2016 or later.

The physician fee schedule rule included more details on the implementation of the physician pay-for-performance program called the value-based modifier. This program will adjust physician practices payment based on both the quality of care and the cost of patients treated. To comply with this program, most practices that include hematologists will have their 2016 payments adjusted based on 2014 performance.

ASH’s summaries of these rules and the comments offered by the Society are available on the ASH website at www.hematology.org/Practice.

ASH Launches New Publication

In addition, the new editor-in-chief admits to being "a huge fan" of The Hematologist and reads every issue. He envisions ASH Clinical News serving as a complement to the "thoughtful and expansive treatments" of scientific content in The Hematologist by providing digestible pieces more along the lines of newspaper or magazine articles. He views the new publication as an opportunity to place the latest basic/translation research in a clinically relevant context that will serve to reinvigorate a portion of the ASH membership who may not have thought about such topics since fellowship.

Recalling his own days as a trainee, when he felt the educational sessions at the ASH annual meeting were "to the head, Dr. Sekeres emphasizes his commitment to making ASH Clinical News accessible to a wide range of audiences. Specifically, he notes the importance of reaching nurses and other physician extenders who are playing an ever-expanding role in hematology and oncology patient care.

"While these clinically oriented practitioners may not have time to read every article in Blood or similar scientifically rigorous journals, my hope is that they will enjoy reading a magazine that explains the research concisely and has at least one section dedicated to their interests."

In addition, he explains that he hopes the magazine will be perceived as having a "fun, hip vibe" and says he would "not shy away from essays that contain humor, nor from considering a venue with a focus on narrative medicine, poetry, photographs, or works of art. People active in humanities and medicine always need another outlet, and having such a section will quickly propel the magazine beyond hematologists."

Another priority for Dr. Sekeres is to include younger writers to light the perception of ASH as an "old boys’ club." He explains that, "We want to portray the opposite with the magazine and emphasize that all voices can be heard."

He sees interactivity with social media being an invaluable tool for engaging with the audience. Along those lines, he welcomes member suggestions for topics and features that would be of interest. Please send ideas to Managing Editor Karen Learner at kleanner@hematology.org.

ASH Active members should check their mailboxes for the first "preview" issue of ASH Clinical News to be mailed in October. (Note that the print edition will be distributed to all ASH members in the United States, Canada, and Mexico. International ASH members will receive complimentary online access.) In addition, a special launch issue will be available on site in San Francisco for all attendees at the ASH annual meeting in December. Beginning in January 2015, the magazine will be published monthly. A companion website and mobile app are also in development.
A D-dimer Cutoff for Excluding PE: One Size Does Not Fit All


Editor’s note: Data published in this article were presented in abstract form by Dr. Righini in the Late-Breaking Abstract Session of the 2013 ASH Annual Meeting in New Orleans.

A D-dimer concentration of < 500 mg/L measured using a high-sensitivity assay system is a robust means of excluding pulmonary embolism (PE) in patients with a low pretest probability of PE, as determined by a validated clinical prediction rule, obviating the need for imaging (e.g., CT-pulmonary angiography) in such patients. The positive predictive value of D-dimer values above this threshold, however, is less robust. D-dimer concentrations ≥ 500 mg/L are identified in 30% to 60% of patients who are suspected of, but ultimately determined to not have PE.1 Because steady-state D-dimer levels increase with age, using a fixed cutoff of 500 mg/L might overestimate the probability of PE in elderly patients and thereby explain, at least in part, the observed high percentage of patients with a D-dimer below the age-adjusted threshold did not undergo imaging (e.g., in a 78-year-old patient, the age-adjusted cutoff would be 780 mg/L). A cutoff of 500 mg/L was retained for patients younger than 50 years. In the current study, this strategy, which was initially derived from three retrospective datasets,2 was tested in a prospective cohort of consecutive patients presenting to the emergency department with suspected PE.

Patients were recruited from 19 centers in Belgium, France, the Netherlands, and Switzerland. The pretest probability of PE was estimated using the Simplified, Revised Geneva score or the two-level Wells score. Patients with a high or likely clinical probability score proceeded directly to imaging. In patients classified as low/intermediate or unlikely probability, a D-dimer test was measured using a high-sensitivity assay system. Patients with a D-dimer value above their age-adjusted cutoff proceeded to imaging, while those with a value below the age-adjusted threshold did not undergo imaging and were not treated with anticoagulation (Figure). The primary outcome was symptomatic venous thromboembolism (VTE) at three months in patients with a D-dimer below the age-adjusted cutoff.

A total of 3,346 patients were enrolled in this study. Of these, the clinical probability was low/intermediate or unlikely in 2,898. A D-dimer < 500 mg/L was obtained in 817 patients. The D-dimer concentration was ≥ 500 mg/L and the age-adjusted cutoff in an additional 337 patients with a pretest probability of PE was estimated using the Simplified, Revised Geneva score or the two-level Wells score. Patients with a high or likely clinical probability score proceeded directly to imaging. In patients classified as low/intermediate or unlikely probability, a D-dimer test was measured using a high-sensitivity assay system. Patients with a D-dimer value above their age-adjusted cutoff proceeded to imaging, while those with a value below the age-adjusted threshold did not undergo imaging and were not treated with anticoagulation (Figure). The primary outcome was symptomatic venous thromboembolism (VTE) at three months in patients with a D-dimer below the age-adjusted cutoff.

This practice-changing study demonstrates that an age-adjusted D-dimer cutoff reliably excludes PE and reduces unnecessary imaging in older patients compared with a fixed cutoff of 500 mg/L. A revised diagnostic approach to patients with suspected PE that incorporates the findings of Dr. Righini and colleagues is shown in the Figure. In addition to age, D-dimer levels increase with surgery and malignancy. A D-dimer cutoff of 500 mg/L has notoriously poor specificity for PE in these settings. As we move away from a one-size-fits-all approach in elderly patients with suspected PE, one wonders whether use of a higher cutoff could also enhance the diagnostic specificity of the D-dimer for VTE in postoperative and cancer patients. Preliminary data are promising.3,4

Endothelial Cells and Bleeding in Myeloproliferative Neoplasms

The Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) — polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF) — are a group of clonal hematopoietic stem cell disorders. Mutations in the Janus kinase 2 (JAK2) gene, especially the JAK2 V617F mutation, are common in these disorders, leading to hyperactive kinase activity and overproduction of myeloid progenitor cells. Patients with ET or PV have a higher incidence of both thrombosis and bleeding. Considerable effort has been devoted to understanding the hemostatic abnormalities in these patients. Increases in intrinsically prohemostatic functions of platelets and neutrophils have been implicated as causal factors for thrombosis. JAK2 V617F+ expressing endothelial cells (ECs) have been reported in a subset of MPN patients, and EC expression was coupled with an increased risk of thrombosis. Additionally, activation of ECs leading to increased production of P-selectin and E-selectin and decreased production of nitric oxide has been identified in some patients. Conversely, the bleeding diathesis in some patients has been attributed to acquired von Willebrand disease, which is due in part to thrombocytosis.

To determine which cell types contribute to hemostatic disorders associated with MPNs, Dr. Leah Erdheiger and colleagues in the laboratory of Ian Hitchcock in the Department of Medicine at Stony Brook University used the FF1 transgenic mouse model to express human JAK2 V617F in specific cell lineages. The FF1 transgenic mouse was developed in the laboratory of Radek Skoda at University Hospital in Basel, Switzerland. It contains human JAK2 V617F in specific cell lineages. The FF1 transgenic mouse model to express JAK2 V617F exclusively in platelets or in hematopoietic/EC cells. Tie2-Cre/FF1 mice did not develop an MPN phenotype, which the authors attributed to the relatively late stage of activation during hematopoietic development of Cre recombinase under control of the P4f promoter.

The authors used a fecal carriage-induced carotid artery occlusion model to compare thrombosis in wild-type, Tie2-Cre/FF1, and Pf4-Cre/FF1 mice. They observed that Tie2-Cre/FF1 mice had a marked increase in time to occlusion compared with wild-type mice. This result was surprising because although increased bleeding is observed in some MPN patients, thrombosis is more common. In contrast, the time to occlusion was normal in Pf4-Cre/FF1 mice. Tail snip bleeding times were also significantly increased in Tie2-Cre/FF1, but not in Pf4-Cre/FF1 mice compared with wild-type mice.

Platelet function studies were performed to assess possible hemostatic abnormalities. Whole-blood aggregometry revealed an increased rate and extent of aggregation in response to epinephrine, collagen, and ADP in Tie2-Cre/FF1 compared with wild-type or Pf4-Cre/FF1 mice. This effect was attributed to thrombocytosis because aggregometry normalized using equal numbers of isolated platelets. No significant differences in expression of platelet integrins (IIIa, IIb, I, v, d, and activated IIb/IIIa were observed between wild-type and Tie2-Cre/FF1 mice. The authors then performed reciprocal bone marrow transplantation experiments to determine if ECs were involved in the bleeding/thrombotic phenotype observed in the Tie2-Cre/FF1 mice. Marrow from Tie2-Cre/FF1 mice was transplanted into irradiated wild-type mice to produce mice in which JAK2 V617F expression was limited to hematopoietic cells. Donor marrow from wild-type mice served as a control. Recipient mice expressing hematopoietic cell-derived JAK2 V617F developed a MPN phenotype characterized by thrombocytosis and neutrophilia. However, the carotid artery occlusion and tail bleeding time assays demonstrated no significant differences between the Tie2-Cre/FF1 and wild-type donor mice. In the reciprocal transplant, wild-type marrow was transplanted into Tie2-Cre/FF1 recipients, producing mice in which only ECs express JAK2 V617F. Wild-type recipients served as a control. Mice expressing EC JAK2 V617F did not develop a MPN phenotype. However, carotid artery occlusion assays showed a significant increase in occlusion time in Tie2-Cre/FF1 recipients, but the tail bleeding time was not prolonged in Tie2-Cre/FF1 recipients. The observation that the hemostatic abnormalities in these mice were less pronounced than in the Tie2-Cre/FF1 mice suggests an interaction between JAK2 V617F expressing hematopoietic cells and ECs.

Because ECs express von Willebrand factor (vWF) and acquired von Willebrand syndrome is observed in patients with MPN, the authors determined whether the Tie2-Cre/FF1 mice displayed characteristics of acquired von Willebrand syndrome. Levels of plasma vWF were normal in Tie2-Cre/FF1 mice. However, Tie2-Cre/FF1 mice displayed a significant reduction in ultra-large vWF multimers. Additionally, whole blood ristocetin-induced platelet agglutination was reduced in Tie2-Cre/FF1 mice, consistent with the defect in vWF function observed in acquired von Willebrand syndrome. Levels of ADAMTS13, which catalyzes the proteolysis of vWF into smaller multimers, were not increased in primary lung ECs isolated from Tie2-Cre/FF1 mice. The shift in vWF multimer distribution seen in Tie2-Cre/FF1 mice was not observed in either of the bone marrow transplant models. Additionally, primary lung ECs isolated from mice expressing JAK2 V617F only in ECs showed increased levels of vWF with a normal multimer pattern compared with controls. These results suggest that expression of JAK2 V617F in both hematopoietic and endothelial compartments contributes to the abnormal distribution of vWF multimers. However, transplant mice whose JAK2 V617F expression was limited to ECs showed a defective response to ristocetin. In contrast, the response to ristocetin was normal in mice expressing JAK2 V617F restricted to hematopoietic lineages.

Together, the results suggest that JAK2 V617F-mediated changes in EC-derived vWF processing contribute to the changes associated with acquired von Willebrand syndrome. JAK2 has been shown to mediate endothelial nitric oxide synthase activity via AKT activation, eNOS in turn has been shown to be involved in a pathways affecting the exocytosis of Weibel-Palade bodies, which store vWF. Additional experiments may reveal other ways in which JAK2 V617F contributes to abnormalities in EC signaling pathways involved in hemostasis.

Thrombosis and bleeding contribute significantly to the morbidity and mortality associated with MPNs, including PV, ET, and primary myelofibrosis. Thrombosis observed in the MPNs was not explained using the murine JAK2 V617F expression model developed in this study. However, the model indicates that JAK2 V617F expression by ECs contributes to the bleeding diathesis associated with MPNs. Additionally, interplay between JAK2 V617F expressing ECs and hematopoietic cells appears to contribute to hemostatic abnormalities.

**Newly Identified Hormone Erythrophore Provides Erythroblasts with Iron on Demand**


Editor's note: Data published in this paper were presented in abstract form by Dr. Kautz at the Plenary Session of the 2013 ASH Annual Meeting & Exposition in New Orleans, California, Los Angeles have identified an erythroid cell hormone, erythrophore (ERFE), that suppresses hepcidin production through pathways other than IL-6/JAK-STAT3-BMP/SMAD and, thereby mediates an acute increase in iron mobilization and absorption during periods of erythropoietic stress (Figure).

After erythropoietic stimulation, differentiating erythroblasts in the bone marrow and spleen rapidly increase ERFE production in an EPO- and STAT5-dependent manner. ERFE is secreted into the circulation and acts directly on the liver to repress hepcidin production, ERFE-mediated hepcidin suppression in turn increases iron availability for new red blood cell synthesis.

The identification of ERFE as an erythropoietic regulator of iron mobilization and absorption through hepcidin suppression has scientific and clinical implications. The hepatic ERFE receptor and its intracellular signaling mechanisms are unknown, but a candidate transcription factor, ATOH8, was first associated with iron metabolism by Dr. Kautz et al. ATOH8 regulates transcription of the gene-encoding hepcidin through a mechanism separate from the BMP/SMAD pathway, and hepatic ATOH8 levels are reduced during erythropoietic stress. In a broader context, ERFE is expressed in skeletal muscle. It is recognized as a C1q/TNF-related protein (CTRP), termed myocitrin (CTRP16), which regulates lipid metabolism. Myocitrin, which is induced after fasting or nutrient administration, acts through PI3-K/akt/mTOR-signaling and hepatic hypertrophy, suggesting a possible role for this pathway in hepcidin regulation.

In clinical hematology, excess iron accumulation complicates diseases with a major component of ineffective erythropoiesis, such as β-thalassemia, in which erythroblast numbers are greatly expanded, but the erythroblasts undergo intraerythroid apoptosis before differentiation. Group expanded erythroblasts in ineffective erythropoiesis produce large amounts of ERFE, and treatment that mitigates ERFE effects would be expected to reduce inappropriate iron absorption. Indeed, a model of β-thalassemia intermedia in which mice also have ERFE gene knockout, Dr. Kautz and colleagues showed that serum hepcidin is normalized and hepatic non-heme iron content is lower while Hgb values are similar when compared with thalassemic mice with normal ERFE activity. Conversely, increased hepcidin in the anemia of chronic inflammation restricts the erythropoietic response by limiting the availability of iron. Development of a therapy that stimulates ERFE production or activity could potentially decrease hepcidin in this setting and thereby ameliorate the iron-restrictive component of the anemia.


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**Pulling Strings Behind the Scenes to Control Red Cell Traits**


Non-coding ironing DNA sequences have lost the connotation of ‘junk DNA’ as their role in gene regulation has become increasingly apparent. Elegant studies by Dr. Ralph Stadhouders and colleagues of Erasmus Medical Center in Rotterdam, The Netherlands, demonstrate how sequence variations in one such intergenic region (HBS1L-MYB) influences the expression of fetal hemoglobin (HbF) and other erythocyte characteristics.

Red blood cell (RBC) parameters measured during a routine complete blood count provide important information for diagnosing and monitoring disease. It has long been known that there is significant variation in RBC parameters in individuals and in population groups, and genome-wide association studies (GWAS) have identified inherited DNA sequence variants linked to these clinically important traits. Of particular interest is the association of several erythrocyth parameters, including erythrocyte count, volume, hemoglobin concentration, and HbF levels with single nucleotide polymorphisms (SNPs) in the q32 region of chromosome 6. More precise localisation studies have revealed that these SNPs cluster in a 24 kb region between the HBS1L and MYB genes, establishing this intergenic area as a quantitative trait locus. The HBS1L gene encodes a member of the GTP-binding elongation factor family, but its function in erythroid biology is unknown. In contrast, the MYB gene plays an essential role in erythropoiesis as the encoded c-MYB protein is a transcription factor that influences expression of a number of erythroid specific genes. Regulation of the MYB gene in erythroid cells has not been completely characterized, but is known to involve microRNA-mediated control and a proximal promoter.

The functional roles of intergenic areas are difficult to assess because they are non-coding elements, but recent bioinformatic and technologic advances have provided new tools to assess chromatin interactions. The authors identified a 24-kb sequence that contained a cluster of core erythroid-specific enhancers located 84-84 and 71-71 kb upstream of the MYB promoter that are involved in long-range control of the MYB gene. Several key SNPs in close proximity to these enhancers attenuated their function, resulting in reduced expression of c-MYB, which correlated with increased expression of HbF. The SNPs changed critical nucleotides in the upstream transcription factor binding motifs, which decreased the association of transcription factors including GATA-1 and the LDB1 complex. The authors postulate that the clusters of transcription factors bound tightly to the MYB active chromatin hub, which promotes transcription. The intergenic SNPs prevent optimal binding of transcription factors and disrupt chromatin looping, destabilizing the hub and reducing transcription of the MYB gene.

The molecular mechanism whereby c-MYB regulates HbF and other erythrocyte traits is still unknown. It is speculated that the cell cycle may be dysregulated by the binding of c-MYB to cell-cycle components, resulting in premature termination of proliferation. Such a process would be expected to produce younger red cells with a higher mean cell volume and increased HbF levels. Alternatively, c-MYB may activate key β-globin repressor genes and thereby exert control on HbF production.

Inducing HbF in adults has therapeutic implications as doing so would ameliorate the severity of β-thalassemia and sickle cell disease. Targeting transcription factors is challenging, but this study provides evidence that intergenic enhancers play a key regulatory role and thus may offer an alternative target. Ingenious genome editing techniques have recently been developed, including the clustered, regularly interspaced, short palindromic repeat (CRISPR)-associated protein (Cas) (CRISPR-Cas) system, which could be used to modify the -84 and/or -71 MYB enhancer sequences to diminish c-MYB expression and thereby augment HbF production.

This study provides insight into the complexity of long-range molecular mechanisms underlying MYB gene expression and establishes a framework for the development of novel therapeutic strategies.

THERESA COETZER, PhD

Dr. Coetzey is a recipient of the South African Nationalists of interest.
nonfatal thrombotic event.1 Although subsequent clinical experiments confirm that heparin died; among the 15 untreated patients, five died and five experienced a hemorrhagic stroke (2.0% vs. 0.2%; p=0.003) were more common in the experimental arm received heparin and a placebo. Both the patient and the treating investigator were blinded to treatment assignment unless rescue thrombolysis was required.3 In the placebo group died (p=0.02). By day 30, the all-cause mortality rates were 2.4 percent in the tenecteplase group, and 3.2 percent in the placebo group (p=0.42). Both extracellular 2 (6.9% vs. 1.2%; p<0.001) and hemorrhagic stroke (2.0% vs. 0.2%; p=0.003) were more common in the tenecteplase group. Pre-specified subgroup analysis suggested that the increased risk of death or thromboembolism with tenecteplase was greater in patients over 75 years of age (odds ratio [OR] = 20.38 if age > 75 years vs. OR ≤ 2.80 if age ≤ 75 years; p-value for interaction = 0.09).

There are several important messages that come from this study of more than 1,000 patients with intermediate-risk PE. First, the addition of tenecteplase to anticoagulation did not effect a statistically significant reduction in overall mortality. Much like another study of hemodynamically stable, intermediate-risk pulmonary embolism. N Engl J Med. 1960;36:1139-1312.

A recent published Pulmonary Embolism Thrombosis Study (PEITHO) was designed to determine whether hemodynamically stable patients with echocardiographic and laboratory markers of increased mortality risk would benefit from adding intravenous (systemic) tenecteplase, a fibrinolytic agent, to anticoagulation. To be included in the trial, a patient had to have right ventricular dysfunction on echocardiography or computed tomography, as well as myocardial injury (defined by an elevated serum concentration of cardiac troponin I or troponin T). The primary outcome measure was death or hemodynamic decompensation within seven days after randomization. Patients in the placebo group died (p<0.01). By day 30, the all-cause mortality rates were 2.4 percent in the tenecteplase group, and 3.2 percent in the placebo group (p=0.42). Both extracellular 2 (6.9% vs. 1.2%; p<0.001) and hemorrhagic stroke (2.0% vs. 0.2%; p=0.003) were more common in the tenecteplase group. Pre-specified subgroup analysis suggested that the increased risk of death or thromboembolism with tenecteplase was greater in patients over 75 years of age (odds ratio [OR] = 20.38 if age > 75 years vs. OR ≤ 2.80 if age ≤ 75 years; p-value for interaction = 0.09).

The stakes are high when a clinician decides whether to administer thrombolytic therapy for intermediate-risk PE. To be included in the trial, a patient had to have right ventricular dysfunction on echocardiography or computed tomography, as well as myocardial injury (defined by an elevated serum concentration of cardiac troponin I or troponin T). The primary outcome measure was death or hemodynamic decompensation within seven days after randomization. Patients in the placebo group died (p<0.01). By day 30, the all-cause mortality rates were 2.4 percent in the tenecteplase group, and 3.2 percent in the placebo group (p=0.42). Both extracellular 2 (6.9% vs. 1.2%; p<0.001) and hemorrhagic stroke (2.0% vs. 0.2%; p=0.003) were more common in the tenecteplase group. Pre-specified subgroup analysis suggested that the increased risk of death or thromboembolism with tenecteplase was greater in patients over 75 years of age (odds ratio [OR] = 20.38 if age > 75 years vs. OR ≤ 2.80 if age ≤ 75 years; p-value for interaction = 0.09).

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The Quelling Cotoss is Over: Vascular Endothelium is the Source of Both Factor VIII and von Willebrand Factor


As a medical student in 1974, I read in the 7th Edition of Wintrobe’s Clinical Hematology that the sites of production of factor V (FVIII) had yet to be defined.1 Roles for the liver, spleen, and reticuloendothelial system had been proposed. Transplantation of a normal liver into a hemophiliac dog resulted in a 50 percent increase in FVIII, while transplanting a hemophiliac liver into a normal dog decreased the FVIII by 50 percent.2,3 These experiments suggested that extracapillar cells also produce FVIII. In the 1980s, immunohistochemical assays showed FVIII antigen in guinea pig liver and spleen. FVIII antigen could be found in normal human liver sinusoidal endothelial cells, as well as in extracts of human lymph nodes, lung, liver, and spleen.4 The localization of antigen in tissues did not, however, distinguish sites of FVIII synthesis from those of storage, and such experiments were subject to misinterpretation due to entrapment of plasma FVIII in tissues. FVIII mRNA was detected in isolated human hepatocytes, as well as in the spleen, the lymph nodes, and the kidney, but not in white blood cells or cultured endothelial cells. More recently, cell sorting studies of microvascular endothelial cells have documented FVIII production.5,6 Von Willebrand Factor, which circulates with FVIII, is known to be synthesized in endothelial cells and megakaryocytes, but definitive proof of the endothelial origin of FVIII in vivo is lacking.7,8 Nonetheless, however, the results of two elegant studies, published in Blood, one from Dr. David Ginsburg’s laboratory at the University of Michigan and the other from Dr. Robert Montgomery’s laboratory at the BloodCenter of Wisconsin, demonstrate that endothelial cells are indeed the primary source of plasma FVIII.

The Michigan group took advantage of the special function of LMAN1, a cargo receptor that is responsible for efficient secretion of factors V and VIII into plasma. LMAN1 mutations in mice reduced plasma concentrations of both factors to 10 to 15 percent of control. Using LMAN1 conditional murine knockout mice, the investigators demonstrated that endothelial cells were the primary site of FVIII biosynthesis, while hepatocytes made no significant contributions to the FVIII plasma pool. These investigators are to be congratulated for solving a long-standing mystery that I first learned of while reading Wintrobe 40 years ago. Upon reflection, it is not surprising that both factors are synthesized by endothelial cells. FVIII is a large protein, requiring a specialized machinery to efficiently pack the molecule into the circulation. Endothelial cells are known to have specialized functions to ensure optimal function of clotting factors. For example, endothelial cells are responsible for the efficient secretion of von Willebrand factor, a multi-functional protein that is phagocytosed by macrophages of the reticuloendothelial system undergoing degradation. During this process, the hemeprotein that dissociates from globin is metabolized to heme, the ferric state of heme with a chloride ligand, and subsequently converted to biliverdin by heme oxygenase-1 (HO-1), a reaction that releases iron and generates carbon monoxide. By studying T cells and monocytes from two groups of transfused SCD patients (alloimmunized and non-alloimmunized), Dr. Zhong and coworkers found that heme-induced differential polarization of CD4+ T-cell subsets. This process was dependent on HO-1 in monocytes suggesting that a degradation product(s) of heme may contribute to FVIII polarization. The difference in polarization was most evident using CD16+ monocyte-enriched fractions, where exposure to heme increased Treg expansion and inhibited Th1 proliferation in non-alloimmunized patients with SCD, but had little effect on Treg/Tfh polarization in the alloimmunized group (Figure). Moreover, following exposure to heme, both higher baseline levels of HO-1 in CD16+ monocytes and a more robust anti-inflammatory T-cell polarization profile was observed in samples from the non-alloimmunized SCD patients compared with those from the alloimmunized group (Figure).

The work by Dr. Zhong and coworkers suggests that the immunoregulatory function of innate immune cells, particularly the responses of CD16+ monocytes to heme, may be compromised in alloimmunized SCD individuals, rendering them unable to induce Treg expansion or inhibit Th1 development, whereas exposure to heme induces anti-inflammatory responses in non-alloimmunized transfused patients. This finding is intriguing but because this was not a prospective, longitudinal study, we are left to wonder whether the differences in innate immune responses precede the development of alloimmunization or whether alloimmunization itself modulates the immune response. We would also like to know which product of heme degradation by HO-1 (e.g., iron, biliverdin, bilirubin) mediates HO-1–dependent T-cell polarization. Addressing these issues could lead to methods for identifying individuals at risk for alloimmunization and suggest potential therapeutic targets to prevent alloimmunization in patients with SCD who otherwise benefit from chronic transfusion programs.

Proposed model of differential heme-induced, HO-1–dependent, CD16+ monocyte-mediated T-cell polarization that might govern risk of alloimmunization in transfused patients with SCD. In addition to the HO-1-dependent effects on CD4+ T-cell subset polarization (detailed in the narrative), heme treatment reduces IL-12 activity in stimulated monocytes from non-immuno-SCD patients (left panel) but not in stimulated monocytes from alloimmunized SCD patient (right panel). As HO-1 contributes to the pro-inflammatory polarization state (low Treg/high Th1 (Teff)) in SCD (right panel), this antagonistic effect on IL-12 activity further supports the heme-induced anti-inflammatory polarization state (high Treg/low Th1 (Teff)) in non-alloimmunized patients (left panel).


GREGORY M. VERCELLOTTI, MD
Dr. Vercellotti holds several inventions of interest.

CHARLES T. QUINN, MD, FRS
Dr. Quinn indicated no relevant conflicts of interest.

Does the Innate Immune System Determine Alloimmunization in Response to Red Blood Cell Transfusion?


Transfusions of red blood cells (RBCs), especially as given as part of chronic transfusion programs, are monoyseqly used as disease-modifying therapy for patients with sickle cell disease (SCD). The increased use of chronic transfusions over the past decade has been driven by expanded indications, with the major contributor being primary stroke prevention in children who are identified through transcranial Doppler ultrasonography screening programs. Also contributing to the increase in transfusions in patients with SCD is the wider availability of methods to limit and monitor transfusional iron loading, including oral iron chelators, automated erythropoietesis, and magnetic resonance imaging to quantify tissue iron. Despite these advances, alloimmunization to RBCs remains a vexing problem, even when extended antigen-matched products (e.g., RH C-, D-, and E-matched units) are used. Depending on the degree of matching, clinically significant alloimmunization may occur in 10 to 30 percent of chronically transfused patients with SCD. Allo-antibodies are problematic because they complicate RBC cross-matching, delay availability of blood products, increase the labor and cost of providing compatible units, shorten RBC survival, and potentially cause hemolytic transfusion reactions (that in some cases can be life-threatening). Occasionally, severe alloimmunization can preclude the use of chronic transfusions altogether, leaving patients with few therapeutic alternatives.

Thus, a key clinical question remains: Why do some chronically transfused individuals with SCD become alloimmunized? Previously identified risk factors for alloimmunization include the degree of antigenic discordance between the recipient and donor, the extent of donor-recipient antigen matching of transfused products, the patient’s age at the time of first transfusion, and the overall transfusion burden. Recent work has also suggested that the genetic complexity of the Rh locus, specifically variant alleles that are in linkage disequilibrium with Rh antibody expression, might contribute to alloimmunization. The current study by Dr. Hui Zhong and colleagues from the New York Blood Center and the University of Pennsylvania investigated the role of the recipient’s immune response to heme. Sesscnet red cells that are photolyzed by macophages of the reticuloendothelial system undergoing degradation. During this process, the heme moiety that dissociates from globin is metabolized to heme, the ferric state of heme with a chloride ligand, and subsequently converted to biliverdin by heme oxygenase-1 (HO-1), a reaction that releases iron and generates carbon monoxide. By studying T cells and monocytes from two groups of transfused SCD patients (alloimmunized and non-alloimmunized), Dr. Zhong and coworkers found that heme-induced differential polarization of CD4+ T-cell subsets. This process was dependent on HO-1 in monocytes suggesting that a degradation product(s) of heme may contribute to FVIII polarization. The difference in polarization was most evident using CD16+ monocyte-enriched fractions, where exposure to heme increased Treg expansion and inhibited Th1 proliferation in non-alloimmunized patients with SCD, but had little effect on Treg/Tfh polarization in the alloimmunized group (Figure). Moreover, following exposure to heme, both higher baseline levels of HO-1 in CD16+ monocytes and a more robust anti-inflammatory T-cell polarization profile was observed in samples from the non-alloimmunized SCD patients compared with those from the alloimmunized group (Figure).

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The Role of Brentuximab Vedotin in Previously Untreated Patients with CD30-Positive T-Cell Lymphoma

**STUDY DESIGN:** In this phase III study, adult patients are randomized 1:1 to receive either conventional CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or 1.8 mg/kg of brentuximab vedotin (BV) plus standard-dose CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) for six to eight cycles, per investigator discretion. To be eligible, patients must have newly diagnosed CD30-positive primary cutaneous T-cell lymphoplastiferative disorder or mycosis fungoides, with fluorodeoxyglucose (FDG)-avid disease by positron emission tomography (PET) and measurable disease of at least 1.5 cm by CT and an Eastern Cooperative Oncology Group (ECOG) performance score ≤2 with adequate organ function.

In this trial, patients entered two phases: a 4-year follow-up, and patients with a complete response rate of ≥80% are eligible for study continuation. The primary endpoint is progression-free survival (PFS) and overall survival (OS) after 4 years for patients who achieve complete response with BV plus CHOP versus CHOP in 121 patients. The secondary endpoint is the complete response rate at the end of two years for patients receiving BV in combination with chemotherapy across different subtypes of CD30-positive T-cell lymphomas with respect to rate of CD30 expression may suggest that further study of BV in other lymphoid malignancies without high-density CD30 expression is warranted.

— Ann LaCasce, MD, MSc

**FGD-PET as a Guide for Consolidation Radiotherapy in Primary Mediastinal Lymphoma**

**STUDY DESIGN:** Adult patients with previously untreated primary mediastinal lymphoma (PMLB) confirmed by immunohistochemistry with a dominant mass in the anterior mediastinum are registered at the time of initial diagnosis and receive a course of standard chemotherapy such as CHOP-14 or CHOP-21, high-dose CHOP, dose-adjusted (DA) EPOCH, ACVBp, YACOP-B, or MACOP-B together with at least six doses of rituximab (R-chemotherapy). Within five to six weeks of completing the chemotherapy, subjects undergo a restaging fluorodeoxyglucose-pet-positron emission tomography (FDG-PET) scan, the outcome of which is centrally reviewed by a panel of experts via a dedicated website. Those with uptake of three or more lesions, or less than the five-point Deauville scale (FDG uptake at or below the level of the liver) will be randomized 1:1 either to undergo consolidation mediastinal field radiotherapy or to receive no further treatment. The randomization is stratified by country, gender, type of chemotherapy given, and FDG-PET score. The radiotherapy dose is 30 Gy given as 15 to 20 daily fractions, commenced within eight weeks of the last administration of R-chemotherapy. Intensity-modulated radiotherapy is allowed to decrease the dose to avoid injuring normal structures including the heart, lungs, and breasts. Patients with a partial response and a positive FGD-PET result (FDG uptake in a residual mass greater than in the liver) will go on to receive either consolidation radiotherapy or other salvage regimen, according to local practice. Follow-up is performed by scheduled CT scans at six, 12, and 24 months and clinical review up to five years.

The primary endpoint is the progression-free survival (PFS) at two years in all the randomized patients. The trial is powered to determine a non劣ior outcome in patients not irradiated, based on a hazard ratio of 0.72 (one-sided a error 0.05), which requires a sample size of 376 randomized patients. Overall survival, safety, and long-term outcomes are secondary endpoints. Two interim analyses are planned. When patients with an end of treatment PST score of three or less have been randomized to observation and followed for six months, the Independent Data Monitoring Committee will review the event rate of randomized patients to determine if there is evidence of early progression in the cohort that did not undergo radiation treatment. A further interim analysis will be performed when 25 percent of the total expected events have occurred, with a critical value ≤0.005 based on a log-rank test comparing PFS times.

**RATIONAL:** Given the young age of many patients with PMBL and the need to avoid long-term toxicity from mediastinal radiotherapy wherever possible, it is important to establish definitively which patients do not require irradiation. Incorporating FGD-PET assessment at the end of chemotherapy as the basis for randomization is one approach to addressing this issue. A previous cohort study by the ELSG of 115 patients with PMBL demonstrated excellent results using conventional R-chemotherapy and mediastinal radiotherapy in most cases, with five-year overall survival and PFS rates of 92 percent and 86 percent respectively and highly significant differences between those having a PFS score of one to three versus four to five at the completion of chemotherapy (Ann Oncol 2012;3:1769-1775).

There are data from a cohort treated with RDA-EPOCH to suggest that, in many patients, consolidation radiotherapy may not be necessary (Dunleavy K et al. N Engl J Med 2013;368:1408-1416), a finding supported by a retrospective study from the British Columbia Cancer Agency where R-CHOP was used as the R-chemotherapy regimen (Savage KL et al. Blood. Ash Annual Meeting Abstracts. 2012;120:303). In light of these findings, it is logical to undergo a prospective study of the safety of omitting mediastinal irradiation in those patients with the most favorable response to chemotherapy.

**COMMENT:** The PFS figures for patients treated with R-CHOP and mediastinal radiotherapy are excellent, but there is a feeling that we may be overusing radiation and that limiting its use to those with residual active disease would be preferable. The complicated RDA-EPOCH regimen seems to result in cures without radiotherapy, but there is reluctance to adopt this regimen widely in the absence of confirmatory results from prospective randomized trials. The difficulty of salvaging PMBL that has relapsed is another cautionary factor. Second-line chemotherapy has a poor record in this illness, so compelling data are needed if the escalation of initial therapy is to be recommended to some patients. The hazard ratio allowed for equivalence in this study is relatively high, but the early safety analyses have been carefully designed.

— Peter Johnson, MD

Dr. Johnson indicated no relevant conflicts of interest.
Acquired aplastic anemia (AA) is a rare disease characterized by progressive and, in the absence of treatment, generally irreversible loss of regenerative bone marrow function, resulting in life-threatening multilineage cytopenias. The incidence is in the range of one to three events per million in Western industrialized countries, and there is no predilection for gender, age, or ethnicity. Clinical observations and mounting experimental evidence suggest an underlying acquired cellular immune attack on the hematopoietic stem cell as the disease etiology in the majority of patients. Both the diagnosis and treatment of children with AA pose unique challenges, which the recently formed North American Pediatric Aplastic Anemia Consortium (NAPeAC), composed of representatives from 22 U.S. and Canadian institutions, aims to address prospectively.

Owing to the resulting high rate of overall and disease-free survival, HLA matched related donor hematopoietic stem cell transplantation (MRD-HSCT) is the preferred treatment for children with severe AA. However, MRD-HSCT is available only to a minority of patients because most do not have a suitable donor. The majority of patients are instead treated with an intensive immunosuppressive therapy (IST) regimen. A recent NAPeAC survey of treatment practices1 showed that, although an antithymocyte globulin– (ATG–) plus cyclosporine A– (CSA–) based combination is nearly universally used, there is broad variation among institutions in the manner of implementation of the regimen. In this article, we highlight some of the most pressing issues related to the diagnosis and care of children with AA that were identified in the NAPeAC report. The results of that study underscore the need for development of evidence-based guidelines for diagnosis and management of children with acquired AA.

Areas of Controversy

Testing for inherited bone marrow failure syndromes

Diagnosis of acquired AA requires exclusion of an underlying constitutional syndrome. Screening for inherited bone marrow failure (BMF) syndromes (e.g., Fanconi anemia, telomere diseases including dyskeratosis congenita, Diamond-Blackfan anemia, or Shwachman-Diamond syndrome) is of particular importance in young patients, as these syndromes most frequently present during childhood, and AA may be the only clinical feature that is apparent at the time of presentation and in the absence of specific laboratory-based testing. Additionally, as the inherited BMF syndromes are not responsive to IST and require adherence to specific HSCT protocols (e.g., directed testing of potential pre-symptomatic sibling donors and use of disease-tailored conditioning regimens), missed diagnoses can have dire consequences. Alternatively, excessive, unnecessary diagnostic testing should be avoided. The NAPeAC survey found testing for inherited BMF syndromes in children at the time of their AA presentation to be variable. For example, whereas most centers (89%) test for Fanconi anemia, fewer (67%) test for dyskeratosis congenita. These and related findings suggest that codification of a standardized diagnostic approach would facilitate future multi-institutional prospective clinical studies.

Infection prophylaxis

Given the profound lymphodepletion that accompanies ATG administration, along with temporary use of corticosteroids, prolonged administration of CSA, and severe neutropenia, the majority of pediatric centers use antibacterial (including against Pneumocystis jirovecii) and antifungal prophylaxis in their patients with AA who undergo treatment with an ATG-CSA-based regimen. However, multiinstitutional prospective trials designed to test the efficacy of supportive care regimens in this patient population are currently lacking.

Growth factor use

The NAPeAC survey found that, in most centers, the use of granulocyte colony-stimulating factor (G-CSF) is restricted to patients with severe AA during neutropenic infections. This limited use of G-CSF may have resulted from thoughtful interpretation of the results of several trials that found no significant impact of G-CSF on survival in patients treated with ATG-CSA-based IST, and that suggested that G-CSF is a risk factor for transformation of acquired AA into myelodysplastic syndrome in patients who failed to respond to IST. Some centers, however, continue to use G-CSF at the start of treatment, highlighting the need for definitive pediatric-based studies designed to address the use of growth factor support in patients with acquired AA.

Duration of IST and post-IST management

Most centers taper the CSA component of the IST regimen once the patient becomes transunion independent and an adequate neutrophil count is maintained. However, given the absence of prospective studies focused on the long-term use of CSA in the treatment of children with acquired AA, carefully designed studies are needed in order to establish an evidenced-based optimal nadir concentration range for CSA; a recommendation for duration of CSA treatment and for tapering CSA; and guidelines for follow-up evaluation and management, especially for partial responders. As relapses are relatively common and clonal evolution can occur in patients many years after treatment, monitoring and management of children with treated acquired AA, who have a life expectancy of many decades, need to be optimized.

Matched unrelated donor HSCT and treatment for patients in whom primary IST fails

With the availability of high-resolution HLA typing and improvements in both conditioning strategies and supportive care and treatment of graft-versus-host disease, results after matched unrelated donor (MUD) HSCT in children with acquired AA have improved markedly. In fact, some small case series suggest outcome parity when compared to MRD-HSCT. Accordingly, some centers now favor MUD-HSCT over repeat IST for patients in whom the initial ATG-CSA-based treatment course fails. Currently however, there is no consensus on treatment of refractory or relapsed disease, and optimal management is a moving target given the development of new therapeutic modalities such as eltrombopag, which NAPeAC hopes to include in future studies.

Treatment of moderate AA

Not all children present with symptomatic, severe disease. Some maintain stable, low-grade cytopenias for months before recovering or progressing to severe AA. Going forward, it will be important to address, in this group of patients, whether preemptive IST treatment confers a more favorable outcome or whether watchful waiting prevents unnecessary exposure to immunosuppressive drugs.

Conclusions

The review of practices across major centers in the U.S. suggests that to optimize the treatment of children with acquired AA, a number of critical issues must be addressed.1 And because of the rarity of the disease, cooperative group studies such as those being initiated by NAPeAC are needed to generate evidenced-based management guidelines. The group is developing a website with resources for physicians and an opportunity to connect with experts in the field. NAPeAC’s long-term goal is to establish not only a unique database and clinical standards of care for children with acquired AA, but also a biospecimen repository that will serve to maximize group-wide resources, including applying the latest genomic technologies to gain a clearer understanding of the pathobiology of this challenging disease. NAPeAC encourages and welcomes participation of groups and individuals interested in joining in the effort to optimally manage children with acquired AA.


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The cellular site of synthesis of coagulation factor VIII has long been debated. These clinical trials that focused on chronic lymphocytic leukemia (Dr. Jennifer Brown and colleagues), mantle cell lymphoma (Dr. Brad Kahl and colleagues), and indolent non-Hodgkin lymphomas (Dr. Ian Flinn and colleagues) showed a reduction in tumor burden in heavily pretreated patients, with some responses lasting up to two years. The toxicity profile was deemed acceptable. These studies pave the way for clinical trials combining idelalisib with other noncytotoxic drugs and provide further support for the prospect of future chemotherapiy-free treatment of these lymphoid malignancies.


Transfusion-related acute lung injury (TRALI) remains the leading cause of transfusion-related fatalities. Using an animal model, Dr. Christopher McKenzie and colleagues reported results consistent with a two-step mediators. Antibody-conjugated TRALI in the first step, an F(ab')2-dependent process, involves stimulation of secretion of macrophage inflammatory protein-2 upon binding of anti-major histocompatibility complex-specific antibody to its cognate antigen on monocytes, thereby recruiting neutrophils to the lungs. This step is not associated with pulmonary edema or mortality. The second step, which induces lung damage, is an Fc-mediated, monocyte-dependent process. In the article by Dr. Christopher Silliman and colleagues, a novel filtration system was used to remove anti-lymphocyte antigen and anti-neutrophil antibodies from plasma and blood samples. This procedure removed the antibodies and their respective lipid priming activity and mitigated disease pathology in an antibody-mediated animal model of TRALI. Conceivably, removal of proinflammatory activity by filtration could reduce the incidence of TRALI or mitigate disease severity.

May 22, 2014

Dr. Sarah Atkinson and colleagues report that there is dynamic variation in hepcidin levels in African children in relation to the malaria season. During the malaria season, active infection and inflammation induce hepcidin, inhibiting iron absorption despite iron loss due to the hemoglobinuria that is a consequence of infection-induced hemolysis. In contrast, at the end of the season, iron deficiency is common and hepcidin levels fall. Iron therapy is thus likely to be more effective during the malaria "off-season."

Annual Meeting Reminders
We hope you will join us for the 56th Annual Meeting in San Francisco, California. If you haven’t already registered for the meeting, keep in mind that the advance registration deadline is November 5, at 11:59 p.m. EST. If you are interested in submitting a late-breaking abstract, the submission website opens October 21 and closes October 28. The selection process for late-breaking abstracts is competitive; a maximum of six abstracts will be selected, regardless of the number of submissions. Reviewers will focus on identifying high-impact abstracts, and an effort will be made to balance the program such that both basic/translational and clinical research are equally represented in the program.
As technology and the Web have evolved, so too have ASH’s online offerings. Now, beyond the ASH website, you can download ASH apps for your smartphone or tablet, follow ASH on Twitter (www.twitter.com/ASH_hematology), and find ASH videos on YouTube (www.youtube.com/user/ASHWebmaster). Our newest offering is ASH on Facebook.

**ASHTest – A New Option for Maintenance of Certification and Continuing Medical Education**

ASH Academy, the eLearning platform for hematologists, now offers multiple-choice tests called ASHTest: Malignant Hematology and ASHTest: Non-Malignant Hematology that provide both maintenance of certification (MOC) and continuing medical education (CME) credit. These 30-multiple-choice-question tests require an 80 percent pass rate in order to claim credit. It is anticipated that a learner answering all questions and reading the accompanying rationales will spend two hours completing each test. All test questions were developed by subject-matter experts using readily available educational material.

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**MARK YOUR CALENDAR**

**September**

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

**October**

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

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

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

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

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

**November**

5 ASH annual meeting advance registration deadline
San Francisco, CA [www.hematology.org/meetings](http://www.hematology.org/meetings)

5 ASH annual meeting individual hotel reservation deadline
San Francisco, CA [www.hematology.org/meetings](http://www.hematology.org/meetings)

6 ASH annual meeting late/on-site registration begins
San Francisco, CA [www.hematology.org/meetings](http://www.hematology.org/meetings)

25 ASH annual meeting registration cancellation deadline
San Francisco, CA [www.hematology.org/meetings](http://www.hematology.org/meetings)

**December**

6-9 2014 ASH Annual Meeting & Exposition
San Francisco, CA [www.hematology.org/meetings](http://www.hematology.org/meetings)

*In order to attend, you must have submitted an application by July 31, and you must have been nominated and invited to attend.

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