Inhibiting EZH2 in Lymphoma: Breaking the Silence of the Genes


The discovery that a large proportion of germinal center lymphomas have mutations in epigenetic regulator genes has been one of the notable revelations from genome sequencing in recent years. This finding has rapidly led to the testing of small molecule inhibitors that might reverse the defective processes caused by these mutations, and this paper from the group at GlaxoSmithKline laboratories describes the results for one such inhibitor, GSK126. The target of the inhibitor is EZH2, an enzyme that is affected by gain of function mutations in up to a quarter of cases of diffuse large B-cell lymphomas (DLBL) and follicular lymphoma. As the enzymatic component of the polycomb repressor complex, EZH2 is involved in transcriptional repression through chromatin condensation by methylation of histone H3 on lysine 27 (H3K27). Thus, EZH2 functions as a histone-lysine methyltransferase.

GSK126 was identified from a high throughput screen of compounds that inhibited the EZH2 catalytic subunit. Following optimization of structure, GSK126 was found to be active at low nanomolar concentrations, inhibiting both wild-type and mutant EZH2 with a high degree of selectivity. Fifty-percent reduction of H3K27 methylation in DLBL cell lines was observed at GSK126 concentrations between 7 and 252 nM. Although the suppression of methylation was not different between EZH2 wild-type and EZH2 mutant lines, proliferation assays showed that six of the seven most sensitive lines bore EZH2 activating mutations. The effects were independent of the presence of BCL2 translocations or p53 mutations and appeared to be mediated by caspase cleavage and apoptosis in some lines but not others, reflecting different degrees of dependence on EZH2 activity. Loss of gene expression indicated de-repression of EZH2 targets by GSK126 in sensitive lines, with the specificity of this effect confirmed using shRNA to knockdown EZH2 expression. The gene de-repression profile was significantly different between cell lines, with sensitive lines showing alterations in pathways involved in cell-cycle regulation, cell death, and control of biologic/cellular processes. Cell lines in which both high EZH2 expression and high levels of H3K27 methylation were present together were the most sensitive to GSK126.

The investigators went on to test the effects of GSK126 in xenograft models, where they showed that the cell lines that had been sensitive in vitro were also responsive in vivo. Mice treated with 50 mg/kg daily showed tumor growth arrest, although larger doses of 150 to 300 mg/kg daily were required to prevent re-growth following withdrawal of the drug. Further studies of weekly schedules suggested that the effective half-life of the drug would permit intermittent dosing. The treatment appeared to be well tolerated at the doses described, and interestingly, there were no signs of bone marrow suppression, despite the many effects of EZH2 on normal hematopoiesis.

Modifiers of epigenetic regulation such as the histone deacetylase inhibitors have so far yielded interesting but modest results in the treatment of B-cell lymphoma, despite some evidence in preclinical studies of synergy with rituximab. As single agents, their activity is limited, and few combination studies have been performed to date. Nonetheless, there is mounting evidence of the fundamental role that epigenetic dysregulation plays in the pathophysiology of germinal center lymphomas, with almost all follicular lymphomas and a substantial proportion of DLBL showing mutations in genes that are involved in histone modification (acetylation or methylation). Mutation of epigenetic regulators appears to be an early event in disease pathogenesis, making the resulting aberrant process a tempting target for specific therapy. The preclinical studies reported by McCabe and colleagues can be seen as proof of concept; however, questions such as whether the presence of EZH2 mutations is essential for response in vivo, or whether looking for specific patterns of methylation in the tumor may be predictive of response, remain to be answered. The results of the first-in-man studies are eagerly awaited.
ASH Offers Complimentary Membership to Students and Residents

During the business meeting at the 2012 ASH Annual Meeting, the membership voted to approve several bylaw changes. Most notable among the changes was the creation of two new membership categories, one to admit medical and graduate students and a second to admit residents. The goal of adding these categories was to create a continuous relationship with ASH for both physicians-in-training and scientists with interest in hematologic diseases.

Members in both categories will receive most of the regular benefits of membership at no cost, including the online version of Blood, The Hematologist, and Hematology (the Education Program) as well as reduced meeting registration fees. Beginning in 2014, nominations for some ASH awards geared toward early-stage trainees, including the new ASH HONORS Award for up-and-coming researchers, will be limited to student/resident members. However, student and resident members will not be able to vote, hold office, or sponsor the abstract or membership application of another individual.

Eligibility for these new categories is currently limited to students and residents enrolled in accredited programs in the United States, Canada, and Mexico. Membership applications will require institutional verification of enrollment and sponsorship from an ASH member. Applications will be approved on a rolling basis throughout the year. The application form and instructions for applying will be available at www.hematology.org/Membership/Categories.aspx. Information on how to apply will be available on this page in the coming months.

North American undergraduate students and graduate students not enrolled in a PhD granting program will still be able to access ASH programs through the North American Student Benefit program.

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:
Karen Learner, Managing Editor
The Hematologist: ASH News and Reports
2021 L Street, NW, Suite 900
Washington, DC 20036
klearner@hematology.org

Fighting for Hematology Research

It is an absolute honor to write as president of ASH, and I am especially honored to be your leader at this complex time when our challenges as hematology clinicians and researchers are huge, but when the opportunities are equally compelling.

I am writing this first column on my plane home from our December meeting, excited by what we just heard. Genomic information is well poised to provide new paradigms for diagnosing hematologic disorders, risk stratification, and treatment. Model systems from primary cell cultures to iP cells to zebrafish to mice are available as tools for discovery. Small molecules and chimeric antigen receptors engineered onto autologous cells have huge therapeutic potential for our patients. I scribbled notes to myself with ideas for new studies and new collaborations. Our science is strong, our Society is strong, and we value each other as colleagues and friends. This is an exciting time to be a hematologist.

But this is also a challenging time, as the funding of hematologic research and the funding of research more broadly are threatened. In a survey of 1,040 presenters of abstracts at our meeting, 63 percent of those from the United States reported that their work was supported by National Institutes of Health (NIH) monies. Should sequestration occur, NIH faces an estimated 8.4 percent budget decrease, and given its ongoing intramural and extramural commitments, this could translate into an estimated 2,400 fewer grants and a payline well below the current payline level of 7 to 13 percent. This means that far less than 7 to 13 percent of the R01 (investigator-initiated research) applications deemed scientifically excellent by review panels would be funded. If sequestration is averted, but an alternative deficit reduction plan in which science is insufficiently appreciated is implemented, equal or potentially more severe cuts are possible.

The NIH funds ideas, and ideas develop in a nonlinear fashion. The path from a hypothesis to a product is often too long, too circuitous, or too risky for pharmaceutical company investment. By funding testable ideas, NIH assures that innovation drives both scientific discovery and the resulting economic enterprise. Similarly, NIH funds research on rare but devastating disorders and on health disparities. The NIH also funds clinical trials and correlative studies that can significantly advance patient care without the potential of influence from financial incentive. Fifty-two percent of international presenters at our annual meeting said that they referenced an NIH-funded study in their own research, documenting the wide reach of NIH support.

By the time you read this column, sequestration may be resolved, and NIH support may be either more precarious or conserved in next year’s federal budget. Yet our advocacy should not stop. A budget is for one year, but ASH’s goal is the long-term sustainability of NIH-funded research. The ASH website is continually updated to reflect the most current political and economic circumstances. I urge you to “Fight 4 Hematology.”

Janis L. Albkowitz, MD

President’s Column

The Hematologist: ASH News and Reports
A New Year Brings Changes and Milestones

The new year marks the beginning of the 10th year of publication of The Hematologist. As we celebrate this milestone, we look forward to another year of discovery, innovation, and challenge, anticipating that change will be the rule, not the exception.

At the Editorial Board Meeting in December 2012, we said goodbye to Ken Anderson (Dana-Farber Cancer Institute) and Diane Krause (Yale University School of Medicine) who both served consecutive terms on the Board of Contributing Editors. Ken and Diane were two of our heavy lifters (sui generis!), and their contributions will be sorely missed. Still, we are grateful for the six years that they unselfishly dedicated to The Hematologist.

To replace Ken and Diane on the Board, we welcome Charles Quinn and Pamela Becker. Charles is associate professor of Clinical Pediatric at the University of Cincinnati College of Medicine and Cincinnati Children’s Hospital Medical Center. His long-standing interest in sickle cell anemia and thalassemia will fill a need of heightening awareness of the progress being made in these bellwether areas of hematology. Charles is the second pediatric hematologist member of the Board (along with Peter Kurre). Pam is associate professor of medicine in the Division of Hematology at the University of Washington School of Medicine. She has clinical and research interests in both benign hematology (inherited and acquired bone marrow failure syndromes) and malignant hematology (acute leukemia). Our readers are certain to benefit from the breadth of Pam’s interests and experience.

Working with Armand Keating over the past year has been a pleasure, and he highlighted in his President’s Column a remarkable number of innovative programs that were launched by the Society in 2012. Dr. Keating has passed the baton into the capable hands of Janis Abkowitz, and we at The Hematologist welcome the opportunity to keep our readers abreast of the direction of the Society and the view of the Executive Committee through Dr. Abkowitz’s iteration of the President’s Column (page 2). Jan’s first column deals with the critical issue of advocacy in support of the National Institutes of Health.

– Charles Parker, MD, Editor-in-Chief

ASH Members Elected to Institute of Medicine of the National Academies of Science

The Institute of Medicine (IOM) has announced the election of 70 new members, including three ASH members. Election to the IOM is considered one of the highest honors in the fields of health and medicine and recognizes individuals who have demonstrated outstanding professional achievement and commitment to service.

The three ASH members elected to the IOM are:

Bruce R. Blazar, MD
Regents Professor, Department of Pediatrics, Hematology-Oncology; Chief, Pediatric Blood and Marrow Transplantation Program; Associate Vice President of the Academic Health Center; Director, Clinical and Translational Science Institute; and Director, Center for Translational Medicine at the University of Minnesota

Carl H. June, MD
Richard W. Vague Professor in Immunotherapy, Department of Pathology and Laboratory Medicine and Director, Translational Research Program at the Abramson Cancer Center at the Perelman School of Medicine, University of Pennsylvania

Andrew J. Schafer, MD
The E. Hugh Luckey Distinguished Professor of Medicine and Chairman, Department of Medicine at Weill Cornell Medical College; Physician-in-Chief, New York-Presbyterian Hospital/Weill Cornell Medical Center
Ask the Hematologist

The Question

What is your approach to the diagnosis and management of mastocytosis?

Defining Mastocytosis

Mastocytosis is characterized by the accumulation and proliferation of neoplastic mast cells in one or more organs (e.g., skin, bone marrow, spleen, lymph nodes, liver, and gastrointestinal tract). Flushing, diarrhea, anaphylaxis, and neuropsychiatric symptoms resulting from mast cell release of bioactive molecules such as histamine, leukotrienes, and various inflammatory cytokines can impose a significant burden on a patient’s quality of life. Such mediator symptoms may be triggered by physical stimuli, exercise, alcohol, NSAIDs, insects, stings, or certain foods. In advanced disease, mast cell infiltration of tissues can lead to organ damage and shortened survival.

The World Health Organization (WHO) includes mastocytosis within the category of myeloproliferative neoplasms (MPN) and divides these disorders into cutaneous and systemic forms. The diagnosis of systemic mastocytosis (SM) is based on consensus diagnostic criteria (Table) and requires one major plus one minor criterion or three minor criteria. Clinical, morphologic, and biologic criteria are used to further classify SM into subtypes that generally reflect clinical severity and prognosis. In indolent SM (ISM), where bone marrow mast cell burden is relatively low, survival is similar to age-matched populations, and clinical issues are usually related to mediator symptoms. Smoldering SM (SSM) is a subtype of SM that reflects SM in transition toward more advanced disease and is characterized by two or more so-called “B-findings” (Figure), including organomegaly, and indicators of a relatively higher mast cell burden (e.g., >30% bone marrow mast cells and serum tryptase level >200 ng/ml). Advanced SM collectively refers to SM subtypes in which organ damage (referred to as “C” findings) is present. This includes the WHO-defined subtypes aggressive SM (ASM), mast cell leukemia (MCL), and SM with an associated hematologic non-mast cell lineage disease (SM-AHNMD). Approximately 90 percent of AHNMDs represent a myeloid neoplasm such as myelodysplastic syndrome (MDS), MPM, MDS/MPN (e.g., chronic myelomonocytic leukemia), eosinophilic disorders, or acute myeloid leukemia (AML). However, mast cell leukemia may be concurrently diagnosed with SM.

Mastocytosis is a challenging disease for many reasons. In the normal state, mast cells are not identified on the peripheral blood differential and represent a small fraction of the myeloid lineage on visual inspection of the marrow aspirate. The idiom “out of sight, out of mind” is apt in the case of SM and, together with its rare incidence, tests the physician to think about this neoplasm in the first place. If present, prototypic mediator symptoms such as flushing, diarrhea, and anaphylaxis may make this task easier. In advanced disease, organ damage such as cytopenias, liver dysfunction, ascites, lytic bone lesions, and hypoalbuminemia with weight loss due to mast cell infiltration of the gastrointestinal tract are sufficiently protean to conceal a unifying diagnosis. Because SM often partners with a myeloid disorder, immunohistochemical (IHC) stains (e.g., tryptase, CD117 [KIT], and CD25) are necessary to identify and quantify neoplastic mast cells in trephine biopsies and can additionally help unmask SM when it lives in the shadow of other neoplasms. Clinical and biologic heterogeneity, even within specific SM disease subtypes, can make it difficult to gauge disease trajectory and prognosis. In patients with an AHNMD, prognosis is usually related to the associated myeloid neoplasm, highlighting the need to fully characterize and stage the concomitant disease.

Diagnosis

In addition to a high index of suspicion, obtaining the expertise of a cadre of subspecialists (hematologists, dermatologists, and allergists/immunologists) as well as hematopathologists versed with the nuances of mast cell disease is important in establishing the correct diagnosis. A combination of clinical, morphologic, immunophenotypic, and molecular studies is required to establish the diagnosis of SM and its subtype. Bone marrow mast cell burden is best quantified by morphologic analysis, and use of the aforementioned IHC stains on the core biopsy is essential. The major criterion for SM requires demonstration of multifocal mast cell aggregates in the bone marrow (or other extracutaneous organ), of which >25 percent are atypical, often spindled-shaped mast cells. In MCL, mast cells account for >20 percent of nucleated cells on the BM aspirate and form a diffuse infiltrate on the core biopsy with or without circulating mast cells. Multi-parameter flow cytometry of the BM aspirate can also be used to quantify mast cells in the marrow and complements morphologic evaluation. The overwhelming majority of SM patients (>80%) carrying the activating KIT D816V mutation, which should be obtained from the bone marrow aspirate or from a core biopsy that is preserved in formalin to avoid degradation of DNA for PCR analysis. Determination of KIT D816V mutation status is critical to the diagnostic evaluation of SM, but it also guides treatment decisions. Although KIT D816V is a imatinib-resistant mutation, this tyrosine kinase inhibitor has been relievantly misapplied and overused in the treatment of SM. However, a small minority of patients (<5%) may exhibit juxtamembrane KIT mutations, which exhibit sensitivity to imatinib in vitro as well as in clinical practice. Such examples illustrate that sequencing the remainder of the KIT gene in SM patients who are negative for codon 816 mutations may prove fruitful. Total serum tryptase levels generally reflect the increased burden of mast cells in patients with SM. A serum tryptase level >20 ng/ml is an additional minor criterion for the diagnosis of SM. Although not always concordant, it is the most useful blood marker to assess changes in mast cell burden in response to cytotherapeutic drug. In advanced disease, staging studies include CT of the abdomen to assess hepato/splenomegaly, lymphadenopathy, and ascites; metastatic skeletal survey to evaluate osteolytic and/or pathologic fractures; and DEXA scans to follow osteoporosis, which is common in SM. Some patients with mediator symptoms who do not fulfill criteria for SM (e.g., only one or two minor criteria and/or exhibit a low burden of atypical mast cells without aggregates on biopsy) have been given the provisional diagnosis of mast cell activation syndrome (MCAS). The diagnostic criteria for this mast cell disorder are still evolving and its natural history is not well-defined.

Treatment

Individuals with symptomatic skin-only disease or SM with mediator symptoms are educated to avoid known triggers and are encouraged to carry an Epipen®, particularly those with a history of anaphylaxis or anaphylactoid symptoms. Antihistamines (H1- and H2-blockers) serve as the foundation for symptom palliation; leukotriene antagonists

Table

<table>
<thead>
<tr>
<th>Major criterion</th>
<th>Minor criteria</th>
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</thead>
<tbody>
<tr>
<td>Multifocal dense infiltrates of mast cells (&gt;15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s)</td>
<td>a. In biopsy sections of bone marrow or other extracutaneous organs, &gt;25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology or, if all mast cells in bone marrow aspirate smears, &gt;25% are immature or atypical.</td>
</tr>
<tr>
<td>Refers to a mastocytosis that is similar to age-matched populations, and clinical issues are usually related to mediator symptoms.</td>
<td>b. Detection of an activating point mutation at codon 816 in KIT in bone marrow, blood, or another extracutaneous organ</td>
</tr>
<tr>
<td>Defining mastocytosis within the category of myeloproliferative neoplasms (MPN) and divides these disorders into cutaneous and systemic forms.</td>
<td>c. Mast cells in bone marrow, blood, or other extracutaneous organs express CD2 and/or CD25 in addition to normal mast cell markers</td>
</tr>
<tr>
<td>World Health Organization diagnostic criteria for systemic mastocytosis*</td>
<td>d. Serum total tryptase persistently exceeds 20 ng/ml (unless there is an associated clonal myeloid disorder, in which case the parameter is not valid)</td>
</tr>
</tbody>
</table>

*Requires 1 major + 1 minor criterion or 3 minor criteria

JASON GOTLIB, MD, MS
Associate Professor of Medicine (Hematology) & Director, Hematology Fellowship Program, Stanford University School of Medicine and Stanford Cancer Institute

Figure

No B or C findings

Presence of 1 or more B findings

Presence of one or more C findings

1. Bone marrow biopsy showing >25% infiltration by mast cells (focal, dense aggregates) and serum tryptase level >200 ng/ml
2. Signs of dysplasia or myeloproliferation in non-mast cell lineages, but insufficient criteria for definitive diagnosis of a hematopoietic neoplasm in SM (B(NR)), with normal or only slightly abnormal blood counts (counts 3. Neutrophilic hyporegenerative myelopathy with impairment of bone function, and/or pituitary hypofunction and/or hypothalamic hypogonadism, and/or lymphopenia or polycythemia or imaging (2/3 cm)
4. Portal hypertension with hypergammaglobulinemia
5. Metastatic disease with weight loss due to SI mast cell infiltrates

The Hematologist: ASH NEWS AND REPORTS
Large Granular Lymphocyte Leukemia: Involvement of the JAK/STAT Pathway in Disease Pathogenesis

PEARLIE K. EPLING-BURNETTE, PharmD, PhD
Senior Member, H. Lee Moffitt Cancer Center, and Professor, University of South Florida, Tampa, FL

Large granular lymphocyte (LGL) leukemia is a rare (but probably under-diagnosed) chronic lymphoproliferative neoplasm that shares overlapping genetic and clinical characteristics with Felty syndrome. Unlike most lymphoproliferative processes that produce symptoms due to either clonal expansion within the bone marrow or infiltration by the neoplastic cells of lymphoid and/or non-lymphoid organs, the clinical manifestations of LGL leukemia (cytopenias [particularly neutropenia] and rheumatic and constitutional symptoms) are a consequence of immune dysregulation and aberrant cytokine production. First described in 1977 by McKenna et al. and shown to be of clonal origin in 1985 by Loughran et al., the disease was identified based on the presence of morphologically atypical lymphocytes with prominent azurophilic cytoplasmic granules. Flow cytometric analysis identified these cells as mature CD8+ T cells that co-expressed CD16 (Fcγ Receptor II). A clinically similar disorder was later described in which the LGL cells had a natural killer cell phenotype (CD56+, CD16−, CD56−). While largely thought to develop as a consequence of a sustained immune reactive process due to chronic antigen stimulation, identification of T-cell receptor (TCR) gene rearrangement in the T-cell variant indicated a clonal origin and in the abnormal T- or Nk-cells in the liver, lung, and spleen led to inclusion by the World Health Organization (WHO) of the disease among lymphoid malignancies. The WHO classification is based on the affected lineage, defined as either chronic lymphoproliferative disorders of Nk cells (CPD-NK) or T-cell large granular lymphocytic leukemia (T-LGL).

A prescient 2001 study showed that STAT3 was activated in peripheral blood mononuclear cells from patients with T-cell LGL leukemia, but the molecular basis of this clinically heterogeneous but morphologically distinct entity remained enigmatic until recently when an international group of investigators identified somatic mutations in the signal transducer and activator of transcription 3 (STAT3) gene in 31 of 77 cases (40%). A subsequent paper extended the initial studies, reporting a mutation frequency of 28 percent (48 out of 170 cases) and documented a similar mutational rate in both the Nk- and T-cell variants. Identification of this somatic mutation confirms the clonal nature of both variants of LGL leukemia and provides new insights into the molecular mechanisms that underlie T-cell and Nk-cell proliferation.

STAT3, a cytoplasmic transcription factor that translocates to the nucleus, was originally identified as an acute phase response factor and a central component of the downstream molecular pathway that mediates signaling induced by the IL-6 family of cytokines that share a common gp130 receptor subunit that initiates the signaling cascade (Figure). Binding of ligand induces receptor dimerization that leads to activation of members of the Janus kinase family (Jak1, Jak2, Jak3, and Tyk2), which, in turn, causes STAT protein dimerization, nuclear translocation, DNA binding, and transcriptional activation of target genes (Figure). The somatic mutations identified by both Koskela et al. and Jerez et al. reside within the Scl homology 2 (SH2) phosphotyrosine-binding domain of STAT3 at the dimerization interface (Figure). Of 49 STAT3 mutations identified by Jerez and colleagues, 80 percent were either Y606F or D661Y (Figure). These mutations result in amino acid substitution in the major protein-protein interaction domain in which an interface is formed with the carboxy-terminal phosphotyrosine 705 (Y705) that is responsible for homo and heterodimerization. Although the specific consequence of the mutation is undefined, constitutive STAT3 DNA binding, increased expression of STAT3-responsive genes, and in vitro functional studies showing both increased transcriptional activity and STAT3-inhibitor-responsive death suggest that STAT3 mutations in LGL leukemia lead to a gain-of-function phenotype that contributes to survival of the leukemic LGL cells.

The first linkage between STAT3 signaling and T-cell leukemia pathogenesis was based on studies of HTLV-1-mediated adult T-cell leukemia (ATL) where constitutive phosphorylation of JAK proteins was shown to mediate tumor growth by HTLV-1. Studies in mice and humans have defined crucial roles for STAT3 in cytokine signaling, embryogenesis, and malignant transformation. Targeted deletion of Stat3 using the Cre-loxP recombination system demonstrated the importance of this transcription factor in T-cell cytokine signaling. Thymocytes from LckCre/Stat3 conditional mice are a consequence of immune dysregulation and aberrant cytokine production.

The STATH pathway

Left, normally binding of IL-6 to its cognate receptor activates the JAK pathway that in turn induces homodimerization of STAT3. The STAT3 homodimer migrates to the nucleus where it initiates transcription of a variety of genes involved in apoptosis, signaling, and proliferation.

Right, in LGL cells harboring a mutation in the SH2 domain, STAT3 may be constitutively active because the mutation supports autodimerization, although this hypothesis remains to be proven definitively.

Bottom, results of microarray analysis show STAT3-responsive genes aberrantly expressed in sorted CD8+ T cells from LGL leukemia patients harboring either a mutant or a wild-type STAT3 gene, suggesting that STAT3 activation is common to these lymphoproliferative disorders, but occurs through alternative mechanisms in STAT3 mutation-negative patients.

**Aberrantly overexpressed genes reported by Koskela and Jerez et al.**

**Aberrantly overexpressed genes reported by only Jerez et al.**

*Figure: ITK inhibitor in lymphoid malignancies. From: Jerez et al. Blood. 2011;118:4148-4157.***

Dr. Gotlib is the chairman of the Study Steering Committee for the Novartis-sponsored global trial of midostaurin in advanced systemic mastocytosis. He also receives funding for administration of the clinical trial and payment for travel expenses from Novartis. Dr. Gotlib will also serve as the principal investigator on a forthcoming trial of brentuximab vedotin in advanced systemic mastocytosis.

(e.g., montelukast) and mast cell stabilizers (e.g., cromolyn sodium) are typically used for refractory mediator symptoms. Osteoporosis is treated with conventional approaches, and radiotherapy and/or IV bisphosphonates may be used to treat osteolyses or pathologic fractures. Patients with advanced SM exhibit shortened survival, and cytoreductive therapy is used in an attempt to reverse organ damage. Interferon-α with or without corticosteroids and 2-chloro-deoxyadenosine demonstrate overall response rates in the range of 30 to 60 percent, including major responses denoting normalization of organ dysfunction. Intensive chemotherapy has been used in MCL with modest benefit, and there is a paucity of published data regarding the utility of stem cell transplantation for advanced SM. The generally short-lived responses and tolerability of these approaches have reinforced the need to enroll patients in clinical trials evaluating novel agents. The tyrosine kinase inhibitors dasatinib and midostaurin exhibit in vitro activity against D816V-mutated KIT, with the latter demonstrating encouraging activity in an ongoing international, multi-center clinical trial. In patients with SM-AHNMD, the clinical approach for such patients has been to treat the SM component as if the myeloid neoplasm were not present and to treat the myeloid neoplasm as if SM were not present. However, in clinical practice, priority should be given to treating the disease component that is contributing to the most urgent clinical concerns. Because KIT D816V or other pathogenetic abnormalities may reside in both the abnormal mast cell and associated myeloid clonal populations, the future availability of agents that inhibit shared therapeutic targets may make the distinction between the two disease compartments less relevant. Next-generation sequencing approaches of sorted cell populations should inform this approach. The recent identification of CD30 expression on neoplastic mast cells provides an opportunity for testing anti-CD30 antibody approaches (e.g., brentuximab vedotin) in advanced SM.
Help ASH Fight for Hematology and Teach Your Elected Officials About NIH

In 2012, ASH launched the aggressive “Fight Now” campaign, which was aimed to protect research funding. The campaign culminated in a call for all attendees of the annual meeting in Atlanta to take action and contact their elected officials in support of biomedical research funding. Several hundred ASH members answered that call and took action to urge Congress to avert the “fiscal cliff” and take a balanced approach to reducing the deficit without further cuts to NIH and other core federal programs.

As this issue of The Hematologist went to press, negotiators had just approved legislation to delay, until March 1, the catastrophic across-the-board cuts to NIH and other federal programs that were scheduled to take effect at the beginning of January as part of the so-called “fiscal cliff.” Unfortunately, NIH and other non-defense discretionary programs are not safeguarded either from future cuts or the new cliff deadline of March 1. To complicate matters further, fiscal year (FY) 2013 funding for NIH has only been provided through March 27, and it will be up to the new Congress to finalize FY 2013 funding after the temporary continued resolution expires.

The nation’s current economic challenges, combined with a large number of new members of Congress who know little about NIH, means that NIH will continue to face potentially devastating budget cuts.

ASH will continue its efforts throughout 2013, but the Society needs your help to protect NIH from further cuts. Visit www.hematology.org/lobby for a complete list of easy and meaningful actions you can take to educate Congress about the impact that inadequate funding has on medical research. Make your voice heard.

A Snapshot of the 113th Congress

As a result of the November elections, there will be few changes to the new Congress. In the House of Representatives, the Republicans have retained the majority and John Boehner (R-OH) will remain as Speaker of the House. The Republican leadership has stated it will continue to focus on cutting discretionary funding and advancing a conservative agenda, including continuing to attempt to repeal the health reform law. In the Senate, the Democrats’ majority was strengthened. The split Congress (Republican majority in the House of Representatives and Democrat majority in the Senate) means partisan fighting will continue. Included in the new Congress are 20 physicians (17 members of the House and 3 Senators).

New Medicare and Medicaid Regulations Benefit Hematologists; ASH Advocacy Improved Physician Payment

ASH’s advocacy efforts were instrumental in making hematologists eligible for enhanced payments within the 2013 Medicare Physician Fee Schedule rule. Payment rates for hematology/oncology services will increase by 2 percent based on the changes made in this rule. In addition, Centers for Medicare & Medicaid Services (CMS) proposed payment for two new codes for transitional care management services provided to patients being discharged from acute, rehabilitation, or long-term acute hospital stays into the community. Primary care providers and specialists can bill these codes for new services within 30 days following discharge. ASH advocacy also influenced the Medicaid rule implementing a provision of the Affordable Care Act stipulating that Medicaid reimbursement for certain primary care services equal Medicare rates in 2013 and 2014. While originally thought to only apply to physicians who are board-certified in family medicine, internal medicine, or pediatrics, the final Medicaid rule included subspecialists in these fields and will result in increased payments for adult and pediatric hematologists.

Headlines from Washington

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As a result of the November elections, there will be few changes to the new Congress. In the House of Representatives, the Republicans have retained the majority and John Boehner (R-OH) will remain as Speaker of the House. The Republican leadership has stated it will continue to focus on cutting discretionary funding and advancing a conservative agenda, including continuing to attempt to repeal the health reform law. In the Senate, the Democrats’ majority was strengthened. The split Congress (Republican majority in the House of Representatives and Democrat majority in the Senate) means partisan fighting will continue. Included in the new Congress are 20 physicians (17 members of the House and 3 Senators).

New Medicare and Medicaid Regulations Benefit Hematologists; ASH Advocacy Improved Physician Payment

ASH’s advocacy efforts were instrumental in making hematologists eligible for enhanced payments within the 2013 Medicare Physician Fee Schedule rule. Payment rates for hematology/oncology services will increase by 2 percent based on the changes made in this rule. In addition, Centers for Medicare & Medicaid Services (CMS) proposed payment for two new codes for transitional care management services provided to patients being discharged from acute, rehabilitation, or long-term acute hospital stays into the community. Primary care providers and specialists can bill these codes for new services within 30 days following discharge. ASH advocacy also influenced the Medicaid rule implementing a provision of the Affordable Care Act stipulating that Medicaid reimbursement for certain primary care services equal Medicare rates in 2013 and 2014. While originally thought to only apply to physicians who are board-certified in family medicine, internal medicine, or pediatrics, the final Medicaid rule included subspecialists in these fields and will result in increased payments for adult and pediatric hematologists.
International Clinical Collaboration Produces High Cure Rate of Acute Promyelocytic Leukemia in Developing Countries

Data demonstrating that the work of the International Consortium on Acute Promyelocytic Leukemia (IC-APl) has improved cure rates in patients with APL in developing countries was recently published in Blood (www.hematology.org/ICAPStudy). By focusing on implementing a standard-of-care protocol adapted to local conditions and resources, this international collaborative effort has succeeded in achieving outcomes comparable to those reported for patients in the United States and Europe where complete response (CR) rates of 90 percent and long-term disease-free survival (DFS) rates of 85 percent are the norm.

Recognizing the need to improve outcomes in developing countries where overall survival (OS) rates for APL are below 60 percent, the ASH International Members Committee founded the IC-APl in 2005. “We chose APL as a model disease for the initiative because of the opportunity to improve patient outcomes in the developing world by collaborating with and learning from colleagues who have already successfully made these strides against this disease,” said Eduardo Rego, MD, PhD, a founding member of the IC-APl and professor of hematology/oncology at the University of Sao Paulo in Brazil. “This initiative aims to build the capacity of local clinicians in the developing world to conduct clinical trials by introducing and fostering clinical and laboratory procedures that represent the standard of care for the treatment of acute leukemia in many countries around the world.”

The participating countries were Mexico in North America and Brazil, Chile, and Uruguay in South America. From June 2006 to September 2010, 183 patients were enrolled in the study. Eligibility criteria included age 15 years or older and a molecularly confirmed diagnosis of APL (demonstration of expression of PML-RAR by PCR performed by a central laboratory). Although treatment was based on the PETHema LPA 2005 protocol, a change to the induction regimen was made, replacing the commonly used, more expensive, and less available anthracycline idarubicin with the less expensive, more readily available anthracycline daunorubicin. Starting on day 1 of the induction cycle, patients received oral ATRA in two daily doses until complete remission with idarubicin given intravenously on days 2, 4, 6, and 8.

Sites registered all cases through the Pediatric Oncology Network Database and collaborated with national coordinators and reference laboratories from each country to confirm the integrity and accuracy of treatment data. Governed by a panel of experts in acute leukemia from Latin America, the United States, and Europe, the group met face-to-face twice a year to discuss each participating country’s progress and to share data. Using the Web-based facility Cure4Kids (kindly provided by the International Outreach Program of the St. Jude Children’s Research Hospital), national coordinators and international APL experts took part in virtual meetings to discuss patient data and clinical and laboratory information, including genetic studies that confirmed the presence of t(15:17) and expression of Pml-RAR transcripts.

Twenty-seven patients (15%) died within the first 30 days of enrollment (defined as early mortality). Based on historical controls, early mortality was reduced by approximately half, and overall survival improved by 30 percent. Of 180 evaluable patients, 153 (85%) achieved CR. For patients entered into the IC-APl study on an intention-to-treat basis, the two-year OS was 80 percent and DFS among patients who achieved a complete remission was 91 percent, with a cumulative incidence of relapse of 4.5 percent.

After five years of follow-up, 75.4 percent of all patients enrolled and 90.2 percent of those who achieved CR remained alive. The most frequent hematologic toxicity was grade 4 neutropenia, which was observed in 3.3 percent. No significant toxicities were reported during maintenance therapy, and no secondary malignancies were reported.

By refining and standardizing diagnostic procedures, implementing a standard-of-care treatment protocol, and collaborating through virtual and face-to-face meetings, overall survival for patients was improved. “We can now close the gap in treatment outcomes between patients in developed countries and those in developing countries,” added Dr. Rego, who also serves as the IC-APl’s Brazilian National Coordinator. “Building on our initial success, we are now expanding this initiative to additional countries where we hope to further integrate education and networking and refine our treatment design to determine if we can further improve outcomes and possibly translate this model to other diseases.” Based on the success of the IC-APl, the consortium’s governing body has voted to expand the collaborative model to address other subtypes of acute leukemia, beginning with acute myeloid leukemia. To reflect this change in scope, the cooperative group is now called the International Consortium on Acute Leukemia (ICAl).

To read more about the IC-APl and now ICAL, please see the article that ran in the November/December issue of The Hematologist titled, “Using Communication Technology to Improve Global Hematologic Care” (www.hematology.org/Publications/Hematologist/2012/9240.aspx).
Exploiting Pathophysiology to Improve Drug Delivery


If you hold a wet ball of sand and rub your hands together, the applied shear stress disperses the sand into its individual grains. Korin et al. in the laboratory of Donald Ingber used this analogy to describe novel particles they call shear-activated nanotherapeutics (SA-NTs). Shear stresses can increase more than 100-fold to greater than 1,000 dynes/cm² at sites of vascular constriction due to stenosis or thrombosis. Platelets are activated by high shear stress in these regions, adhere to the surface of narrowed vessels, and contribute to the pathologic process. The authors used the idea of this phenomenon to target SA-NTs to constricted blood vessels. To do this, they fabricated SA-NTs as platelet-sized, ~4 µm aggregates composed of ~0.2 µm biodegradable poly(lactic-co-glycolic acid) “nanoparticles” (NPs). Flow experiments revealed that SA-NTs remained intact at physiologic levels of shear stress but broke up into their constituent NPs at greater than 100 dynes/cm². Using a microfluidic device containing a constricted lumen lined with cultured endothelial cells, the authors demonstrated that released NPs accumulated on cells downstream but not upstream of the constriction.

The authors constructed SA-NTs containing fluorescent NPs coated with the fibronectin fragment, recombinant tissue plasminogen activator (tPA). The properties of the tPA-SA-NTs were studied in a ferric chloride-induced model of mesenteric artery thrombosis in mice. Vessel narrowing in this model produced shear stresses of 450 dynes/cm² as measured by optical Doppler velocimetry. When injected intravenously after vascular injury, tPA-SA-NTs accumulated at sites of thrombosis and produced thrombolysis. When injected intravenously before vessel injury, tPA-SA-NTs produced a significant increase in vessel occlusion time compared with an equal dose of soluble tPA.

The authors also injected preformed fibrin clots into their constricted lumen microfluidic device and found that tPA-SA-NTs produced clot lysis significantly faster than an equal dose of soluble tPA. Fibrin clots also were injected into mouse lungs in an ex vivo ventilation-perfusion system. tPA NPs localized selectively at regions of vascular occlusion. Lysis of the fibrin emboli by the tPA-NPs resulted in normalization of pulmonary artery pressure levels at a dose which an equivalent amount of soluble tPA had no effect. The dose of soluble tPA required to normalize pulmonary artery pressures (5 µg/ml) was 100-fold greater than the effective dose of tPA-NPs. Finally, the authors produced an in vivo pulmonary embolism model by infusing fluorescent fibrin clots intravenously into mice. When infused intravenously after embolization, IPA-SA-NTs resulted in a reduction of both total clot area and clot number. Additionally, a fatal pulmonary embolism model was developed by injecting larger fibrin clots that lodged in the main pulmonary arteries. Mice were then infused with tPA-SA-NTs or a control vehicle. All control animals died within one hour of embolization (n = 7), whereas six out of seven tPA-SA-NT mice survived.

The study by Korin et al. provides proof-of-principle that a physical phenomenon - shear stress-dependent accumulation of biologically active agents - can be exploited to target drugs to thrombotic sites. Local delivery of drug-laden SA-NTs may lower required doses and decrease adverse events such as bleeding. This approach is potentially applicable to the clinical use of IPA and other antithrombotic agents.

Blood flowing left to right encounters vascular narrowing due to a thrombus. Shear-activated nanotherapeutics (blue aggregates) break up into nanoparticles (blue particles) due to high shear stress at the site of the thrombus.

Don’t Be So Negative About Negative Results


Among the stalwarts of treatment for multiple myeloma are the proteasome inhibitors (PIs), particularly bortezomib. However, development of drug resistance is inevitable, predicatedly accompanied by its dreaded negative impact on clinical outcome. The primary function of proteasomes is maintenance of cellular homeostasis through enzymatic degradation of unneeded or damaged proteins. Proteasomes are multi-protein complexes, located in both the cytoplasm and the nucleus, that are often depicted schematically as having a cylindrical or barrel shape. Proteasomes are composed of α- and β-subunits. The active sites of protein degradation (mediated by chymotrypsin-like, trypsin-like, and post-glutamyl peptide hydrolyzing activities) are located in the β-subunits (designated proteasome I, PSMB) facing the inside of the barrel. The α-subunits, located on the top and bottom of the proteasome, detect ubiquitinated proteins targeted for degradation, thereby serving as the gatekeepers for entry into barrel. Bortezomib inhibits hydrolysis of ubiquitinated proteins as a consequence of binding to the β5 subunit. Direct as to how bortezomib resistance is acquired is a topic of intense investigation.

When in the course of treatment patients become PI-resistant is variable. One hypothesis to explain this variation is that those who develop resistance early in the course of treatment have existing genetic sequence variants (polymorphisms or mutations) that mediate the resistance while those that develop resistance later acquire the causative sequence variants through somatic mutation. In support of this hypothesis, studies have shown that PSMB5 sequence variants can arise when tumor cell lines are cultured with bortezomib. The purpose of the current study, led by David Lichter from Millennium Pharmaceuticals in Cambridge, MA, was to determine if sequence variants in PSMB genes might account for drug resistance or have an impact on clinical outcome in patients with myeloma. To investigate this hypothesis, Lichter and colleagues sequenced the coding regions of PSMB genes in pre- and post-treatment samples from patients who participated in the phase III Assessment of the Proteasome Inhibitor for Extending Remissions (APEX) trial of single-agent bortezomib compared with high-dose dexamethasone for treatment of relapsed myeloma. Twelve new PSMB sequence variants were identified, but no association was found between the frequency of these PSMB single nucleotide polymorphisms (SNPs) and clinical response to either bortezomib or dexamethasone nor was overall survival or time to disease progression found to correlate with PSMB SNP allelic frequency. Allelic and genotype frequency of non-synonymous SNPs (those sequence variants that result in an amino acid change in the protein product of the gene) in pre- and post-treatment multiple myeloma samples did not differ significantly from the frequency of those SNPs in the general population and no unique non-synonymous substitutions were observed in the post-treatment samples. Moreover, sequence variants occurred at similar frequencies in pre- and post-treatment samples. Together, these results suggest that treatment with bortezomib (or dexamethasone) does not exert a selection pressure that favors outgrowth of myeloma cells with mutant PSMBs and that the observed new sequence variants represent germline polymorphisms rather than somatic mutations. Notably, the PSMB sequence variants that were identified in preclinical models of bortezomib resistance were not detected in patient tumor samples collected after clinical relapse following bortezomib therapy. Thus, alternative mechanisms must underlie bortezomib resistance.

While the outcome of this study can be viewed as negative (i.e., a mechanism for bortezomib resistance was not identified), the rigorous experimental design, the use of primary tissue samples from well-characterized patients enrolled in a phase III clinical study, and the relatively large number of samples analyzed make the results compelling. Thus, sequence variants that directly affect the structure/function of PSMBs can be confidently excluded as the cause of bortezomib resistance, and the search for other mechanisms for loss of sensitivity to PIs can proceed. Just as the mechanism for resistance to JAK2 inhibitors (heterodimerization of JAK2 with other JAK family members) differs from the mechanism that underlies resistance to the tyrosine kinase inhibitors used to treat CML (somatic affecting mutations of the ATP binding and solvent pocket of BCR-ABL), we can imagine that the basis of bortezomib resistance will be novel and that elucidating this mechanism will provide both fascinating new insights into the sinister but elegant nature of tumor biology and an empiric approach to pharmacologically subverting the drug resistance.

The Hematologist: ASH NEWS AND REPORTS
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dr. flaumenhaft indicated no relevant conflicts of interest.

in 1979, weiss et al. described a patient who suffered a moderate to severe bleeding disorder and impaired platelet procoagulant activity. the disorder was termed scott syndrome after mrs. m.a. scott, the patient described in the report. a few years later, in 1985, rosing et al. showed that scott syndrome was secondary to the inability of platelets to express phosphatidylserine on their surface following activation. they subsequently demonstrated a scramblase activity responsible for reversing the asymmetric distribution of phospholipids in the plasma membranes of hematopoietic cells. like most mammalian membranes, resting hematopoietic cells express neutral phospholipids (e.g., phosphatidylcholine) on their outer leaflet, while phospholipids with negatively charged head groups (e.g., phosphatidylserine, phosphatidylethanolamine) are sequestered in the inner leaflet. upon activation, a scramblase activity catalyzes the translocation of neutral lipids to the inner leaflet and negatively charged phospholipids to the outer leaflet (figure). this scramblase activity appeared to be missing in patients with scott syndrome. but what was the protein responsible for this activity?

despite the description of several putative scramblases, the identity of the molecular defect in scott syndrome remained a mystery for 25 years following publication of the rosing paper. in 2010, suzuki et al. identified a constitutively active mutant form of tmem16f in a subclone of a mouse b-cell line that expressed excessive phosphatidylserine on its surface. depletion of tmem16f from a mouse b-cell line inhibited activation-induced phospholipid scrambling. in addition, a patient with scott syndrome was found to carry a mutation in the tmem16f gene at a splice-acceptor site, leading to premature termination of the protein. two additional patients with scott syndrome were subsequently found to have mutations in the tmem16f gene. so, tmem16f is the elusive scramblase, right?

a recent paper by lily yeh jan’s group at university of california, san francisco, indicates that the answer may not be so simple. the investigators generated a tmem16f knockout mouse. the mouse demonstrated impaired, though not absent, expression of phosphatidylserine in response to ca²⁺ ionophore. bleeding time was increased in the tmem16f-deficient mice and thrombus formation in response to fcc⁴ was impaired. in short, the mouse looked a lot like a patient with scott syndrome. unexpectedly, however, the authors found that tmem16f is not a calcium-activated, cl⁻-specific channel like other tmem16 family channels, but is instead a ca²⁺-activated nonselective cation channel. heterologous expression of this unusual receptor in hek293 cells failed to induce any obvious scramblase activity. endogenously expressed tmem16f in megakaryocytes was more sensitive to ca²⁺ than tmem16f heterologously expressed in hek293 cells. furthermore, scramblase activity was observed in tmem16f-deficient platelets when exposed to high calcium ionophore concentrations. these observations suggest that additional regulatory factors in hematopoietic cells contribute to tmem16f-dependent scramblase activity. thus, tmem16f may act as part of a larger scramblase complex, rather than as the sole scramblase in hematopoietic cells.

the exposure of phosphatidylserine on the surface of activated platelets has long been known to provide a surface for the assembly and activation of clotting proteins. however, the putative scramblase responsible for surface expression of phosphatidylserine has been difficult to pin down. the recent discovery that tmem16f is the defective channel in patients with scott syndrome suggested that this channel may represent the scramblase. yet the nature and function of this channel remained undetermined. the study by yang et al. now demonstrates that tmem16f is an unusual ca²⁺-activated nonselective cation channel that is necessary but apparently not sufficient for phospholipid scramblase activity.

3. suzuki j, umeda m, simo pi, et al. calcium-dependent phospholipid scrambling by tmem16f. nature. 2010;468:834-838.

the scramble for the scramblase

yang h, kim a, david t, et al. tmem16f forms a ca²⁺-activated cation channel required for lipid scrambling in platelets during blood coagulation. cell. 2012;151:111-122.

Figure

Tmem16f: scramblase or scramblase activator?

Tmem16f is a Ca²⁺-activated nonselective cation channel that is required, but may not be sufficient, for scramblase activity in hematopoietic cells. Neutral phospholipids are indicated in white. Phospholipids with negatively charged headgroups are indicated in red.
Plasma exchange has greatly reduced the morbidity and mortality of thrombotic thrombocytopenic purpura (TTP). Yet, even when combined with corticosteroids and/or rituximab (Table), not all patients respond and complications related to the exchange procedure remain. Thus, better therapeutic approaches are needed.

In both the congenital and acquired forms of TTP, deficiency of the metalloprotease ADAMTS13 results in decreased or absent cleavage of high-molecular-weight multimers of von Willebrand factor (VWF). These unprocessed ultra-large (UL)-VWF multimers spontaneously bind to platelets forming platelet-rich thrombi that occlude the microvasculature (a pathologic process called thrombotic microangiopathy). It is this process that causes the end-organ damage that accounts for the clinical manifestations of TTP (i.e., thrombocytopenia, microangiopathic hemolytic anemia, renal insufficiency, and mental status changes).

Taking advantage of the known pathology of TTP to develop novel approaches to therapy, two teams of investigators independently reported that anti-VWF antibodies prevent signs of the disease in a baboon model of acquired TTP. Experimentally, TTP was induced by injecting animals with 3H9, a monoclonal antibody that blocks ADAMTS13, thus mimicking the disease in a baboon model of acquired human TTP. Callewaert and colleagues evaluated the efficacy of nanobody ALX-0681. Nanobodies are modeled after heavy-chain-only lgs found in camels, llamas, alpacas, and other members of the family Camelidae and are much smaller (12-15 kDa) than standard antibodies (150-160 kDa). ALX-0681 is a bivalent humanized nanobody that targets the A1 domain of VWF that binds to platelet GP1b, the VWF receptor on platelets (Figure). Given concomitantly with 3H9, ALX-0681 prevented the onset of TTP, and given subsequent to antibody-mediated induction of TTP, ALX-0681 promoted rapid resolution of thrombocytopenia and hemolytic anemia.

Feyts and colleagues investigated the effects of the humanized murine monoclonal anti-VWF antibody GBR600 and showed it was effective in both preventing and reversing the thrombocytopenia in the baboon model of TTP. The efficacy of GBR600 was shown to correlate with the capacity of the antibody to inhibit VWF activity.

The clinical modulation of TTP by anti-VWF monoclonal antibodies demonstrated by Callewaert et al. and Feyts et al. provides an exciting new approach to treat TTP, specifically by blocking the interaction between UL-VWF multimers and platelet GPIb in order to prevent formation of pathologic platelet thrombi; Importantly, neither brain CT scans nor post-mortem analysis revealed signs of bleeding in the animals treated with the nanobody ALX-0681.

| Table |
|-----------------|-----------------|-----------------|-----------------|
| **Mechanisms of TTP Therapies** | Provides ADAMTS13 Activity | Reduces Anti-ADAMTS13 Antibody | Suppresses Immune Response | Blocks VWF Binding to GPIb |
| Plasma Exchange | + | + | - | - |
| Steroids | - | - | + | - |
| Rituximab | - | - | + | - |
| ALX-0681 | - | - | + | - |
| GBR600 | - | - | + | - |

*Based on studies in animals.*

How applicable are these studies of antibody-induced TTP in baboons to humans in the human disease? First, it is important to note that the baboon model may represent early TTP only, as the targeted inhibition of ADAMTS13 by the monoclonal antibody 3H9 results in thrombocytopenia and hemolytic anemia, but no end-organ disease, including absence of neurologic dysfunction and renal insufficiency in the animal model. Secondly, ADAMTS13 deficiency is alone sufficient to cause thrombocytopenia and microangiopathic hemolytic anemia in the baboon model, with no requirement for exogenous triggers of disease, as is typical in human TTP. Thirdly, while starting anti-VWF therapy early may eliminate signs of the disease, recognition of human TTP may be delayed until neurologic symptoms develop or until clinical symptoms become sufficiently severe for patients to seek medical attention. Thus, it is unclear if the timing of the experimental treatment is informative from a clinical perspective.

If disease can be averted by anti-VWF therapy, better diagnostic tests to recognize disease earlier would be ideal. Alternatively, if the therapy is safe and inexpensive, early treatment might be practical and desirable in patients with suspected, but unproven, TTP. Clearly, additional studies are needed to define the extent to which anti-VWF monoclonal antibodies can affect the natural history of TTP in humans and to determine when in the course of the disease anti-VWF antibody treatment is effective and if it is sufficiently effective as monotherapy or best used in conjunction with standard therapy centered on plasma exchange.

mall non-coding RNA, including microRNA (miRNA), regulate gene expression through either degradation of messenger RNA or suppression of translation. The capacity of miRNA to regulate lineage commitment, differentiation, and self-renewal of hematopoietic stem and progenitor cells (HSPC) is becoming increasingly recognized. Studies of their physiologic impact are often hindered by the abundance of miRNA target transcripts and the diverse functional effects that aberrant expression of these regulators produce. Understanding miRNA regulation in HSPCs is further complicated by the fact that the majority of miRNAs are normally expressed at very low copy numbers. Existing studies of miRNA in HSPCs, therefore, have often taken a candidate approach in which a small number of miRNAs and their targets are analyzed using in vitro model systems. Interpretation of these studies is frequently compromised by the absence of in vivo functional validation.

In the current paper, Adams and colleagues from Yale University School of Medicine use an elegant in vivo screening method that lets cell function guide discovery of miRNAs involved in HSPC regeneration. Their functional screen hinged on the transduction of hematopoietic cells with a pool of retroviral vectors encoding a set of HSPC-relevant miRNA. The flanking sequence of each vector was tagged with a genomic sequence that served as a unique barcode for recovery and identification of cells expressing a particular transgenic. Cells, transduced at limiting efficiency to favor single-copy integration, were then transplanted into irradiated recipients. Once stable hematopoietic function had been regained after transplantation, the animals were challenged with a chemotherapy agent, 5-fluorouracil (5-FU). The hypothesis being tested posited that regaining the subsequent recovery from 5-FU-induced stress, stem cells expressing a particular provirus-encoded miRNA would be predominant in the repopulated marrow, while those expressing other miRNAs would be relatively underrepresented. Because each animal provides a screen for several HSPC clones, each of which carries a unique barcoded miRNA, a moderately sized library of transcripts (135) could be evaluated in a relatively small number of mice (15) by correlating changes across three independent transplant experiments.

Based on specific miRNA representation in animals during recovery from 5-FU, the authors earmarked a group of miRNAs for further study. MicroRNAs that were overexpressed in transplanted animals contributed to hematopoietic recovery after injury, while those that were underrepresented negatively impacted this process. Focusing on the latter class of suppressive miRNAs, miR-153-2, miR-150, and miR-652 were selected for further characterization. Through a series of competitive repopulation studies of wild-type marrow and HSPCs transduced to overexpress candidate miRNAs, they identified miR-153-2, miR-150, and miR-652 as inhibitors of myeloid and platelet recovery, with miR-150 overexpression resulting in the greatest suppressive effect. The authors then painstakingly validated the results using a number of different in vivo and in vitro experimental approaches. They demonstrated decreased colony-forming capacity and increased apoptosis after 5-FU treatment in cells that overexpressed miR-150, which was partially phenocopied in a model of a well-established target transcript of miR-150, c-myb. Collectively these experiments convincingly implicate a specific miRNA (i.e., miR-150) in suppression of bone marrow recovery after chemotherapy-mediated injury.

This work is notable on at least three levels. First, inspired by genetic approaches in other model systems, the functional in vivo screen for miRNA circumspects many of the difficulties inherent in examining miRNA-mediated stem cell regulation. It allows investigators to sift through the large number of potential targets that emerge from conventional in silico screens and focus their efforts on those that are the most likely to be relevant to the phenotype of interest. This method offers gains in efficiency and stringency compared with broad observational studies. Second, the screen can be readily adapted to identify miRNAs that are involved in regulation of hematopoietic stem cell regulation in vivo. A variety of benign and malignant conditions. Third, even when viewed as a proof-of-concept report, the identification of miR-150 as a negative regulator of hematopoietic recovery after chemotherapy-mediated marrow injury is significant. This finding, along with emerging data on the role of miR-150 in leukemogenesis, provides the rationale for additional study.


Keeping Quiet in the Niche: E-Selectin Disrupts Hematopoietic Stem Cell Sleep


Patients undergoing chemotherapy for hematologic malignancies frequently ask, “How do hematopoietic stem cells (HSCs) recover after the toxic insult so that the blood counts return to normal?” The clinician usually describes how quiescent HSCs can robustly awaken to reconstitute red cells, white cells, and platelets and simultaneously self-renew, creating another quiescent HSC. This process provides a mechanism for both expansion of hematopoiesis and self-renewal of the quiescent HSC pool, assuring maintenance of hematopoiesis. The majority of adult HSCs are quiescent, maintained in the G0 phase of the cell cycle, and in this dormant state, stem cells are protected against myeloablative agents such as chemotherapy or radiation. Once the toxic insult is obviated, the quiescent stem cell pool is activated and reconstitution of hematopoiesis ensues.1 HSC preservation occurs in specialized niches in the vascular/endothelial and osteoblast/endosteal bone marrow microenvironment. Multiple cell-cell adhesive interactions with specific molecular receptors, cytokines, and ligands are required to ensure HSC multi-potential self-renewal and niche self-renewal. In the current paper, Winkler and colleagues from Mater Medical Research Institute, South Brisbane, Queensland, Australia, report that vascular-specific intercellular adhesion molecule 1 (VCAM), E-selectin, regulates HSC dormancy, self-renewal, and chemoresistance and suggests that hematopoietic recovery following chemotherapy could be modulated through E-selectin blockade.

E-selectin is a member of the selectin family (P-selectin and L-selectin being two other members) of cell adhesion molecules that function as lectins (proteins that bind carbohydrates). E-selectin is expressed by cytotoxic-activated endothelial cells, whereas P-selectin is expressed by both platelets and endothelial cells, and L-selectin is expressed by leukocytes. The investigators used wild-type and E-selectin knockout mice (Sele−/−) to show that the HSC cycle time is decreased when E-selectin is knocked out. This work is notable on at least three levels. First, inspired by genetic approaches in other model systems, the functional in vivo screen for miRNA circumspects many of the difficulties inherent in examining miRNA-mediated stem cell regulation. It allows investigators to sift through the large number of potential targets that emerge from conventional in silico screens and focus their efforts on those that are the most likely to be relevant to the phenotype of interest. This method offers gains in efficiency and stringency compared with broad observational studies. Second, the screen can be readily adapted to identify miRNAs that are involved in regulation of hematopoietic stem cell regulation in vivo. A variety of benign and malignant conditions. Third, even when viewed as a proof-of-concept report, the identification of miR-150 as a negative regulator of hematopoietic recovery after chemotherapy-mediated marrow injury is significant. This finding, along with emerging data on the role of miR-150 in leukemogenesis, provides the rationale for additional study.

The authors do not propose a mechanism by which E-selectin promotes HSC proliferation. A commentary by Malcolm Moore that accompanies the article hypothesizes a potential role for disruption of TGF-β-mediated inhibition of HSC proliferation. Use of E-selectin antagonists could reduce the myelosuppressive effects of chemotherapy, but on a cautionary note, tumor cells appear to utilize E-selectin ligands for homing, in which case use of such antagonists may induce quiescence and chemoresistance in malignant cells.2

Refining our Understanding of the Genetic Variation in CLL


Over the past decade, numerous studies utilizing comparative genomic hybridization techniques, single nucleotide polymorphism (SNP) arrays, and, most recently, whole-genome and whole-exome sequencing have elucidated new findings that bear relevance to the biology and natural history of chronic lymphocytic leukemia (CLL). What is critical to any such study is the quality of the test material, meaning that samples should be collected at a uniform time from a cohort being managed uniformly and that test samples should be paired with germline material.

The above criteria were met in a study by Edelmann et al. who recently reported one of the most comprehensive assessments of genomic copy number variation in symptomatic, but as yet untreated, CLL patients (patients who were enrolled in a clinical trial, the German CLL8 study, of first-line therapy but had not begun treatment at the time the peripheral blood samples used in the current study were collected). The experimental design was based on SNP-array analysis that was used to assess copy number alterations (CNAs) using DNA prepared from CD19+ mononuclear cells from 393 patients. Unlike many other previously published analyses, the current study included a strategy for identifying tumor-specific abnormalities. In this case, paired samples were analyzed in 144 patients, with DNA from CD19+ mononuclear cells serving as the non-tumor control. To validate accuracy, Edelmann and colleagues compared the SNP-array results with those obtained from interphase cytogenetic analyses using four standard fluorescence in situ hybridization (FISH) probes. Those comparisons confirmed that all of the genomic deletions and additions observed by FISH were reproduced by the SNP-array. Further, the frequency of non-FISH-defined CNAs was low (0.55) and appeared independent of IgVH mutational status and FISH-defined genomic abnormalities other than del(17p13.1), mutant TP53, and del(11q22.3) where the frequency was 1.06. In total, two-thirds of the cases carried no CNAs other than those detected by standard FISH. Collectively, these findings suggest that genomic instability is not a common component of the pathophysiology of CLL.

From the SNP-array analysis came characterization of both minimally deleted regions in the common karyotype abnormalities and identification of less frequent genomic aberrations previously undocumented. High-resolution analysis of the common 13q14 deletion suggested that in addition to deletion of microRNAs miR-16-1 and miR-15a, deletion of two long non-coding RNA genes, DLEU1 and DLEU2, likely contribute to disease pathogenesis. Most frequent among the previously unidentified genomic alterations was del(15q15.1), which was present in 4 percent of patients, with the smallest deleted region found within the MGA gene locus. Subsequent nucleotide sequencing revealed somatic mutation of MGA in one of 59 patients. MGA is part of the network of MAX and MAX-interacting proteins that are involved in regulatory mechanisms involved in cell proliferation, differentiation, and apoptosis, suggesting that deletion of MGA may contribute to CLL pathogenesis in a small percentage of patients. However, del(15q15.1) had no prognostic significance relative to overall survival. Evidence of chromothripsis (extreme chromosome reorganization thought to occur in a one-step catastrophic event and defined in this study as the presence of at least 10 switches between 2 or 3 copy number states on an individual chromosome) was identified in a small subset of patients and negatively impacted on both progression-free survival and overall survival. However, the majority of the patients with evidence of chromothripsis had unmutated IgVH and high-risk genomic aberrations, including one-third with a concomitant TP53 mutation. The current study did not include analysis of samples at the time of relapse to assess the contribution of clonal evolution to treatment failure. Such studies will likely be a future focus as the treatment phase of the CLL8 study matures and more samples from patients who have relapsed become available.

The importance of this study centers around both analysis of DNA samples from a clinically uniform cohort of symptomatic CLL patients who were treatment-naïve and the use of paired, non-tumor DNA that allowed confirmation of the tumor specificity of the observed CNAs. Outside of those that were also detected by standard FISH, relatively few CNAs were independently identified by SNP-array analysis, suggesting a minor role for genomic instability in the pathogenesis of even symptomatic patients who are treatment-naïve. CNAs were modestly more frequent among patients with high-risk genetic features, and this group was also more likely to have evidence of chromothripsis. However, chromothripsis was found in only a small portion of the study group (17 of 353), suggesting that catastrophic DNA rearrangement is uncommon in CLL. Rigorous studies such as those of Edelmann and colleagues, when coupled with next-generation sequence analysis, will further elucidate the pathology of CLL and identify genetic abnormalities that can be targeted for therapy.

JOHN C. BYRD, MD
Dr. Byrd indicated no relevant conflicts of interest.

Turning the Page: The ASH Annual Meeting 2012

(Cont. from page 1)

...identify and characterize the genetic basis of acute myeloid leukemia (AML). Dr. Ley went on to describe the results of experiments from his laboratory that used whole-genome sequencing to investigate the genetic basis of AML. Those landmark studies have demonstrated that an initiating somatic mutation in conjunction with one or two other cooperating somatic mutations, arising in a hematopoietic stem cell, together account for the proliferative/survival advantage that underlies the clonal expansion characteristic of AML. Dr. Ley and colleagues have also shown that accumulation of additional somatic mutations in the original initiating clone leads to development of subclones, some of which are resistant to standard AML chemotherapy. It is this Darwinian process that accounts for the treatment failure that has plagued the field for decades. Dr. Ley’s work has important clinical implications, providing both an approach to more precise disease classification and new insights into genetic characteristics that influence prognosis and management. The overarching aim of these studies is to use the genetic signature to tailor therapy for each individual patient who has AML. While reaching this goal seems futuristic, the dedication and imagination embodied in the work of Dr. Ley and colleagues inspire us to believe that impossible really is just a matter of opinion. I’m confident that Dr. Thomas would agree.

The Plenary Scientific Session further exemplified how we are turning the pages on several aspects of hematology. A very practical issue, both clinically and monetarily, which confronts clinicians on daily basis, is the prophylactic use of platelet transfusions. Dr. Simon Stanworth, representing an international team of collaborating investigators presented “The Effect of a No-Prophylactic Versus Prophylactic Platelet Transfusion Strategy on Bleeding in Patients with Hematologic Malignancies and Severe Thrombocytopenia (TOPPS trial): A Randomized Controlled, Non-Inferiority Trial.” The study included 609 adult patients who were undergoing treatment for hematologic malignancies. Both patients receiving chemotherapy only and those receiving high-dose chemotherapy with hematopoietic stem cell rescue were included in the study. The trigger point for prophylactic transfusions was a platelet count <10 x 10^9/L. The study demonstrated that patients who were enrolled on the prophylactic transfusion arm had significantly fewer bleeding episodes and a longer time between study enrollment and their first bleeding event. Another important and practical clinical question addressed in the Plenary Session was whether there is benefit in maintaining hematocrits lower than 45 percent in patients with polycythemia vera (PV). Dr. Tiziano Barbui presented data from the first randomized study comparing the relationship between hematocrit and the incidence of thromboembolic complications in patients with PV. The comparison groups were those whose hematocrits were maintained below 45 percent versus those whose hematocrits were maintained between 45 and 50 percent. The results of the study showed that the patient group whose hematocrit was maintained below 45 was four times less likely to experience a thromboembolic complication. A colleague commented to me that the two clinical studies described above confirmed what we already knew. However, I can think of many trials where we “knew” what the results were going to be, and we turned out to be wrong. More importantly, the results of these two trials provide us with rigorous data upon which to base treatment decisions.

The Poster Hall gets a lot of foot traffic during the annual meeting.
The remaining presentations in the Plenary Session transitioned from old questions to relatively current questions and suggested new questions to be addressed by future studies. We heard Dr. Francesco Lo-Coco present the results of an international phase III study that supported use of the combination of arsenic trioxide and all-trans retinoic acid as first-line treatment for non-high-risk patients with acute promyelocytic leukemia. Dr. Dan-Avi Landau showed how knowledge of the past can be used to anticipate the future. In this case, the molecular characteristics of malignant subclones were shown to predict clinical outcome in patients with chronic lymphocytic leukemia. A newly discovered participant in the pathobiology of myeloid neoplasms was introduced to us by Dr. Hideki Makishima. The focus of this presentation was SETBP1, the gene that is mutated in the congenital disorder, Schinzel-Giedion syndrome (characterized by skeletal deformities, mental retardation, and neuroepithelial tumors). SETBP1 was found to be somatically mutated in subgroups of patients with CML, secondary AML, and blast-phase CML, and in patients with AML arising out of myelodysplastic syndromes. SETBP1 mutations were shown to be acquired in the process of leukemic evolution. Like normal cells, malignant cells have to be able to repair DNA damage in order to survive. Dr. Kimberly Cramer, representing investigators from the United States, Poland, and the United Kingdom, described how advantage can be taken of addiction to a particular type of DNA repair mechanism to eradicate leukemic stem cells. In this case, homologous recombination repair of DNA double-strand breaks in CML cells was shown to be dependent on RADS2, and targeting the DNA binding site of RADS2 using a small-molecule inhibitor induced synthetic lethality in the tumor cells. This strategy holds promise for treating other types of neoplastic disease in which the malignant cells are addicted to RADS2, as normal cells have other mechanisms for homologous recombination repair and are therefore unaffected by RADS2 inhibition.

The breadth of the meeting is astounding, and at times overwhelming, but in the end, most participants leave the sessions inspired, optimistic, and invigorated. Credit for the success of the annual meeting goes in large part to the vision of the program chairs and to the leadership of last year’s ASH President, Dr. Armand Keating, whose interest in the biologic diversity of regenerative medicine provided the focus of the Presidential Symposium. Of course many of our clinician members practice oncology as well as hematology. The Society recognizes both this important demographic and the close association between ASH and the American Society of Clinical Oncology (ASCO) by organizing a combined seminar series that is reciprocated at the annual ASCO meeting. This year’s ASH/ASCO Joint Symposium, co-chaired by Dr. Keating and ASCO President Dr. Sandra M. Swain, was titled “Clinical Oncology Update: Studies from the 2012 ASCO Annual Meeting.” Attendees were rewarded with an expert overview of recent development in clinical oncology through a series of four lectures. Progress in the management of hematologic malignancies was highlighted by the presentation of abstracts that reported the results of clinical trials that investigated the efficacy of brentuximab vedotin in CD30+ lymphomas, carfilzomib and pomalidomide in multiple myeloma, and bratum and chronic lymphocytic leukemia.

Recognizing the diversity of interests of the membership, meeting sessions focused on such varied topics as a “toolbox” for quality improvement in practice, hematology in pregnancy, news in teaching hematology in medical school, and epigenetics in hematopoiesis. It is noteworthy that a decade ago epigenetics was viewed largely as an arcane area of investigation with little apparent clinical relevance. At this year’s meeting, a special symposium was devoted to the subject, and we learned not only of the progress that has been made in understanding how DNA modification regulates normal hematopoiesis but also of the role of aberrant epigenetic regulation in the pathogenesis of hematologic malignancies. These discoveries have led to the development of targeted therapies, some of which are now in use or are in clinical development, reminding us again of how fundamental research provides the basis for creation of novel approaches to therapy. Coagulation held center stage in a Special Symposium on the Basic Science of Hemostasis and Thrombosis, and Dr. Alan Burnett masterfully reviewed progress in the treatment of AML in the Ham-Wasserman Lecture. We were reminded of the extraordinary intricacy and the beauty and power of the immune system in the Ernest Beutler Lecture delivered jointly by Dr. Bruce Blazer and Dr. Carl June.

My experience as this year’s ASH News Daily editor was made easier by some remarkable people including Dr. Joe Mikhail, last year’s editor, who showed me the ropes and taught me how to have fun with a hard task and by my fantastic team (“the Fab Five”) on the editorial board: Jose Bufill, MD; Jenna Goldberg, MD; Matt Hsieh, MD; Marc Kahn, MD, MBA; and Andy Leavitt, MD. Importantly, I want to thank the dedicated ASH staff, particularly Tiffany Reid, Jen Hamilton, and Karen Learner for their expert support. For those unable to attend this year’s ASH annual meeting or for those who would like to read any articles they missed or reread those they particularly enjoyed, all four copies of 2012 ASH News Daily are available at www.hematology.org/Publications/ASH-News-Daily/8148.aspx. I hope to see all of you in New Orleans, and next year I will just get to enjoy reading the 2013 ASH News Daily. In the words of Mr. Seeger, it is time for me to “turn the page.”
Edward Donnell Thomas (1920-2012)

Don Thomas wanted to solve big problems. When asked how he chose to work on marrow transplantation, Don explained that while completing his training in hematology, he asked himself what the largest problems in the field were. He first considered hemoglobinopathies, with the idea that they might be cured if the switch from Hb F to Hb A could be prevented, but he didn’t know how to attack the problem. So he turned to what he considered the second greatest problem, leukemia. It was the early 1950s, and he learned of the experiments of Leon Jacobsen and others showing that marrow of normal mice could be destroyed by radiation but animals could recover if infused with marrow from a syngeneic donor. With that knowledge, he became convinced of the clinical potential of marrow transplantation. He wasn’t alone: Joseph Ferrebee at the Imogene Bassett Hospital in Cooperstown, NY, was also inspired by that same concept, and in 1955, after completing his training, Don moved to Cooperstown to work with Dr. Ferrebee. There, in 1957, they made the first attempts to treat patients with total-body irradiation and chemotherapy followed by intravenous infusion of normal donor marrow. Transient marrow grafts were seen in a few patients, but none lived more than 100 days. Similar attempts by many others also failed, either because grafts were rejected or because grafts rejected their new host. In retrospect, since nothing was known about histocompatibility matching, these results were not surprising, but they certainly were discouraging, leading most to abandon the field.

But not Don. Whether persistent or stubborn, he remained convinced of marrow transplant’s potential and began experiments using an outbred canine model. Most transplants between littermates failed, but occasionally a dog would become somehow genetically matched. In 1963 he moved to Seattle and, with colleagues, began developing methods for canine histocompatibility typing. They also worked out appropriate doses of irradiation to assure engraftment and found that post-transplant methotrexate helped prevent graft-versus-host disease. By the late 1960s, they could reliably engraft canine littersmates, and with the knowledge gained from those rigorous animal studies, together with advances in human histocompatibility typing, Don returned to the clinical challenge of human marrow transplantation, 12 years after his first failures.

He assembled a team of nurses, technicians, and physicians and, in 1969, began clinical trials of matched sibling marrow transplantation for advanced leukemia. The initial study patients were very sick with life expectancies measured in weeks. The team went to extraordinary lengths to support their patients, housing them in sterile laminar flow rooms, asking staff members to donate platelets, administering prophylactic granulocyte transfusions, developing with Bob Hickman methods to allow intravenous alimentation, and creating homemade anti-thymocyte globulin by inoculating and subsequently phlebotomizing horses (Don performed this procedure himself). Despite these efforts, most patients died, but a few survived. In 1975, Don published results that showed a plateau in the survival curve demonstrating that a minority of patients (13 of 100) with otherwise incurable leukemia had, in fact, been cured. These results led to exploration of the use of transplantation earlier in the disease course, while patients were in remission, and soon cure rates in excess of 60 percent were being reported. This success led the Seattle team to explore transplantation as therapy for other hematopoietic diseases, including thalassemia, sickle cell anemia, myelodysplasia, lymphoma, and myeloma. In the late 1970s, the Seattle group performed the first successful, unrelated donor transplant for leukemia. With expanded indications and increased donor availability, the use of transplantation grew steadily; this year approximately 65,000 transplants will be conducted and the cumulative number performed since Don began his work will surpass 1 million.

Don was an extraordinary person, extremely bright, with a remarkable memory and real curiosity. If you were working in an area that he didn’t fully understand he would come by and modestly ask for a lesson. He was quiet and soft-spoken, letting others lead most discussions. And yet, he projected a remarkable aura of authority. When he did talk, everyone listened. Papers and proposals were expected on time and carefully done. Shoddy work or sloppy thinking was not acceptable. At faculty meetings, someone might make a joke but no one would laugh, at least not until Don did, and then everyone would laugh, until Don stopped, and then everyone stopped. Yet, he was modest and quick to deflect praise to his coworkers to whom he was very loyal, and those of us who were the recipients returned that loyalty.

Two aspects of his career stand out to me. First, Don truly believed in the idea of marrow transplantation and was willing to bet the house on it. While other cancer centers were developing broader clinical programs, Don focused almost the entire clinical efforts of the Fred Hutchinson Cancer Research Center on marrow transplantation. Virtually every recruit for well over a decade was dedicated to the general topic. A second defining aspect of Don’s approach to tackling the complexity of marrow transplant was his appreciation that it would take a team to achieve his vision. The group Don assembled included nurses, administrators, lab techs, and medical subspecialists focused on the spectrum of complications of marrow transplantation, including infectious diseases and pulmonary and gastrointestinal disorders. Don firmly believed that it took such a team to achieve the clinical outcomes that led to the eventual success of marrow transplantation.

When Don learned he was awarded the Nobel Prize, one of the first places he went was to the transplant unit’s nursing station to thank the nurses (who he called his secret weapon) for their contributions.

As hard-working and focused as Don was, he also understood that there was life outside of work. When recruiting faculty to Seattle, he used to jokingly ask if candidates were skiers or fishermen, because he wanted to balance the two to ensure that enough people would be at work both winter and summer. He and his wife Dottie were avid hunters and fishermen, and every meal we shared at Don’s house featured elk, moose, or steelhead trout. He particularly enjoyed having young faculty over and pointing out pictures of Dottie with a shotgun in one hand and several dead ducks in the other, commenting with a smile that he sure hoped their paper was going to be turned in on time.

“Don truly believed in the idea of marrow transplantation and was willing to bet the house on it.”

– Frederick R. Appelbaum, MD, Executive Director, Seattle Cancer Care Alliance; Clinical Research Division, Director, Fred Hutchinson Cancer Research Center; Professor, Medical Oncology Division, University of Washington School of Medicine

Editor’s Note: Professor Thomas, a past president of ASH, died this past October in Seattle. He was 92. Dr. Thomas shared the 1990 Noble Prize in Physiology or Medicine with Dr. Joseph E. Murray, who pioneered solid organ transplantation, performing the first kidney transplant in 1954. Notably, Dr. Murray died this past November at the age of 93.

The E. Donnall Thomas Lecture and Prize was created by ASH in 1992 in recognition of the singular impact that Don Thomas’ work had on hematology and to acknowledge the many contributions that he made to the Society. The E. Donnall Thomas Lecture and Prize, awarded annually, is intended to recognize pioneering research achievements in hematology that have represented a paradigm shift or significant discovery in the field.
Stimulating Erythropoiesis, But Doing It Differently Than EPO Does

**STUDY TITLE:** A Phase 2a Study to Evaluate the Pharmacokinetics, Pharmacodynamics, Safety, Tolerability, and Pharmacodynamics of Sotatercept (ACE-011) for the Correction of Anemia in Subjects With End-Stage Renal Disease on Hemodialysis

**CLINICALTRIALS.GOV IDENTIFIER:** NCT0144574

**COORDINATORS:** Celgene Corporation and Acceleron Pharma, Inc.

**PARTICIPATING CENTERS:** 26 hemodialysis centers in the United States

**ACCRUAL GOAL:** 43 subjects with ongoing recruitment

**STUDY DESIGN:** This phase II, randomized, single-dose, double-blind, placebo-controlled study, followed by a multiple-dose, single-blind, placebo-controlled, dose-escalation study with evaluations of the anemia, safety, efficacy, tolerability, and pharmacodynamics of sotatercept in the correction of anemia in subjects with end-stage renal disease on hemodialysis. The study has two parts. In part 1, subjects are randomized to receive either a single 0.1 mg/kg subcutaneous dose of sotatercept or matching placebo in a 3:1 sotatercept-to-placebo ratio. In part 2, subjects will be randomized to one of three dose groups (0.3 mg/kg, 0.6 mg/kg, or 0.9 mg/kg) in a 3:1 ratio of sotatercept to placebo. For each dose group, sotatercept will be injected subcutaneously once every 28 days for up to eight treatments. The primary endpoint of the study is characterization of the pharmacokinetics and pharmacodynamics of sotatercept. Notable secondary endpoints include the magnitude and rate of increase in hemoglobin and changes in blood pressure.

**RATIONALE:** Sotatercept is a recombinant, chimeric protein consisting of the extracellular domain of human activin receptor type IIa and the Fc domain of human IgG1. It binds activin A and other members of the transforming growth factor-β superfamily of cytokines, thereby inhibiting their functional activity. In a previous clinical study, sotatercept produced dose-dependent increases in hemoglobin, hematocrit, and RBCs, and reticulocytes in healthy women of all ages. Because sotatercept does not bind the erythropoietin (EPO) receptor and acts more rapidly than EPO, its erythropoiesis-stimulating activity likely involves non-EPO-dependent erythroid lineage cells. This clinical trial is one of several examining the erythropoietic effects of sotatercept in chronic anemias including Diamond-Blackfan anemia, transfusion-dependent β-thalassemia, and myelofibrosis.

**COMMENT:** Other than androgenic steroids with their significant systemic side effects, medications that stimulate erythropoiesis activate EPO receptors on erythroid progenitor cells. EPO receptors are activated directly by recombinant EPO, EPO-related drugs such as darbepoetin and polypeylene glycol-conjugated EPO, or EPO-mimetic peptides. EPO receptors can be activated indirectly via agents that increase endogenous EPO production through stabilization of the hypoxia-inducible transcription factors HIF-1α and HIF-2α. Drugs that activate EPO receptors have serious potential side effects, specifically aggravation of hypertension and induction of thrombosis. Thus, there exists a need to develop non-EPO-dependent mechanisms for treating anemia.

Erythropoietic stimulation via mechanisms that do not involve EPO receptors would have specific advantages in underproduction anemias such as the anemias of myelodysplasia, thalassemias, and narrow failure syndromes that are characterized by endogenous EPO concentrations that are elevated but ineffective. The other ongoing trials of sotatercept may provide information about stimulating erythropoiesis in underproduction anemias with elevated endogenous EPO levels, however, the current trial in renal failure patients on hemodialysis is unique as the markedly reduced capacity of these patients to produce endogenous EPO from their diseased kidneys means that erythropoietic responses to sotatercept (if observed) would be due to EPO-independent mechanisms. Whether the adverse effects of drugs that activate EPO receptors might be averted with sotatercept is of interest because many older patients with anemias also have underlying hypertension and vascular disease. Hypertension, albeit controlled, is common in renal failure patients, and the effect of sotatercept on blood pressure is a relevant secondary endpoint of the study. The anemia of renal failure is often multifactorial with contributions from EPO insufficiency and absolute and functional iron deficiency. Additionally, renal failure patients on hemodialysis have features of anemia of chronic inflammation, a process that is relatively resistant to EPO administration. If sotatercept effectively stimulates erythropoiesis in renal failure patients with the anemia of chronic disease, then less EPO may be required for their care, and sotatercept alone may benefit patients without renal failure who have the anemia of chronic inflammation.

– Mark J. Koury, MD

**Difficult But Not Impossible:**

**A New Approach to Treatment of Myelodysplastic Syndromes/Chronic Myelomonocytic Leukemia**

**STUDY TITLE:** ONTIME (ON 01910. Na Trial In Myelodysplastic Syndromes); Randomized Study of ON 01910. Na in Refractory Myelodysplastic Syndrome Patients With Excess Blasts

**SPONSOR:** Onconova Therapeutics, Inc.

**COLLABORATOR:** The Leukemia & Lymphoma Society

**CLINICALTRIALS.GOV IDENTIFIER:** NCT01241500

**PARTICIPATING CENTERS:** 77 study sites in the United States and Europe (Belgium, France, Germany, Italy, and Spain)

**ACCRUAL GOAL:** 270 patients

**STUDY DESIGN:** Eligible patients are 18 years of age; have a Zubrod performance status of 0-2; at least one bone marrow aspirate and biopsy with a CMML by French, American, British, or World Health Organization criteria (MDS patients must have 5-30% marrow blasts and white blood cell count < 5 x 10⁹/L; those with CMML must have 10-20% marrow blasts and a WBC count < 1 x 10⁹/L); are ineligible for or have relapsed after stem cell transplant; and have received at least six cycles of azacitidine or four cycles of decitabine within the last 12 months. Eligible patients need to have had a stable WBC for four weeks prior to enrollment, have responded and then relapsed. Eligible patients need to have had a WBC for four weeks prior to enrollment, have adequate renal and hepatic function (creatinine < 2.0 mg/dL, total bilirubin < 1.5 mg/dL, and transaminases < 2.5 times the upper limit of normal), should not receive other therapies for MDS except stable doses of filgrastim or an erythropoiesis-stimulating agent, and have had the MDS/CMML diagnosis reconfirmed within six weeks of study entry.

**Eligible patients are randomized in 2:1 ratio to receive either open-label rigosertib (n=180) at a fixed dose of 1800 mg/24 hours, administered as a 72-hour continuous intravenous infusion every two weeks until week 16 and every four weeks thereafter, or best supportive care (BSC) (n=90).**

**Dr. Koury indicated no relevant conflicts of interest.**

**Rigosertib is a styryl sulfone multi-kinase inhibitor with inhibitory effects against the phosphatidylinositol 3 kinase (PI3K) and the ability to induce oxidative stress (Chapman CM et al. Clin Cancer Res. 2012;18:799-901). Rigosertib also induces arrest of mitosis at the G2-M phase that may be mediated through inhibition of Polo-like kinase 1, possibly indirectly (Chen AW et al. Cancer Chemother Pharmacol. 2009;65:177-86). Although only small numbers of patients with MDS were treated with rigosertib before enrolling on the randomized trial, in some treated patients, the proportion of both blasts and hematopoietic cells with chromosomal aneuploidy was reduced (Olters MD et al. Leukemia. 2012;36:982-93). In a series of 60 patients with MDS enrolled in four phase I/II rigosertib trials, of whom 38 had previously received a HMA, eight patients (13%) experienced hematologic improvement, while among 51 patients with excess blasts (31%) had a median survival of 11 months. The most common causes of death include infection and hemorrhage.**

**Almost all patients with MDS will either fail to respond to an HMA or will eventually progress during HMA therapy. If rigosertib is shown to improve survival compared with BSC/LDAC in these patients for whom an HMA has failed, it is likely to become an established second-line therapy for higher-risk MDS in transplant-ineligible patients. While the frequent continuous intravenous administration that is necessary due to the short half-life of the current compound is inconvenient for patients, an oral formulation of rigosertib is in development (Raza A et al. Blood. ASH Annual Meeting Abstracts. 2011;118:3822). Fatigue and gastrointestinal upset (nausea, cramping, diarrhea) are the most commonly reported adverse events in rigosertib-treated patients.**

**COMMENT:** New drugs for MDS are greatly needed, especially for higher-risk patients who fail to achieve complete remission. A stem cell transplant is not an option. Rigosertib appears to suppress MDS clones in some cases and may delay disease progression, including AML evolution, though true disease remission appears uncommon. Nonetheless, hematopoietic improvement, adverse events, and overall survival are the most common causes of death include infection and hemorrhage.

**The clinical value of stable disease in MDS is currently unclear, but it has been hypothesized that a survival benefit could be achieved even in patients who do not experience blast reduction during active therapy, via a delay in clonal evolution and disease progression. This hypothesis remains unproven, and the rigosertib randomized trial will help test this assertion.**

– David P. Steensma, MD

**Dr. Steensma’s site is participating in the rigosertib randomized trial, but he has not received funding or salary support as part of this effort and has no other relevant disclosures.**
ASH Guides Mobile App – Now Featuring Guide for von Willebrand Disease

ASH Guides is a mobile app that features all of the Society’s clinical quick-reference guides. The app now includes the Evaluation and Management of Heparin-Induced Thrombocytopenia (HIT), Immune Thrombocytopenia (ITP), and von Willebrand Disease. Later in 2013, ASH will introduce additional mobile versions of the Society’s entire Clinical Quick Reference Guide collection, including recommendations for use of Epoetin and Darbepoetin, and the newest Quick-Reference Guide on Anticoagulant Dosing and Management of Anticoagulant-Associated Bleeding Complications in Adults. The app is currently available for iOS, Android, and Blackberry devices. To install the app, simply search for “ASH Guides” in your device’s app store. For more information about the guides and app, go to www.hematology.org/Practice/Guidelines/2934.aspx.

WHAT'S ON THE WEB

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), find ASH videos on YouTube (www.youtube.com/user/ASHWebmaster), and visit ASH on Facebook at www.facebook.com/AmericanSocietyofHematology.

MARK YOUR CALENDAR

January

25-26 Highlights of ASH
New York, NY www.hematology.org

Highlights of ASH
Dallas, TX www.hematology.org

February

1 Applications will be available for the ASH-AMFDP award
Washington, DC www.hematology.org/awards

1-2 Highlights of ASH
Miami, FL www.hematology.org

Highlights of ASH
San Francisco, CA www.hematology.org

6 Application deadline for Research Training Award for Fellows*
Washington, DC www.hematology.org/awards

22 Application deadline for ASH HONORS award
Washington, DC www.hematology.org/awards

28 Deadline to request assistance in selecting a mentor and/or creating a budget for the ASH Visitor Training Program (Note: Application deadline is May 2.)
Washington, DC www.hematology.org/awards

March

1 Application deadline for ASH Clinical Research Training Institute**
Washington, DC www.hematology.org/awards

8 Application deadline for the ASH Minority Medical Student Award Program (MMSAP)
Washington, DC www.hematology.org/awards

23-24 Highlights of ASH in Asia
Shanghai, China www.hematology.org

April

4 ASH Mentor Award nomination packages due
Washington, DC www.hematology.org/awards

12 Deadline to claim CME credits and print a CME certificate for the 54th ASH Annual Meeting
Washington, DC www.hematology.org

25-26 Highlights of ASH in Latin America
Santiago, Chile www.hematology.org

*In order to submit an application for the Research Training Award for Fellows, you must have submitted a letter of intent by December 14, 2012.

**In order to submit an application for the Clinical Research Training Institute, you must have submitted a letter of intent by January 8, 2013.

For additional meeting dates and award deadlines, go to www.hematology.org/Calendar.