The case of a 31-year-old man with thrombocytopenia
and targetable lesion are eligible to receive novel therapy
on a marker-negative subtype. Biopharmaceutical
companies are participating in the trial and providing
the investigational agents for this study. The first
investigational agents include AG-221, an IDH2 inhibitor;
tospleenib, a spleen tyrosine kinase (SYK) inhibitor;
samalizumab, a monoclonal anti-CD200 antibody; and BI
836058, a monoclonal anti-CD33 antibody.

Patients aged 60 years and older with newly diagnosed
AML are eligible for this study and can receive
targeted therapy for as long as they are responding, or
alternatively, they can undergo hematopoietic stem cell
transplantation if they achieve favorable responses.
Primary outcome measures for this trial are determining
the feasibility of completing all molecular, genetic,
immunophenotypic, and/or biochemical studies needed
to classify disease subtype and assign therapy within
seven days. The clinical response rate to each novel
therapy is also a primary outcome measure. Patient-
reported outcomes will be included as an important
exploratory objective.

RATIONALE: Despite the use of intensive cytotoxic
chemotherapy and hematopoietic stem cell
transplantation during the past 40 years, the prognosis
of older AML patients has not improved and has lagged
behind that observed in younger patients. Historically,
AML has been classified by routine cytomorphology
and screening for select chromosomal alterations. Recently,
however, a growing number of genetic alternations have
been identified, highlighting the disease heterogeneity
and the challenges in developing a common treatment
approach for all patients. Moreover, emerging evidence
suggests that molecularly targeted therapies, such
as FLT3 or IDH inhibitors, may be effective in distinct
AML subtypes, and this has provided the rationale
for the personalized medicine approach in this trial.
**Presidential Column**

Way to Make a Statement: Government Policy and ASH

If you are a long-time ASH member, or a fan of the Headlines from Washington column here in *The Hematologist*, you are aware that our advocacy efforts on Capitol Hill are core to what ASH does as a society. Member volunteers, leadership, and staff take part in Hill visits, write congressional representatives, and advocate on behalf of hematology as a field, and scientific progress in general. This work is central to ASH’s mission, and our efforts continue to grow.

For major policy events that are seen as having an immediate and sweeping effect upon hematologists and their patients, ASH also delivers statements, sometimes in cooperation with other medical societies. The most recent joint statement issued (see page 3) expressed ASH’s serious concern — along with other hematology and oncology groups — that the Administration’s executive order, limiting or denying U.S. entry to noncitizens, would have a devastating impact on some of our own constituents, many of whom represent some of the best minds in the field, from across the globe. As the statement notes, “we respectfully call on the Administration to consider the negative impact of its executive order on our nation’s ability to attract the world’s best scientific and clinical talent to participate in the fight against cancer and blood diseases, irrespective of their country of origin. This includes those immigrants who are inspired by the opportunity to bring their scientific curiosity and intellect to our country.”

It is undeniable that we are living in a politically contentious time. Therefore, we felt that it might be helpful at this time to offer some insight into what our policy statements are or, more precisely, what they are not:

ASH is committed to encouraging and stimulating dialogue on all issues that affect our work, our patients, and scientific progress. To this end, our statements on U.S. government policy are not an attempt to speak for all members of ASH or any specific group; it would be impossible to be nimble if we took the time to survey every member before weighing in on timely matters, such as the recent executive order. Rather, our statements reflect ASH’s core principles of advocating for an environment that supports the advancement of hematology research and the best care of our patients.

Policy statements are not aligned with, or preferential toward, any political party. As a nonpartisan organization, ASH has made policy statements during times of both Democratic and Republican Administrations, and has both criticized and praised either, based upon the merits of their specific policies and their effects on our members and their interests as professionals in the field. In the Society’s work on Capitol Hill, it has been critical that we work with both sides of the aisle, and to this point, we have awarded the Public Service Award to Representative David McKinley (R-WV) in 2016, and to Representative Danny K. Davis (D-IL) in 2015. Therefore, it is critical that statements from ASH always be an impartial expression of concerns and a means for creating awareness.

Lastly the statements we release do not emerge from a vacuum. We hear from our members on a regular basis, so oftentimes, the statements we make might be the product of feedback we receive — for example, we received a number of calls and email messages concerning the executive order on travel restrictions prior to and since the statement release. Statements from ASH are always considered from the perspectives of hematologists, and, wherever possible, our opinions are supported by evidence and assessments from expert stakeholders. One area where this is evident is ASH’s statements related to NIH funding levels. Maintaining a multi-channel dialogue between members, leadership, and staff is a part of what makes our society work. We are committed to openness, and we welcome all opinions on every issue, at any time. The health-care profession and public policy have a complex and enduring connection, making it difficult to separate politics from practice. ASH will continue to use its voice when issues arise affecting hematologists, and we encourage members to do the same.

Kenneth C. Anderson, MD

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**The Beat AML Trial**

(Cont. from page 1)

The trial will be limited to older adults as this is a group that has historically fared poorly with traditional cytotoxic chemotherapy.

**COMMENT:** This trial is a unique collaboration between patients, researchers, pharmaceutical companies, laboratories, and regulatory agencies to develop a precision medicine approach for the treatment of AML. If successful, this trial could facilitate the approval of new drugs of promise. This trial will also capture patient-related outcomes, which will provide an essential perspective on this novel treatment approach. Challenges will include obtaining the information needed for treatment assignment in a short window of time in a multicenter setting and the feasibility of a seven-day waiting period before starting definitive therapy. However, this study represents an innovative and very exciting treatment paradigm in a disease setting where effective targeted therapy requires close alignment with disease subtype.


Note: ASH has partnered with the Leukemia & Lymphoma Society to help spread the word about this pivotal trial.

~ Elizabeth Raezt, MD, and Tibor Kocoszovics, MD

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**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:
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ASH and Other Societies Issue Joint Statement on Administration’s Executive Order on Immigration

As the world’s leading organizations representing laboratory researchers, physician-scientists, clinicians, the nation’s cancer centers, and patient advocates committed to improving care for patients with cancer and blood diseases, ASH and five other medical societies have issued a statement regarding the Administration’s Executive Order on immigration.

In the statement, the organizations voiced their concern that restricting travel will interfere with scientific collaboration by limiting the exchange of ideas, practices, and data across cultures. “Our nation depends on the contributions of the greatest minds from around the world to maintain the high quality of our biomedical research enterprise and health care services.” They also highlighted the importance of in-person meetings and other global exchanges as they provide incomparable opportunities for collaborations and information-sharing.

The organizations ended the statement calling on the Administration to “…consider the negative impact of its executive order on our nation’s ability to attract the world’s best scientific and clinical talent to participate in the fight against cancer and blood diseases, irrespective of their country of origin.” Read the full statement online via www.hematology.org/Newsroom/Press-Releases/2017/7076.aspx.

Apply for the 2017 ASH Medical Educators Institute

The ASH Medical Educators Institute (MEI) provides a “boot camp” in teaching techniques, medical education scholarship, and career development for hematologists who are new to or in the early phases of medical education careers. In order to create future leaders in hematology education, MEI looks to build skills and position junior faculty for successful careers as clinical educators and mentors to future generations of hematologists. This year, the institute will accept 20 participants and will consist of three core activities:

- A fall workshop in Washington, DC, which begins with a mandatory kick-off dinner on October 17, followed by a three-day workshop at ASH Headquarters. ASH will pay the costs of travel to the program, lodging, and meals for accepted applicants.

- A webinar series taking place over the following 12 months.

- A mentorship component whereby faculty for the program will be available to participants as mentors for the completion of a scholarly project and for career development.


Awards Roundup

ASH provides an array of awards and programs to support hematologists in all stages of their careers and to recognize those who have significantly advanced the field of hematology.

1. ASH Harold Amos Medical Faculty Development Program (ASH-AMFDP): This award provides four years of research support, a complimentary ASH membership, and the opportunity to attend the ASH annual meeting for the four years of the award. The deadline to apply is March 15, 2017. Visit www.hematology.org/Awards/Career-Training/406.aspx for eligibility requirements and application information.

2. Scholar Awards: This award focuses on the period between completion of training and the establishment of an independent career for hematologists and provides partial salary and additional support during this critical period. The letter of intent is due in early May and the full application deadline is mid-July. For more information, visit www.hematology.org/Awards/Career-Training/6657.aspx.

3. Visitor Training Program (VTP): The VTP is meant to help build hematology capacity in developing countries in order to improve patient care. The program provides funding for hematologists or hematology-related healthcare professionals in the developing world to receive training on a specific topic or technique for up to 12 weeks. Upon completion of the training in the clinic or laboratory of an ASH member, participants return to implement the skills and knowledge acquired at their home institution. The application is due April 17, 2017. For eligibility requirements and application information, visit www.hematology.org/Awards/Career-Training/436.aspx.

For information about all awards offered by ASH, visit www.hematology.org/Awards.

Two New “Conversations With Innovators” Videos

Two new videos in “The Hematologist: Conversations with Innovators” series are now available on YouTube, featuring Drs. Kenneth Ataga and Lindsey A. George from the University of North Carolina and Children’s Hospital of Philadelphia, respectively. In the first video, Dr. Ataga talks about his work studying crizanlizumab to prevent pain crises in patients with sickle cell disease (see page 11). In the second video, Dr. Gerge discusses her program’s findings on sustained Factor IX expression in hemophilia B patients after gene therapy. To view the full series, visit www.hematology.org/Thehematologist/Multimedia.
A 31-year-old otherwise healthy man was referred to hematology clinic for evaluation of long-standing thrombocytopenia. He has had thrombocytopenia since his teenage years with a platelet count in the 60 to 90 × 10^9/L range. He had no history of prior bleeding episodes and had never required therapy for his cytophenia. The remainder of his complete blood count showed a normal white blood cell count (4.4 × 10^9/L) and mild anemia (hemoglobin of 11.2 g/dL; MCV of 97.0 fL). The reticulocyte count was 4.45 percent with an absolute count of 158.1 × 10^9/L. The peripheral blood smear is shown in the images below:

What is the diagnosis?

A. Immune thrombocytopenia purpura
B. May-Hegglin Anomaly
C. Sitosterolemia (Phytosterolemia)
D. Wiskott-Aldrich syndrome

For the solution to the quiz, visit The Hematologist online, www.hematology.org/Thehematologist/Images.
1. The treating clinician should provide relevant clinical data or ensure that this is readily accessible by the pathologist. 

2. The treating clinician should provide relevant physical examination and imaging findings or ensure that these are readily accessible by the pathologist.

3. The pathologist should review recent or concurrent complete blood counts (CBCs) and leukocyte differentials and evaluate a peripheral blood smear.

4. The treating clinician or pathologist should obtain a fresh bone marrow aspirate for all patients suspected of acute leukemia, a portion of which should be used to make bone marrow aspirate smears for morphologic evaluation. If performed, the pathologist should evaluate an adequate bone marrow trephine core biopsy, bone marrow trephine touch preparations, and/or marrow clots, in conjunction with bone marrow aspirates.

5. In addition to morphologic assessment, the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis, appropriate molecular genetic and/or FISH testing, and flow cytometric immunophenotyping. The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia, T-ALL (including early T-cell precursor leukemia), B-ALL, and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis.

6. For patients with suspected or confirmed acute leukemia, the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of ALL.

7. The treating clinician or pathologist may use cryopreserved cells or nucleic acid, formalin fixed, non-decalcified paraffin-embedded (FFPE) tissue, or unstained marrow aspirate or peripheral blood smears obtained and prepared from peripheral blood, bone marrow aspirate or other involved tissues for molecular or genetic studies in which the use of such material has been validated. Such specimens must be properly identified and stored under appropriate conditions in a laboratory that is in compliance with regulatory and/or accreditation requirements.

8. For patients with ALL receiving intrathecal therapy, the treating clinician should obtain CSF. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.

9. The treating clinician may obtain a CSF sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist.

10. For patients with suspected or confirmed ALL, the pathologist may use flow cytometry in the evaluation of CSF.

11. For patients who present with extramedullary disease without bone marrow or blood involvement, the pathologist should evaluate a tissue biopsy and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the bone marrow.

12. For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD.

13. For pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13;q22); ETV6-RUNX1, t(9;22)(q34;q11.2); BCR-ABL1, KMT2A (MLL) translocation, AMP1, and trisomy 4 and 10 is performed.

14. For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(9;22)(q34;q11.2); BCR-ABL1 is performed. In addition, testing for KMT2A (MLL) translocations may be performed.

15. For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnosis, prognosis, and/or therapeutic management that includes, but is not limited to: PAX5, KIT, JAK2, and/or IKZF1 for B-ALL and NOTCH1 and/or FBXW7 for T-ALL. Testing for overexpression of CRLF2 may also be performed for B-ALL.

16. For pediatric and adult patients with suspected or confirmed AML of any type, the pathologist or treating clinician should ensure that testing for FLT3-ITD is performed. The pathologists or treating clinician may order mutational analysis that includes but is not limited to: IDH1, IDH2, TET2, WT1, DNMT3A, and/or TP53 for prognostic and/or therapeutic purposes.

17. For adult patients with confirmed core binding factor (CBF) AML (AML with (8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22) / (16;16)(p13.1;q22); CBFB-MYH11, the pathologist or treating clinician should ensure that appropriate mutational analysis for KIT is performed. For pediatric patients with confirmed core binding factor AML (AML with (8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22) / (16;16)(p13.1;q22); CBFB-MYH11, the pathologist or treating clinician may ensure that appropriate mutational analysis for KIT is performed.

18. For patients with suspected APL, the pathologist or treating clinician should also ensure that rapid detection of PML-RARA is performed. The treating clinician should also order appropriate coagulation studies to evaluate for DIC.

19. For patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities, the pathologist or treating clinician should also ensure that mutational analysis for NPM1, CEBPA, and RUNX1 is also performed.

20. For patients with confirmed acute leukemia, no recommendation is made for or against the use of global/gene specific methylation, mRNA expression, or gene expression analysis for diagnosis or prognosis.

21. For patients with confirmed mixed phenotype acute leukemia, the pathologist or treating clinician should ensure that testing for t(9;22)(q34;q11.2); BCR-ABL1, and KMT2A (MLL) translocations is performed.

22. All laboratory testing performed for the initial work-up and diagnosis of a patient with acute leukemia must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements.

23. If after examination of a peripheral blood smear, it is determined that the patient will require immediate referral to another institution with expertise in the management of acute leukemia for treatment, the initial institution should, whenever possible, defer invasive procedures including bone marrow aspiration and biopsy to the treatment center to avoid duplicate procedures, associated patient discomfort, and additional costs.

24. If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded as they become available.

25. In the initial report, the pathologist should include laboratory, morphologic, immunophenotypic, and, if performed, cytochemical data, on which the diagnosis is based, along with a list of any pending tests. The pathologist should issue addenda/amended reports when the results of additional tests become available.

26. The pathologist and treating clinician should coordinate and ensure that all tests performed for classification, management, predicting prognosis and disease monitoring are entered into the patient’s medical records.

27. Treating clinicians and pathologists should use the current World Health Organization (WHO) terminology for the final diagnosis and classification of acute leukemia.
The Question
What genetic markers and risk stratification algorithms do you use to guide individualized treatment of juvenile myelomonocytic leukemia (JMML)?

Our Response
JMML is a rare and aggressive myeloproliferative/myelodysplastic neoplasm of infants and young children. It is characterized by leukoerythroblastosis on peripheral blood examination and fewer than 20 percent blasts in the bone marrow. Clinical features frequently include hepatosplenomegaly, fever, rash, poor growth, and susceptibility to infections. Several congenital syndromes predispose to JMML, including neurofibromatosis type I (NF1) and CBF (Casitas B-lineage lymphoma) syndrome.1 Genetic mutations in NF1, NRAS, KRAS, RRAS, RRAS2, FPN11, and CBL are all hypothesized to increase signaling through the Ras/MAP kinase pathway, which is the most frequent genetic alteration that can be identified in approximately 95 percent of patients.2 Additionally, patients with Noonan syndrome and germline FPN11 mutations can also develop a JMML-like myeloproliferative disease during childhood. Interestingly, patients with germline FPN11 and CBL mutations who develop a MPN have high rates of spontaneous regression.3 Similarly, patients with NF1, RRAS, NRAS mutations experience spontaneous resolution of their disease.4 Unfortunately, identifying these patients at diagnosis has proven exceptionally difficult. In contrast to patients who experience self-resolving disease, overall outcomes in this disease after hematopoietic stem cell transplantation (HSCT) are quite poor, with event-free survival of less than 50 percent, primarily due to relapse.5 Updated diagnostic criteria for JMML, which are included in the revised 2016 World Health Organization classification, consist of a combination of clinical, laboratory and genetic/cytogenetic features (Table 1).6

How do you risk stratify JMML?
Recently, whole exome sequencing has identified additional genetic alterations contributing to relapse, including upregulation of JAK-STAT signaling, epigenetic modification through the PRC2 components such as DNM3TA, EH2H and ASXL1, and altered RNA splicing.7,8 Mutations in a hotspot region of SETBP1 appear to be the most single common cause of relapse.9 The most frequent cytogenetic abnormality, occurring in approximately 25 percent of patients with JMML, involves several groups have recently demonstrated that an increased number of somatic alterations correlates with a poor prognosis in JMML.10

In patients who meet criteria for JMML, we stratify based on age, driver mutation, and the presence of secondary genetic alterations including monosomy 7 (Figure). Given the high rate of spontaneous resolution in patients with CBL syndrome, these patients frequently can be observed without therapy. Intervention with chemotherapy is typically only indicated for CBL patients who become symptomatic (splenomegaly, failure to thrive, recurrent infections, etc.). Patients harboring NRAS mutations, particularly infants without secondary genetic alterations, can be closely monitored for signs of spontaneous resolution or treated with low-dose chemotherapy. Some case reports have also utilized azacytidine.11 All other patients should be considered for chemotherapy and then proceed to HSCT with the best available donor.

What conventional treatment approaches are employed in JMML?
One unresolved question in JMML is the role of pretransplant chemotherapy. In nearly every other pediatric leukemia, it is known that a lower disease burden at time of transplantation is associated with improved outcomes. However, in JMML, many clinicians proceed directly to HSCT without decreasing disease burden with pretransplant chemotherapy. Another common treatment approach is to bridge to HSCT with oral imatinib or low-dose cytarabine.12 This approach reduces spleen size and white blood cell count but rarely reduces the ability of the bone marrow to generate hematopoiesis. The fraction of the underlying mutation (unphased data). In contrast, we have observed that more intensive treatment with fludarabine and high-dose cytarabine is capable of eradicating somatic mutations by deep sequencing prior to HSCT. Whether this leads to improved long-term outcomes is still unknown, but our institutional bias is to offer this well-tolerated regimen to patients planning to undergo HSCT.

A recent clinical trial, Children’s Oncology Group (COG) ASCT221 (NCT01824603), randomized patients to receive HSCT conditioning with busulphan and fludarabine, or busulphan, cyclophosphamide, and melphalan. It was closed prematurely after patients treated with busulphan and fludarabine were noted to have increased rates of disease progression. Therefore, we therefore recommend using busulphan, cyclophosphamide, and melphalan for all patients undergoing HSCT until we can identify patients appropriate for a reduced-toxicity regimen.

What is the role of novel targeted therapies in the current treatment algorithm?
There are two novel approaches currently in clinical trials in the United States and Europe. Nearly 100 percent of JMML patients harbor a Ras pathway alteration. Preclinical models using genetically engineered mice with Ras pathway mutations suggest that MEK inhibition could be an effective approach in this disease.13 The Children’s Oncology Group will therefore be sponsoring a clinical trial (ADVL1521) to test the efficacy of the oral MEK inhibitor, trametinib, in patients with relapsed and refractory JMML. As all monotherapy regimens are likely to be limited by the development of resistance, one of the secondary objectives of this trial is to identify mechanisms of resistance to rationally plan future combinatorial treatments.

There are several cases of reports of complete remissions achieved with the hypomethylating agent azacytidine in JMML.14 In one recent clinical trial, patients treated with relapsed and refractory JMML who have previously achieved remission with azacytidine subsequently responded to fludarabine and cytarabine.15 The optimal pretransplant therapy is likely to be a combination of cytotoxic chemotherapy, MEK inhibition, hypomethylating agents, and other targeted agents. It is important to note that there has never been a randomized trial of pretransplant regimens in JMML. Due to the rarity of this disease, an international multi-institutional trial is the only feasible approach to carrying out such a trial. Recently, response criteria have been proposed for evaluating outcomes to non-transplant therapies and HSCT in the context of clinical trials, which should help with comparison of different treatment modalities.16 Lastly, as disease courses vary dramatically from spontaneous resolution to rapid progression after HSCT, a robust risk-stratification algorithm based on multiple biomarkers will be critical to predict these divergent outcomes and inform treatment decisions for patients with JMML.

What are future directions for treatment and what questions remain?
Several additional agents that are undergoing preclinical testing include Ras mimetics, SHP-2 antagonists, anti-GM-CSF antibodies, and CAR-T cells targeting JMML-specific antigens.

The optimal pretransplant therapy is likely to be a combination of cytotoxic chemotherapy, MEK inhibition, hypomethylating agents, and other targeted agents. It is important to note that there has never been a randomized trial of pretransplant regimens in JMML. Due to the rarity of this disease, an international multi-institutional trial is the only feasible approach to carrying out such a trial. Recently, response criteria have been proposed for evaluating outcomes to non-transplant therapies and HSCT in the context of clinical trials, which should help with comparison of different treatment modalities.16 Lastly, as disease courses vary dramatically from spontaneous resolution to rapid progression after HSCT, a robust risk-stratification algorithm based on multiple biomarkers will be critical to predict these divergent outcomes and inform treatment decisions for patients with JMML.

Dr. Stiegitz and Dr. Koegel indicated no relevant conflicts of interest.

12. Chang T, Krivickas L, Theobald EH, et al. Sustained MEK inhibition improves outcomes to non-transplant therapies and HSCT in the context of clinical trials, which should help with comparison of different treatment modalities.16 Lastly, as disease courses vary dramatically from spontaneous resolution to rapid progression after HSCT, a robust risk-stratification algorithm based on multiple biomarkers will be critical to predict these divergent outcomes and inform treatment decisions for patients with JMML.

The Hematologist: ASH NEWS AND REPORTS

6
New Congress Tasked With Finalizing 2017 Budget for NIH, Other Federal Agencies

The new Congress and administration have been sworn into office but still have many tasks ahead. Chief among them is how to keep the government running for the remainder of the fiscal year. The continuing resolution (CR) that currently funds the government, including the National Institutes of Health (NIH), will expire on April 28. As this issue of The Hematologist went to press, the Senate passed a proposed budget resolution which would proceed with finalizing the fiscal year (FY) 2017 budget. The new Congress is faced with either extending the current CR for the rest of the fiscal year or passing appropriations bills to fund the government at the new levels until FY 2017 ends on September 30. ASH and other members of the biomedical research community have strongly urged Congress to finish the appropriations process, noting how the budget ambiguity resulting from a CR leaves NIH and researchers in limbo and how the prospect of a flat or reduced final budget would delay progress.

While ASH continues to closely monitor FY 2017 and await a final budget agreement, it is important for your legislators to hear from you about the impact that a loss of funding has on your research and the patients you treat. Contact your congressional delegation by visiting the ASH Advocacy Center at www.hematology.org/Advocacy. Write your legislators and ensure that a final bill is passed with adequate increases to the NIH and other important public health programs.

HHS Releases Final Rule to Enhance Protections for Participants in Research

On January 18, the U.S. Department of Health and Human Services (HHS) and 15 other federal agencies issued a final rule to update regulations that safeguard individuals who participate in research. Most provisions in the new rule will go into effect in 2018. The current regulations, which have been in place since 1991, are often referred to as the “Common Rule.” They were developed at a time when research was conducted predominantly at universities and medical institutions, and each study generally took place at a single site. Since then, research with human participants has grown in scale and become more diverse.

The new rule is intended to strengthen protections for people who volunteer to participate in research, while ensuring that the oversight system does not add inappropriate administrative burdens, particularly to low-risk research. In response to concerns expressed by many of the commenters, the final rule contains a number of significant changes from the proposed rule including the removal of a provision that would have required researchers to obtain consent before using a participant’s non-identified biospecimens. The final rule maintains the current practice with respect to oversight of these specimens. Other important elements in the final rule include:

- The requirement for consent forms to provide potential research subjects with a better understanding of a project’s scope, including its risks and benefits, so they can make a more fully informed decision about whether to participate.
- Requirements, in many cases, to use a single institutional review board (IRB) for multi-institutional research studies. The proposal from the NPRM has been modified, however, to add substantial increased flexibility in now allowing broad groups of studies (instead of just specific studies) to be removed from this requirement.
- For studies on stored identifiable data or identifiable biospecimens, researchers will have the option of relying on broad consent obtained for future research as an alternative to seeking IRB approval to waive the consent requirement. As under the current rule, researchers will still not have to obtain consent for studies on nonidentified stored data or biospecimens.
- The establishment of new exempt categories of research based on the level of risk they pose to participants. For example, to reduce unnecessary regulatory burden and allow IRBs to focus their attention on higher risk studies, there is a new exemption for secondary research involving identifiable private information if the research is regulated by and participants protected under the HIPAA rules.
- Removal of the requirement to conduct continuing review of ongoing research studies in certain instances where such review does little to protect subjects.
- Requirement that consent forms for certain federally funded clinical trials be posted on a public website.

The finalization of the rule comes after the Obama Administration released a Notice of Proposed Rulemaking (NPRM) on the “Common Rule” in September 2015. ASH solicited feedback on the NPRM from the ASH Committee on Government Affairs and the Committee on Scientific Affairs, and submitted final comments to the government on January 6, 2016. Additional information on the Common Rule revisions may be found on the HHS website at www.hhs.gov/cmb/policy/privacy/transctions/ finalized-revisions-common-rule.

ASH Continues Efforts to Support Oral Parity Legislation in Several States

ASH and coalition partners saw several successes in 2016 with Alaska and Pennsylvania approving oral chemotherapy parity legislation, bringing the total number of states that have passed these critical laws to 42 plus the District of Columbia. ASH will continue to work with the State Patients Equal Access Coalition (SPEAC), with the goal of having this life-saving legislation signed into law in Tennessee, Michigan, and North Carolina this year. Much like the federal legislation that ASH continues to promote (and which is expected to be reintroduced in the first half of 2017), the state bills would ensure equitable insurance coverage for oral and intravenous anti-cancer treatments by fixing a critical loophole in many insurance plans. While intravenous cancer treatment is typically paid for as part of a health plan’s medical benefit, patient-administered anticancer drugs, including oral and self-injectable drugs, are often only covered as a prescription benefit, leaving many patients responsible for extremely high and unmanageable copays.

If you live in any of the states mentioned and want to get involved in ASH’s advocacy efforts, please contact ASH Government Relations Coordinator Foster Curry at fcurry@hematology.org, for more information.
Learning to Count Beyond Four


Hematopoietic stem cells (HSCs) have a remarkable capacity for lifelong production of blood cells; however, they are not immune to aging. Surprisingly, as a mouse ages, there is actually an accumulation of phenotypically defined HSCs, with up to five times as many found in old mice compared to young mice.1-6 Despite the increase in number, hematopoietic output shifts toward a myeloid bias, and the repopulating ability of old HSCs is only about one-quarter as efficient as young HSCs. While most of the published work has used mouse models, a retrospective analysis by the National Marrow Donor Program assessing donor characteristics on recipient outcome found that age was the only donor trait significantly associated with overall and disease-free survival. Recipients who received a graft from a donor older than 45 years had significant decreases in five-year survival, demonstrating clear defects in HSC function as a result of aging.

Evidence of the ability of stem cells to count their divisions creates many additional questions to these questions are likely to significantly advance the field and our understanding of long-term stem cell function. Understanding the mechanisms of this counting function may help aid in developing further therapeutic strategies. Intriguingly, the normal maximal amount of serial transplantations in the mouse model is about four to five serial transplantations, suggesting that the workarounds may even have its own counting mechanism. Answers to these questions are likely to significantly advance the field and our understanding of self-renewal.

In the past decade, studies using advanced high-throughput sequencing technologies have provided insights into the genetic basis of myelodysplastic syndromes (MDS). This work has confirmed that 1) the bone marrow cells in patients with MDS (regardless of blast count) are clonally derived; 2) founder and multiple subclones exist simultaneously, (i.e., clonal heterogeneity); and 3) mutations in several genes predict poor overall survival in patients independent of established clinical risk factors. Several of these studies have attempted to define the clonal architecture of MDS and the genetic changes that underlie its progression to secondary acute myeloid leukemia (sAML). This work has largely been restricted to studying the allelic burden of a limited number of driver mutations at a single time-point or performing comprehensive mutation profiling of serial samples from a small number of patients, potentially obscuring the clinical utility of this type of testing in diagnosis and dynamic prognostication and therapy selection.

To further clarify the relationship between molecular changes and disease progression and clinical outcomes in MDS, Dr. Hideki Makishima and colleagues analyzed somatic mutations in a very large cohort of MDS patients, including many serially collected patients. Clonal architecture and dynamics were investigated by whole exome sequencing (WES) and targeted sequencing of 699 patients with MDS and sAML; 122 patients were analyzed longitudinally. These and published data sets (n = 2,250) were interrogated to determine the differential roles of driver mutations in disease progression. They found that higher-risk MDS was characterized by a higher number of mutations with increasing diversity and a larger clone size. Additionally, the number of cases with heterogeneity was significantly higher in sAML compared with lower-risk MDS.

In WES analysis of serial samples, evolution of a new dominant clone that swept out other subclones was common during disease progression and was frequently accompanied by newly emerging subclones. In most cases, leukemic transformation was marked by acquisition of new mutations without clone sweeping.

To clarify the relationship between driver mutations and disease progression, the authors compared the frequencies of major driver mutations in low- and high-risk MDS, as well as sAML. Mutations in genes designated as type 1 (FLI1, PTPN11, WT1, IDH1, NPM1, IDH2, and NRAS) were significantly enriched in sAML compared with high-risk MDS. When they compared high- and low-risk MDS, mutations in TET2, RUNX1, ASXL1, ZRSR2, and TET2 (designated type 2 mutations) were significantly enriched in high-risk MDS. SF3B1 mutations were strongly enriched in low-risk MDS, compared to high-risk MDS. In longitudinal samples, type 1 mutations were more likely to be newly acquired or to increase in clone size. In contrast, type 2 and other mutations decreased in their clone size or were even lost in the second sampling. As shown in the Figure, patients with type 1 mutations (group I) had a significantly shorter time to progression to sAML compared to patients with type 2 mutations who lacked type 1 mutations (group II) and those lacking type 1 and type 2 mutations (group IV). Patients with SF3B1 mutations and lacking type 1 and type 2 mutations (group IV) also had a significantly shorter time to progression than group I patients. In a multivariate analysis, the group I category remained an independent negative predictor of overall survival, together with other factors, including complex karyotype, IPSS score, −7/del(7q), age, and del(20q). SF3B1 mutations were mutually exclusive of other splicing factor mutations, and type 1 and type 2 mutations, potentially reflecting unique biology among this clinically distinct MDS subtype. They observed a global trend of co-occurrence of type 1 and type 2 mutations, with TET2, and NPM1 acting as exceptions and present mutually exclusively of other type 1 and type 2 mutations. Interestingly, type 1 mutations had significantly lower variant allele fractions compared to type 2 mutations in the patients with both categories of mutations, suggesting that type 1 mutations were acquired during disease progression after previous type 2 hits.

In demonstrating that MDS disease progression is accompanied (and potentially mediated) by distinct molecular events, this work suggests that screening for these specific mutations might be useful to predict clinical outcome or allow therapeutic intervention at critical time points. Proof of this clinical utility requires further study, including elucidation of the underlying pathophysiology represented by these genetic findings.


Kaplan-Meier curves for progression free survival (n = 429) A) and overall survival (n = 1,347) B) of patients with type 1 mutations (group I), with type 2 mutations (group II), and with type 1 and type 2 mutations (group III) of patients with type 1 or type 2 mutations (group IV), and other patients with no type 1, type 2 or SF3B1 mutations (group IV). Reprinted by permission from Macmillan Publishers Ltd: Nature Genetics doi:10.1038/ng.3742, copyright 2016.
The Epitranscriptome in Leukemia: A Role for the N⁶-Methyladenosine RNA Demethylase FTO in Acute Myeloid Leukemia


Within the last decade, there has been intense interest in the role of epigenetic alterations, the reversible modifications of DNA nucleotides and amino acids of histone tails, in normal and malignant hematopoiesis. In contrast, there has been very little evaluation of modifications of RNA, which were initially described more than 30 years ago. Analogous to the “writers,” “erasers,” and “readers” of the epigenome, similar proteins that alter and bind RNA modifications have been described (Figure). Collectively RNA modifications are now referred to as the “epitranscriptome” because they can be dynamically placed and removed, just as epigenetic marks on DNA and histones.

The dearth of studies of RNA modifications in cancer is dated by the fact that 1) there have been no techniques to map the locations of modifications in RNA until recently, and 2) the proteins that read or alter RNA modifications are rarely mutated in cancer. Moreover, the most common RNA modification, N⁶-methyladenosine (m⁶A), constitutes only 0.1 to 0.4 percent of adenosine nucleotides in mammalian cells. Thus, the modest abundance of RNA modifications also has limited efforts to understand the functions of RNA modifications.

Following the description of techniques to map m⁶A deposition throughout the transcriptome, Dr. Zejian Li and colleagues now identify that the m⁶A demethylase FTO (fat mass and obesity-associated protein) promotes acute myeloid leukemia (AML). The authors found that FTO is unique among m⁶A methyltransferases and demethylases in that its expression is increased in AML over normal hematopoietic cells (Figure). Through a series of functional experiments enforcing expression of wild-type FTO relative to enzymatically dead versions of FTO or RNAi-mediated downregulation of FTO in AML cells, the authors found that overexpression of FTO promotes leukemogenesis of cells bearing fusions of MLL or PML-RARA as well as normal karyotype AML with FLT3-ITD and NPM1 mutations.

Consistent with FTO’s role as an m⁶A demethylase, FTO overexpression in AML cells was associated with global m⁶A downregulation. Prior studies have postulated numerous roles for m⁶A in mRNA metabolism including roles in 5’ capping, 3’ polyadenylation, splicing, nonsense-mediated decay, and nuclear export of mRNAs. Through correlation of transcriptome-wide maps of m⁶A deposition (via m⁶A-Seq) with gene expression (via RNA-seq) in cells with and without FTO overexpression, the authors found the somewhat unexpected finding that 80 percent of FTO target mRNAs are negatively regulated by FTO. These data suggest that, at least in the context of AML, FTO-mediated m⁶A demethylation is a negative regulator of mRNA expression. The authors specifically focused on two m⁶A methylated genes which appeared to be regulated by FTO, ASB2 and RARA, given prior knowledge of the importance of these genes to MLL- and PML-RARA-rearranged AML. They found that m⁶A methylation regulates expression of these genes by targeting the 3’ untranslated regions (UTRs) of both ASB2 and the 3’ and 5’ UTRs of RARA (Figure). In fact, modulating the levels of ASB2, RARA, or the enzymes affecting m⁶A of ASB2 and RARA transcripts regulated differentiation of APL cells to reticuloid.

We are only at the beginning of our understanding of the epitranscriptome in the context of normal and malignant hematopoiesis. Further efforts to map the locations of modifications and to understand the function of readers, writers, and erasers of RNA modifications through deletion and overexpression of these proteins in hematopoietic cells will be critical. Moreover, given the development of therapies targeting so many epigenetic modifiers, similar efforts to develop chemical probes and drugs targeting epitranscriptomic modifiers may be biologically enlightening as well as potentially therapeutically important.

Testosterone Therapy: Risk Factor for Venous Thromboembolism?


In 2011, 2.9 percent of men more than 40 years old in the United States were taking some form of testosterone replacement therapy, either as intramuscular intradermal, subcutaneous, or oral preparations.1 In 2017, that number is likely higher, given aggressive advertising campaigns.2 “IT” as a lifestyle intervention for testosterone to prevent cardiovascular disease (CVD) and for use in the “low T” paradigm has been adopted by many urologists.3 Alternatively, the availability of testosterone preparations to increase the “spring in your feet” [as advertising materials promise].4 In 2015, a superb New England Journal of Medicine “Perspective” article concluded that “to date there is no definite evidence that increasing serum testosterone concentrations in men (with age-related hypogonadism) is beneficial and safe.”5 Due to the lack of conclusive data, it has not been seen whether testosterone is prothrombotic, and, if it is, whether it is prothrombotic per se and thus, in all patients on testosterone replacement therapy, or only in patients who develop testosterone-induced erythrocytosis. These issues are relevant for decision making in two clinical scenarios:

1. The patient who develops a venous thromboembolic event (VTE) on testosterone replacement therapy: Did the testosterone contribute to the VTE development? Should testosterone in this situation be viewed as a true risk factor and the patient, therefore, be treated only short-term (such as 3 months) with anticoagulation if testosterone is discontinued?

2. The patient who has a history of VTE and is no longer on anticoagulation: Can testosterone replacement therapy safely be given?

A recent article reports the results of a well-done, very large population-based case-control study from the United Kingdom that investigated a) whether testosterone is a risk factor for VTE, and b) how the risk changes depending on how long a man has been taking testosterone. Patients with a first VTE event (deep vein thrombosis and pulmonary embolism) were identified via a comprehensive validated algorithm by use of hospital discharge diagnoses, causes of death, and medical records. Testosterone exposure was obtained by prescription writing history. Individuals were defined as being either unexposed or currently exposed to testosterone, and the latter group was stratified into two groups in respect to how long the man had been on testosterone: up to six months before the VTE or more than six months before event. Fifty matched controls without VTE in the source population were identified for every VTE patient. The study population consisted of 192,415 patients with VTE and 960,830 controls.

The main findings include: 1) The adjusted rate ratio of VTE for current versus no testosterone treatment before VTE was 1.29 (95% CI, 0.94-1.66). 2) In the first six months of testosterone therapy, the rate ratio of VTE was 1.63 (95% CI, 1.12-2.37); after more than six months, testosterone replacement therapy was 1.00 (95% CI, 0.68-1.47). 3) Stratification by route of administration yielded similar rate ratios for intramuscular, transdermal, and oral testosterone use. The authors appropriately conclude that “starting testosterone therapy was associated with an increased risk of VTE, which peaked within six months and declined thereafter.” An additional conclusion is that the VTE risk in the first six months is only small.

How do these findings influence my medical practice? First, in the patient diagnosed with VTE who started testosterone therapy within six months of the VTE, I now list testosterone as a contributing, albeit only mild, VTE risk factor. In the patient who had been on testosterone for more than six months prior to the VTE, however, I conclude that the testosterone did not contribute to the VTE. Second, with respect to duration of anticoagulation decisions, 1) I would view the patient with testosterone-associated, but otherwise unprovoked VTE who had been on testosterone for more than six months as having a truly unprovoked event, and thus recommend long-term anticoagulation; and 2) it is unclear how long the man who developed a VTE within six months of having started testosterone should be treated with anticoagulation. Recent discussion with several of my academic colleagues has not yielded a consensus – management suggestions range from short-term anticoagulation, as the transient VTE risk factor testosterone has been removed; to empiric use of a D-dimer with discontinuation of anticoagulation if a patient is D-dimer negative on anticoagulation and still negative four weeks later, but continuation or restarting of anticoagulation if the D-dimer is initially positive or turns positive at four weeks; to long-term anticoagulation. I favor the D-dimer approach. Given the lack of data on how such a patient is best managed, treatment decisions are not evidence-based. They also need to take the patient’s preference into consideration.

Finally, unfortunately it remains unknown whether testosterone leads to VTE in patients who do not develop erythrocytosis. Whether the drug is prothrombotic per se, independent of the hematocrit. This important question was not addressed in this study.


Enhancing CAR T Cell Activity in Follicular Lymphoma: Targeting BTLA Through Engineered Soluble HVEM Delivery


A nt-CD19 directed chimeric antigen receptor (CAR) T-cell therapy has yielded high response rates and durable responses in a select group of patients with diffuse large B-cell lymphoma (DLBCL) or Hodgkin lymphoma (NHL); however, patients either progress or relapse after this therapy. Resistance is not well understood but may be related to loss of the target tumor antigen or to aspects of the tumor microenvironment. Defining resistance mechanisms will allow for enhancement of this therapy for lymphoma patients.

Mutations in HVEM (herpes virus entry mediator; TNFSF71) are among the most frequent mutations in germin center lymphomas. Dr. Michael Boice and colleagues investigate the function of HVEM on follicular lymphoma (FL) lymphomagenesis. Under normal circumstances, HVEM binds to the B and T lymphocyte attenuator (BTLA) receptor and inhibits the B cell. Loss of HVEM, through somatic mutations, and loss of BTLA, through inactivation of the histone methyltransferase KMT2D, appear to be mutually exclusive events that are seen in 20 to 30 percent and more than 50 percent of FL, respectively. Furthermore, this incidence does not change with disease transformation, suggesting that these events occur early in oncogenesis. To assess the role of HVEM and BTLA in the development of FL, Dr. Boice and colleagues introduced short hairpin RNAs (shRNAs) against HVem or empty vector controls into vavPBcl2 hematopoietic progenitor cells (HPCs) and injected them into lethally irradiated mice. Knockdown of HVem resulted in a significant acceleration in lymphoma development, as well as increased phosphorylation of SYK, BTK, and BLNK, evidence of activation of the B cell receptor (BCR) pathway. Knockdown of BTLA in the same FL model system yielded similar results.

The microenvironment is important for both the development and persistence of FL. Follicular dendritic cells (FDCs), follicular reticular cells (FRCs) and follicular T helper cells (Tfh) are all known to support the malignant FL cell. Compared to a group of control lymphomas, lymphoma cells that are deficient in HVEM produced significantly more TNFα, IL10, and IL17, which are essential activators of FDCs and FRCs. These levels are decreased upon treatment with soluble HVEM (soHVEM). Evaluation of the tumor microenvironment in HVEM-deficient tumors (both derived from cell lines and from primary FL tumors) revealed enhanced FDC networks and FRC activation, as well as increased levels of CXCL13, which is known to be produced by FDCs and FRCs and to attract Tfh. Indeed these tumors were associated with increased numbers of Tfh compared with control lymphomas, and with increased levels of TNFα produced cytokines. SoHVEM, therefore, may be able to rescue HVEM-deficient, BTLA-intact B-cells and inhibit growth of HVEM-deficient, BTLA- intact lymphoma. To test this, Dr. Boice and colleagues treated the B1L11 B cell lymphoma cell line with soHVEM or empty vector after stimulation of the BCR with IgM. Both soHVEM and ibritinib had similar inhibitory effects on BTK phosphorylation following BCR stimulation compared with controls, but this effect of soHVEM required intact BTLA. Similar results were seen in BTLA-expressing primary FL cell lines, regardless of whether the cells express HVEM or not. SoHVEM caused growth inhibition and induced apoptosis of BTLA-positive lymphoma cell lines in vitro as well as in vivo in mouse xenograft models. Finally, they treated xenografted DoHH2 lymphomas, which express BTLA but carry a homzygous HVEM deletion, with anti-CD19 CAR T-cells as well as anti-CD19 CARs engineered to express soHVEM. The latter resulted in enhanced cell killing.

The identification of targetable and recurrent genetic mutations in HVem in FL that lead to enhanced B cell activation and survival through both BCR activation and through the maintenance of a permissive microenvironment has therapeutic potential for a subset of FL patients. SoHVEM alone is unlikely to yield clinical benefit given both the difficulty in delivering the peptide drug to the bone marrow, and the genetic and immunologic complexity of the disease. Combining therapy with CAR T cells takes advantage of the powerful anti-lymphoma effect of CAR T-cells and the ability to deliver the drug directly to the target. This is an elegant way to enhance CAR T cell therapies while targeting additional vulnerabilities of the lymphoma cell and its environment. Unlike the antibody directed approaches that are nearly universally blocked by disease antigen CD19; these modified CAR T-cells will only be of additional benefit to a genetically defined subgroup of patients. This system, however, can serve as a prototype for lymphomas with additional or alternative targets.

CARON JACOBSON, MD
Dr. Jacobson indicated no relevant conflicts of interest.
Relapsed CLL: A Problem, Not Yet Solved


Patients with chronic lymphocytic leukemia (CLL) and their doctors have seen the therapeutic landscape change rapidly in the past few years, with the introduction into practice of the kinase inhibitors ibrutinib and idelalisib, which block specific signalling pathways downstream of the B cell receptor (BCR), and more recently the BCL2 inhibitor, venetoclax. With durable responses now being common in many patients with previously relapsed CLL, it is timely that research continues to focus on the minority for whom treatment of CLL remains particularly problematic—those with CLL that has deletion of the short arm of chromosome 17, which bears the TP53 gene and is associated with a poor prognosis (median survival 29 months).

Dr. Susan O’Brien and colleagues conducted a single-arm phase II study of ibrutinib in patients with deletion 17p CLL that was progressing after previous treatment (relapsed or refractory CLL). Such patients exhibit poor responses and survival after chemoimmunotherapy. The patient population was moderately heavily treated, with more than one-third having had three or more prior lines of therapy. Using the standard dose of 420 mg once per day, 145 patients were followed for a median of 28 months. The overall response rate was 83 percent, and the 24-month progression-free survival (PFS) estimate was 63 percent, indicating durable benefit for some patients, but with an ongoing risk of progression. Fifty percent of patients had permanently discontinued ibrutinib, with progression being the most common reason (24% of all patients) and toxicity the second most frequent (17%).

Dr. Anthony Mato and colleagues conducted a retrospective cohort study at multiple major U.S. centers to describe the outcomes of patients who discontinued treatment with either ibrutinib or idelalisib, addressing questions about the reasons for cessation of therapy and the efficacy of subsequent treatment with the alternative kinase inhibitor (i.e., ibrutinib if first treated with idelalisib, and vice versa). The group identified 178 patients who discontinued a BCR pathway inhibitor. The median time to discontinuation was five months (range, 0.25 to 41 months). Toxicity and disease progression were the two major reasons for discontinuation. Thirty-eight patients received subsequent treatment with the alternative kinase inhibitor (either alone or in combination), with 50 percent achieving an objective response. The response rate (66%) and durability of response (median not reached) was higher in patients who had discontinued the original kinase inhibitor due to intolerance rather than disease progression (40% response rate; median PFS, 7 months). Thirteen received a BCL2 inhibitor, with 76 percent responding; 12 received chemoimmunotherapy, with 25 percent responding; however, durability of benefit with these approaches was not reported. Overall, the median survival for discontinuers was 26 months, with progressors due to Richter transformation faring worst and those with intolerance having a much better prognosis.

Together, these two papers highlight that while kinase inhibitors are a major advance in therapy for CLL, challenges remain, especially in patients at higher risk for relapse (e.g., deletion 17p CLL), and those who experience progression on a kinase inhibitor. The prospective data generated by Dr. O’Brien and colleagues confirm the benefit of ibrutinib in patients with deletion 17p CLL. The estimated two-year progression-free survival is superior to those historically observed for chemoimmunotherapy where median PFS is typically less than six months. However, with 50 percent of patients discontinuing for progression or toxicity, the dilemma becomes how to treat them. The work of Dr. Mato and colleagues is informative, despite limitations in its retrospective design and the inherent risk of selection bias. Where patients fail ibrutinib- or idelalisib-based therapy because of intolerance, a treatment with the alternative kinase inhibitor, alone or in combination, seems to be a reasonable option, as responses may be durable. Where progression is the cause of treatment failure, then only a minority will respond to another kinase inhibitor and benefits may be short-lived.

Dr. Linkins indicated no relevant conflicts of interest.

LORI-ANN LINKINS, MD, MSC (CLIN EPI), FRCPC

Antepartum LMWH Is Not the Answer for Most Pregnant Women With a History of Placenta-Mediated Complications


Placental complications cause considerable neonatal morbidity and mortality, in addition to being emotionally devastating for parents. These complications include pre-eclampsia, birth of small-for-gestational-age neonates, placental abruption, and placental insufficiency. Microvascular and macrovascular thrombosis of the placenta are considered contributing causes of these complications,1 and hence, potentially modifiable with anticoagulant therapy.

Dr. Marc Rodger and colleagues reported the results of their meta-analysis of randomized controlled trials comparing low-molecular-weight heparin (LMWH) with nothing or with aspirin alone in pregnant women with a prior history of one or more placenta-mediated complications. Eight randomized controlled trials enrolling a total of 563 pregnant women were included in the analysis. The dose, type, and timing of LMWH varied, as did the use of aspirin. The primary outcome measure was a composite of early-onset (<24 weeks) or severe pre-eclampsia, birth of a small-for-gestational-age neonate (<5th percentile), late pregnancy loss (≥20 weeks), or placental abruption leading to delivery. The majority of the study population were Caucasian with a mean age 30.9 years, and 42 percent had documented thrombophilia (not all participants were tested).

LMWH did not produce a statistically significant reduction in the primary composite outcome, with 14 percent (62 of 444) of participants who received LMWH experiencing placenta-related complications compared with 22 percent (95 of 433) who did not receive LMWH (difference, −8 percent; 95% CI, −13.3 to 1.4; p=0.09). The only subgroup that showed a statistically significant reduction with LMWH were women with a previous history of placental abruption (8% vs. 25% incidence of recurrent abruption; difference, −16.7%; 95% CI, −23.0 to 10.4; p=0.0001).

The key message from this meta-analysis is that LMWH is not the answer for placenta-mediated complications we had hoped it would be. Given the multifactorial nature of these complications, this finding is not surprising. Furthermore, while some may point out that this meta-analysis is underpowered to exclude any benefit of LMWH, it is important to consider the magnitude of that potential benefit. For the majority of subgroups, the highest estimated absolute risk reduction is 10 percent. Although the rate of complications due to LMWH was very low, having a pregnant woman inject daily for nine months is a nontrivial burden for delivery for delivery is not a small “ask” for what appears to be a small benefit. It is possible that specific high-risk groups, such as women with previous placental abruption, might benefit from LMWH during future pregnancies; however, this needs to be decided on a case-by-case basis. Studies in pregnant women are very challenging to conduct, so more definitive answers in this subgroup may be a long time coming.

In conclusion, it is worthy to note that the findings of Dr. Rodgers and colleagues support the movement away from conducting thrombophilic work-ups in pregnant women who experience placenta-mediated complications (with the exception of screening for antiphospholipid antibodies in women with three or more pregnancy losses before 10 weeks gestation).2 Nearly half of the women included in their meta-analysis had documented thrombophilia, and documented thrombophilia, yet there was no difference in the effect of LMWH within these subgroups. Thrombophilia testing causes anxiety; has insurance implications; and, based on evidence provided by this meta-analysis, falsely reassures patients that a modifiable cause for their recurrent pregnancy complications has been found.


ASH NEWS AND REPORTS
Masitinib Offers a Novel Treatment for the Symptoms Associated With Indolent Systemic Mastocytosis


Most cells are perhaps not the most comfortable topic of discussion for hematologists. In fact, if we were asked to talk for one full minute on the topic of a mast cell, I’m not sure how many of us could continue beyond 40 seconds. I wonder how one of hematology’s founding fathers, Paul Ehrlich, who wrote his own PhD thesis on mast cells, would feel about the apparent lack of knowledge regarding this topic. Indeed, he gave the cell its name – Myeloid from the Greek term for breast – a choice that was taken to represent his view of the nourishing nature of these cells within connective tissue. Yet this cell is surely one of our own – derived from a hematopoietic precursor which expresses CD34, CD13, and the KIT tyrosine kinase – and is characterized by the expression of histamine and heparin. The physiological role of the mast cell is still being explored, but encompasses myriad functions related to allergy, trophic support of developing tissue, and innate immunity.

Clonal proliferations of mast cells are not common, but their clinical consequences can be dramatic and severe. Systemic mastocytosis (SM) refers to the accumulation of mast cells in the bone marrow or other internal organs. The major diagnostic criterion for the disease is the presence of aggregates of at least 15 mast cells in bone marrow or another extracutaneous organ. Four diagnostic minor criteria include: more than 25 percent atypical (spindled) mast cells in the mast cell aggregate; aberrant mast cell immunophenotype with expression of CD25 and/or CD2; D816V or another activating point mutation in the KIT gene, and an increased serum tryptase level of more than 20 ng/ml and one minor, or three minor criteria, are sufficient to make a diagnosis of SM. The KIT D816V mutation is found in 90 percent of patients and drives excessive accumulation of the tumor cells. Inhibitors with activity against wild-type KIT and the KIT D816V mutant protein are either under review by the health regulatory agencies (e.g., midostaurin) or in early-phase clinical trials (e.g., BLU-285) as treatment for patients with advanced SM.

This clinical study reports on the outcome of a phase III, randomized, double-blind, placebo-controlled trial using masitinib in the treatment of patients with severely symptomatic forms of systemic mastocytosis. At the outset it should be noted that these patients had indolent SM (include the subvariant smoldering SM), and did not include the postradiotherapy SM or SM with organ dysfunction. As such, these patients can generally expect a normal life expectancy, though around one third of patients within this category experience severe symptoms due to mast cell mediator release and represent the type of patients that might benefit from this therapy. Masitinib is an inhibitor of the wild-type KIT protein, but not the common D816V variant. Importantly, it also inhibits the Lyn and FYN proteins, which are mediators of mast cell survival and mediator release.

During the accrual period from 2009 to 2015, the authors studied 135 patients (although only 108 formally satisfied the WHO criteria for systemic mastocytosis) and were randomized to oral masitinib (6 mg/kg daily in two daily doses) or placebo, with allowance that optimal symptomatic medications could be continued. All the patients reported severe symptoms within the four categories of pruritis, flushing, depression or fatigue; it is noteworthy that the latter two criteria are relatively unappreciated in this disorder. These problems relate to mast cell mediator release and it was no surprise that first-line therapies for the patients had included agents to control mediator symptoms such as antihistamines, proton pump inhibitors, sodium cromoglicate, antidepressants, and leukotriene antagonists.

The primary end-point of the study was somewhat unusual and was based on a 75 percent or more improvement in any one of the aforementioned four major symptom groups within the first 24 weeks of treatment. Importantly, 18.7 percent of patients who were taking masitinib achieved this endpoint compared to 7.4 percent of patients in the placebo group. Of interest, at week 24, there was a 20.2 percent absolute mean change in the serum tryptase level from baseline between the two groups in an intent-to-treat analysis: a decrease of 18.0 percent in the masitinib arm versus an increase of 15.8 percent for the placebo group – an absolute difference of 28.2 percent (p = 0.021). Masitinib-associated clinical benefits were generally sustained during a two-year extension period. Although treatment was generally well tolerated, there was an excess incidence of diarrhea, rash, and asthenia in 9 percent, 8 percent, and 4 percent of patients, respectively, in the masitinib group. In addition, 24 percent of patients in the masitinib arm discontinued therapy because of an adverse event, compared with 10 percent in the placebo arm. For these patients with lower tumor burden who have a normal life expectancy, these side effects need to be weighed against the potential benefits of the drug in mitigating SM-related symptoms.

This is the first phase III randomized study in SM, and focuses attention on our current need of patients with indolent forms of SM who have refractory mediator symptoms despite the use of conventional medications. The biologic basis for masitinib’s impact on mast cell activation symptoms remains ambiguous since the drug does not inhibit the canonical KIT D816V protein found in most patients. It is postulated that depletion of wild-type mast cells or inhibition of KIT-independent mechanisms of mast cell activation (e.g., via signaling through Lyn and FYN) may be relevant in such patients, but biomarker studies are needed to validate this hypothesis.

Peripheral Blood Genomic Interrogation in Myeloma: Developing a Blood Biopsy to Replace the Bone Marrow Biopsy


A dry groll for cancer diagnostics is the "liquid biopsy" – the ability to diagnose, profile, and follow a patient’s cancer from a sample of peripheral blood rather than an invasive sample such as the bone marrow biopsy. Bone marrow aspiration and biopsy can be inconvenient and uncomfortable, making repeat sampling to follow genetic evolution impractical. In solid tumors, liquid biopsies are starting to become a reality. For example, in lung cancer, Roche’s epidermal growth factor receptor mutation test of circulating-free tumor DNA from plasma is now an approved test.1 In multiple myeloma, Dr. Jens G. Lohr and colleagues recently presented the early stages of a comprehensive assay of individual circulating myeloma cells isolated from peripheral blood.2 From a routine sample of only 6 mL of peripheral blood, the authors developed a robust pipeline for isolating very rare circulating myeloma cells (1 in 106 cells) using a combination of CD138+ CD45- cell enrichment and single-cell microfluidic isolation. Using this approach, they could isolate at least 12 circulating myeloma cells from 24 patients. They then performed targeted sequencing at 35 loci known to be commonly mutated in myeloma, and found that they could call the same mutations as previously identified by conventional genotyping of the bone marrow. Interestingly, they also detected some mutations in the circulating tumor cells that were not detected in the corresponding bone marrow myeloma cells, suggesting that some bona fide “driver” mutations occur in a greater fraction of the myeloma cells in the blood than in the bone marrow. Circulating tumor cells may therefore be a more accurate representation of the tumor repertoire than the sampling from an individual bone marrow site. Furthermore, the investigators could isolate and genotype circulating tumor cells in patients with low tumor burden, such as those responding to treatment and in a patient with monoclonal gammapathy of undetermined significance (MGUS). Finally, in addition to identifying mutations, the investigators also could perform transcriptome profiling at the single-cell level.

Overall, Dr. Lohr and colleagues have developed a new tool for exploring the complexity of multiple myeloma through circulating myeloma cells, thereby overcoming the logistical burden of bone marrow biopsies and permitting analysis to be performed at multiple time points. This innovation empowers investigators to address a range of fundamental questions in myeloma. Resistance to treatment and relapse are a core problem in myeloma care, and this has been attributed to “clonal tiding,” where multiple heterogeneous clonal populations may evolve with treatment. Liquid biopsies described here may allow for more thorough dissection of this problem in real time and identify new mechanisms of resistance. Its sensitivity may also permit a better understanding of the biology behind minimal residual disease. Similarly, it may be used at earlier stages of disease, such as MGUS and smoldering myeloma, prior to the evolution of symptoms and where treatment may be more effective when the disease burden is lower. Importantly, this technology, in addition to providing a comprehensive profile of a patient, has also added the benefit of convenience and comfort – something all patients will welcome. Other options such as circulating DNA assays are simultaneously under investigation and will allow liquid biopsies to be used more broadly.


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Dr. Egan, Dr. Ye, and Dr. Lohr are co-authors of the article that is the basis of this Diffusion.
Professor Niels Borregaard (1951-2017)

Professor Niels Borregaard joined Blood as an Associate Editor on January 1, 2016. Just a few months later, we were shocked to hear that he was diagnosed with lung cancer; consequently, within six months of beginning his term, he had to resign from his editorship. He was disappointed as he thoroughly enjoyed his work, and we accepted his resignation with great regret. Niels was an editor par excellence, who came to us with considerable experience. He was the editor in chief of the European Journal of Haematology for 14 years (2000-2014), and was also a section editor for the Leukemia Journal (1996-1999). We were delighted when Niels joined the Blood team because he consistently demonstrated an affinity for editorial work and scientific publishing. He immediately became an active part of the team, devoting considerable time and eagerly sharing his views and ideas with us. He was a helpful, unbiased, and balanced editor with a different perspective as an editor and his open-minded personality carried the signature of his broad expert knowledge. He was an active and proactive member of the Blood editorial team. His death comes as a huge shock to us. The immunotherapy vaccine that was especially developed for him apparently came too late.

Niels was a clinical hematologist with a background in neutrophil research and myelopoesis. He was based in Copenhagen, Denmark, and he completed his MD in Aarhus, where he also completed his doctoral thesis on the activated neutrophil. Between 1982 and 1984 he worked with Drs. Robert A. Clark and Alfred I. Tauber in Boston. In 1988 he was appointed professor of hematology at the Rigshospitalet, University of Copenhagen. At Lund University in Sweden he received the honor “Dr. Honoris Causa in Medical Sciences.” Niels published more than 200 articles in the field of neutrophil cell biology and received several awards for his work.

We at Blood will always regret the short tenure of his invaluable input and wisdom. The international hematology community and Blood have lost a generous colleague and a wonderful friend.

Niels is survived by his wife and four children.

On behalf of the Blood Journal:
– Bob Löwenberg, Editor-in-Chief
– Nancy Berliner, Deputy Editor
– Nina Hoffman, Senior Director, Publishing
– Glenn Landis, Editorial Director
**JANUARY 5, 2017**


Dr. David J. Cantor and Gregory David pinpoint Sin3B, a component of the mammalian Sin3-HDAC corepressor complex, as an essential factor that regulates quiescence and differentiation of long-term hematopoietic stem cells.


CD19-targeted therapies based on T cells expressing CD19-specific chimeric antigen receptors or CD19/CD3-bispecific T-cell-engager have potent antitumor activity in patients with CD19-positive lymphoma and leukemia. Dr. Friederike Birag and colleagues identify a novel mechanism of CD19-targeted immune escape.


Dr. Taehyung Kim and colleagues provide evidence that chronic myeloid leukemia (CML) can arise from a preexisting Philadelphia chromosome-negative hematopoietic clone already harboring additional gene mutations, which is consistent with the concept of preexistent clonal hematopoiesis in CML.


Dr. Stephanie G. I. Polderdijk and colleagues design and evaluate a therapeutic inhibitor of activated protein C. They produced a recombinant variant of s-cotrypsin incorporating three residue changes within the P2-P1' sequence of its reactive loop. This variant exhibits the desired high specificity profile and inhibitory efficiency toward activated protein C.

**JANUARY 12, 2017**


In a plenary e-Blood paper, Dr. Florian Beck and colleagues present a phosphoproteomic analysis to characterize the profiles of ADP-stimulated platelets. They delineate a complex dataset that can be used to develop assays to monitor platelet activation and to identify novel pharmacologic modifiers of platelet function.


In a Blood Forum article, Dr. Patrick T. McGann and colleagues discuss the crushing burden of sickle cell disease in sub-Saharan Africa and offer approaches to improve survival through newborn screening and early interventions.


Dr. Jeongheun Yoon and colleagues extend chimeric antigen receptor (CAR) T-cell technology and the immunosuppressive properties of regulatory T cells (Tregs) to the suppression of factor VIII (FVIII) inhibitors by the creation of a FVIII-specific CAR Treg that effectively suppresses murine splenocyte response to FVIII in vitro and in vivo.

**JANUARY 26, 2017**


and


Two plenary papers in this issue establish that iron homeostasis is regulated by two BMP family proteins produced uniquely by hepatic endothelial cells. Dr. Philipp-Sebastian Koch and Susanna Canali, and colleagues used targeted knockout mice to demonstrate that Bmp2 and Bmp6 are critical independent regulators of hepcidin in the control of normal iron homeostasis.

**FEBRUARY 9, 2017**


Dr. Kamilla S. Josefsdottir and colleagues demonstrate that antibiotic-induced bone marrow suppression is mediated through contraction of the gut microbiome that can be partially rescued by fecal microbiota transfer.


and


Two papers in this week’s issue use phenotypic and genotypic data in large databases to establish that sickle cell trait is not a risk factor for cardiovascular events. This should help lay to rest the ongoing controversy about the impact of sickle cell trait on cardiac fitness.
When More (Oxygen) Is Not Better

EVAN HALL, MD,1 SCOTT WENDROTH, MD,2 AND JASON GOTLIB, MD, MS3

1. Fellow in Hematology/Oncology, Stanford University School of Medicine/Stanford Cancer Center, Stanford, CA
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A 58-year-old Asian woman with a history of inclusion body myositis on high-dose corticosteroids and methotrexate was noted to be hypoxic (oxygen saturation 86% by pulse oximetry) in an outpatient clinic. She had recently been started on dapsone for PCP prophylaxis. Her vital signs were otherwise stable. She reported fatigue, but no cough, fevers, or other symptom concerning for infection. Further laboratory work-up revealed a white blood cell count of 18 × 10^9/L, hemoglobin of 6.9 g/dL (MCV 106 fL), and platelet count of 182 × 10^9/L. Reticulocytes made up 8 percent of her red blood cells. Her hemoglobin was 12 g/dL two weeks prior. She reported no history of bleeding. Despite an increasing concentration of supplemental oxygen (up to 15 L/min by facemask), she remained with a saturation of 87 percent. Chest x-ray was clear, and CT pulmonary embolism (PE) protocol did not reveal a PE. A peripheral blood smear was obtained and is shown in the Figure below. Which of the following tests is most likely to yield the diagnosis?

A. ADAMTS13 activity and inhibitor level
B. Glucose-6-Phosphate Dehydrogenase (G6PD) screen
C. Blood methemoglobin level
D. Coombs test
E. DIC panel

For the solution to the quiz, visit The Hematologist online, www.hematology.org/Thehematologist/Images.

Figure

Put your fellow readers to the test, and send us your Image Challenge submissions! Email case descriptions and image files to the Managing Editor at jlllorens@hematology.org.

ERRATUM: In the January/February 2017 issue, on page 16 in the Image Challenge “Blasts of a Different Sort,” author Jenny Hoffman, MD, of Stanford University School of Medicine, was inadvertently excluded from the byline. We apologize for this error, and the article has been corrected online.