Diffusion

than expected frequency in filler sequences, as would
Dipurines and dipyrimidines were observed at a higher
content at a level consistent with TdT’s known bias with
filler sequences were found to be enriched for G/C
To lymphoid cells, TdT is also known to be expressed in a
Although canonically thought of as restricted in expression
(TdT), the DNA polymerase responsible for untemplated
inferences to suggest an unusual mechanism: activity in
To explain the frequent presence of filler in
microhomology nor filler.
with the remaining 24 percent containing neither overt
microhomology was not observed in a majority (62%) of
researchers hypothesized that if this mechanism were
for sequence-level evidence of polymerase slippage. The
sequences has long been speculated as a culprit for
DNA polymerase slippage near repetitive (microhomology)
NPM1
cases.
are more restricted to AML and occur in 35 percent of
acute lymphocytic leukemia (ALL), while
NPM1
mutations
in nucleophosmin 1 (NPM1)
2

in women with hematologic
abnormalities in acute myeloid
leukemia (AML) confer
distinct clinicopathologic
characteristics and are the foundation of modern
AML subclassification systems. Among the most
common recurrent genetic driver mutations in AML are
activating internal tandem duplications (ITD) within the
juxtamembrane domain of the type 3 receptor tyrosine
kinase FLT3 (FLT3-ITD) and exon 12 frameshift
mutations in nucleophosmin 1 (NPM1). FLT3-ITDs are present in 25
percent of all AML cases and are also found in a subset of acute
lymphoblastic leukemia (ALL), while NPM1 mutations are
more restricted to AML and occur in 25 percent of cases.
NPM1-mutated AML is generally associated with a favorable
prognosis, while the presence of a FLT3-ITD generally
confers a poor prognosis, especially at a high
(>0.5) FLT3-ITD allele fraction.

Although the molecular events underlying the formation
of NPM1 mutations and FLT3-ITDs are poorly understood,
DNA polymerase slippage near repetitive (microhomology)
sequences has long been speculated as a culprit for FLT3-
ITDs. A study published recently by Dr. Julian Borrow and
colleagues analyzed 900 FLT3-ITDs from 271 patients
for sequence-level evidence of polymerase slippage.
The researchers hypothesized that if this mechanism were
responsible for ITD formation, the sequences immediately
flanking the 3’ and 5’ ends of the duplication would
include short (1-6 base pairs), homologous sequences
(microhomology), which is indeed seen in a minority
of cases (Figure 1A compared to 1B). Unexpectedly,
microhomology was not observed in a majority (60%) of
ITDs. In fact, 38 percent of all ITDs contained an additional
sequence of unknown origin (nontemplated “filler”)
with the remaining 24 percent containing neither overt
microhomology nor filler.

To explain the frequent presence of filler in FLT3-ITDs,
the authors use a series of mathematical deductions and
inferences to suggest an unusual mechanism: activity in
the myeloid cells of terminal deoxynucleotidyl transferase
(TdT), the DNA polymerase responsible for untemplated
additions of nucleotides to V, D, and J exons during
immunoglobulin and T-cell receptor rearrangement.
Although canonically thought of as restricted in expression
to lymphoid cells, TdT is also known to be expressed in a
subset of AML at diagnosis and in myeloid progenitors.
Supporting the involvement of TdT in FLT3-ITD formation,
filler sequences were found to be enriched for C/G
content at a level consistent with TdT’s known bias with
filler sequences of a length consistent with TdT activity.
Dinucleotides and dinucelidines were observed at a higher
than expected frequency in filler sequences, as would

How FLT3-ITDs and NPM1 Insertions Maneuver Into AML Using the Lymphoid Enzyme TdT to Initiate Replication Slippage


JOHN C. LAMACCHIA, MD, PHD, AND ANNETTE S. KIM, MD, PHD

Growing the Data Hub

The Data Hub is open for data contributions toward two initial diseases, multiple myeloma (MM) and SCD. We have already integrated a critical mass of data in these diseases that will allow the research community to answer questions of scientific interest. To date, the Data Hub has integrated data from six academic centers throughout the United States and Europe, representing thousands of patients with MM and SCD. We anticipate that this data repository will grow exponentially in 2020. The Data Hub also recently announced the contribution of the ASPRE and ENDEAVOR data sets from Amgen, representing more than 1,300 patients with MM on therapeutic clinical trials, and the FISCO registry data set from Novartis, representing observational data from 500 patients with SCD.

In 2020, as the SCD CTN is launched, the plan is for data from patients with SCD at all participating CTN sites to be incorporated into the Data Hub. These data will represent consecutive patients with SCD who are seen at these sites — not limited to those on clinical trials — and will include a significant proportion of the U.S. population with this disease. Though MM does not have a CTN within the ASH RC, we plan for a major expansion of U.S. sites contributing prospective MM data to the Data Hub in 2020.

Reframing Our Data Models

For data within the Data Hub to inform research and practice, data must be high quality and fit for purpose. We ensure this through the development of disease-specific data models for SCD, MM, and others yet to come. Once initial disease-specific models are developed and published, we will iteratively refine, refine, and update new releases of these models over time.

As we use the term, a “data model” refers to a collection of attributes that explain how we identify, categorize, and make data available for use. Data models contain high-priority data elements for each disease through a process that recognizes that some elements are critical for research and practice, while not all elements can be collected for each patient every time. Data elements have specific definitions, structure, sourcing, frequency, and context of collection. We house our data within a standardized architecture but can acquire and integrate data through a variety of channels.

As these harmonized data models are developed, we anticipate publication and

Update on the ASH Research Collaborative Data Hub

WILLIAM A. WOOD, MD, MPH
Associate Professor of Medicine, Division of Hematology/Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC

On behalf of the ASH Research Collaborative (ASH RC) and the Data Hub, I am pleased to provide the hematology community with an update on our progress to date and plans for 2020. The ASH RC is a non-profit organization established by ASH in 2018 to foster collaborative partnerships that accelerate progress in hematology, with the goal of improving the lives of people affected by blood diseases. As a major initiative within ASH RC, the Data Hub aims to create the largest shared information resource within the global hematologic community. ASH RC also contains a SCD Clinical Trials Network (SCD CTN), an ambitious project designed to accelerate the development and evaluation of therapies in a large proportion of the United States population affected by SCD. Through the Data Hub, SCD CTN, and projects still to come, the ASH RC will transform research and practice in malignant and nonmalignant hematologic diseases throughout the world, for the benefit of patients and the hematology community.

ASH RESEARCH COLLABORATIVE
Accelerating Progress in Hematology

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(Cont. on page 12)
President’s Column

Improving the Poor Prognosis of Grant Applications

During the Fall of 2018 I was getting really worried. Despite submitting numerous grant applications, nothing was being funded, and I could glimpse the need to winnow down my research staff in the not-too-distant future. I was already making some shifts to conserve funds, much as a cell goes into starvation mode and shifts its metabolism, to try to survive until times got better. And then I received a couple of email notices telling me that grants had been funded. Just like that, I went from the depths of despair into the sweet light of a few more years of guaranteed funding. But it was a powerful reminder about what it would feel like to not have research funding. I know many colleagues who are facing the same unpleasant situation right now, and with a poor prognosis for any particular grant application, there’s little I can honestly say that’s reassuring.

The National Cancer Institute just announced that their 2020 funding level for R01s will increase from the 8th percentile to 10th percentile for new grants and competitive renewals and to the 15th percentile for early-stage investigators. The National Heart Lung and Blood Institute’s 2020 funding pay lines are at the 16th percentile for R01 grants and the 26th percentile for early-stage investigators. While other Institutes have not yet released their numbers, the numbers we do know are better than those in recent years; but most National Institutes of Health (NIH) applications still will not be funded. Given the effort it takes to write an NIH application, these success rates are extremely discouraging. ASH is continuing its Bridge Grant program for investigators who are revising and resubmitting NIH grants, to try to retain hematology investigators. ASH has distributed more than $17 million through the program since its inception, with more than 70 percent of the recipients subsequently being awarded an R01 from NIH within three years. Foundations and other funding sources such as the Patient-Centered Outcomes Research Institute (PCORI) continue to support research but are also highly competitive. While we should celebrate the recent consistent increases in the NIH budget, the reality is that we are still below the buying power of 2003, and the drought from 2003 to 2015 (Figure) has probably forced many people to leave research and discouraged others from even trying to pursue a research career. I don’t have any hard data to back up my assertions; I’m just reporting the anxious pulse I feel of fellows and faculty who don’t see a path to enter or remain in academic research.

This issue of The Hematologist is filled with articles describing advances in our understanding of hematology and improvements in care, built upon solid basic and clinical research conducted by hematologists. ASH continues to lobby for consistent and robust research funding to keep the pipeline of breakthroughs gushing. If it takes years or decades for research to reach the prescription pad and for young investigators to reach their peak of productivity, then the effects of a 12-year dry spell may not be felt for another decade. It is important that legislators and the public be reminded that their investment in research is long-term, but the payoffs are potentially huge. So if you are conducting funded research and giving a presentation or an interview, please make sure that you not only thank your funding sources (e.g., “I’d like to thank my funding sources for their support”) but that you explicitly tell your audience, “…without which, this work would not have been possible.”

Stephanie J. Lee, MD, MPH

Figure 1. National Institutes of Health (NIH) Funding, FY1994-FY2020
Program Level Funding in Current and Projected Constant (FY2020) Dollars.


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The Hematologist Board of Contributing Editors Welcomes Three New Additions in 2020

The spring season reminds us of the many transitions all around, and this certainly applies to The Hematologist. In December 2019 in Orlando, I shared ASH’s deep gratitude to three outgoing Contributing Editors: Drs. Caron Jacobson, Lori-Ann Linkins, and Stephan Moll.

Dr. Jacobson, of the Dana-Farber Cancer Institute, used her expertise and insight to explain novel treatments for lymphomas and the latest data on CAR T-cell therapy at a time when application of this therapy was rapidly expanding. Dr. Linkins, whose home base is McMaster University, contributed a wealth of knowledge in the research of deep vein thrombosis, bleeding disorders, and much more. Lastly Dr. Stephan Moll was our resident expert in the area of hemostasis and thrombosis. Not only will we miss his in-depth reviews on therapeutic advancements, but Dr. Moll’s willingness to report on unique professional opportunities (such as being called to consult on a lemur with a possible vascular event) lent humor and variety to our publication. Without a doubt, each of these experts has made a positive impact on the mission of The Hematologist. One of the great joys of this work is being able to interact with so many varied professionals and to learn from their work, expertise, and experience, and I have passed on the thanks of ASH and our readership to each of these individuals.

This publication could not happen without the dedication and commitment of our Contributing Editors. Of course, with each farewell, there is always a welcome, and we are thrilled that the ASH Executive Committee approved the nominations of three new Contributing Editors — Drs. Damon Houghton, Frederick Locke, and Eric Tseng.

— Laura C. Michaelis, MD

Dr. Damon Houghton is assistant professor of medicine in the Department of Cardiovascular Disease, Division of Vascular Medicine, and the Department of Internal Medicine, Division of Hematology/Oncology, at the Mayo Clinic in Rochester, Minnesota. He is a specialist with training in hematology and vascular medicine, and his primary research interest involves optimizing the care of patients with, or at risk for, venous thrombosis. His clinical practice consists of a hybrid of thrombophilia, coagulation, and vascular medicine.

Dr. Frederick Locke is vice chair and associate member of the Department of Blood and Marrow Transplant and Cellular Immunotherapy, and co-leader of the Immunology Program at Moffitt Cancer Center. His clinical and translational research is focused on cellular therapies for lymphoid malignancies. He is lead investigator for several multicenter CD19 chimeric antigen receptor T cell trials, and his lab evaluates immune responses in the context of cellular therapies.

Dr. Eric Tseng is a hematologist in the Division of Hematology/Oncology at St. Michael's Hospital and assistant professor at the University of Toronto. His practice focuses on nonmalignant hematology and thromboembolism. Dr. Tseng completed adult hematology training at the University of Toronto and a thromboembolism fellowship at McMaster University. He is involved in knowledge translation activities through ASH and Thrombosis Canada. His academic interests include quality improvement initiatives related to the diagnosis and prevention of venous thromboembolism and the implementation and evaluation of hematology training programs.

New ASH Clinical Practice Guideline on Sickle Cell Disease Now Available

The Society has released a new ASH Clinical Practice Guideline on Sickle Cell Disease (SCD). The ASH Clinical Practice Guideline on SCD-Related Transfusion Support is part of a series of five SCD guidelines ASH is developing to provide updated treatment guidelines that reflect the newest evidence about the disease to help the medical community better treat people with SCD.

You can access the full guidelines on the Blood Advances website (www.ashpublications.org/bloodadvances/article/4/2/227/440607).

For additional information on other ASH Clinical Practice Guidelines, visit www.hematology.org/guidelines.

ASH and The Leukemia & Lymphoma Society Team Up to Connect Patients With Blood Cancers to Clinical Trials

ASH and The Leukemia & Lymphoma Society (LLS) Clinical Trial Support Center (CTSC) are collaborating to expand access to LLS’s unique free service providing clinical trials navigation and support to patients with blood cancer and their families. With only 5 to 8 percent of adult cancer patients enrolling onto clinical trials, this collaboration aims to bridge this gap and connect more patients to appropriate clinical trials. Through this collaboration, ASH member physicians and their care teams, along with patients and caregivers, receive one-on-one support from CTSC Nurse Navigators and have direct access to them for the duration of the search, enrollment process, and while patients are on the trials.

In a new podcast from The Hematologist (available on SoundCloud and iTunes), Dr. Gwen Nichols from LLS and Dr. Jennifer Holter-Chakrabarty of the ASH Task Force on Immunotherapy discuss this great new collaboration and explain how to use the portal. Visit www.hematology.org/TheHematologist/Multimedia to listen.

The ASH Portal to access the CTSC Nurse Navigators is now live, and you can access it by visiting www.hematology.org/CTCTrialNavigation.
Fertility Preservation for Women With Hematologic Malignancies

ALISON W. LOREN, MD, MSCE
Associate Professor of Medicine; Vice Chair, Faculty Development, Department of Medicine; Director, Blood & Marrow Transplant Cell Therapy & Transplant Program, Raymond and Ruth Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

THE RESPONSES

Hematologists should address with all patients the possibility of reproductive harm associated with treatments for blood cancer.1–3 Standard-of-care options for preserving fertility in women include oocyte or embryo cryopreservation.4–5 Experimental options such as ovarian tissue cryopreservation6 or ovarian in vivo maturation7 may be appropriate in some scenarios; ovarian tissue cryopreservation is currently the only option available to prepubertal girls.

Pursuit of oocyte or embryo cryopreservation requires a period of 10 to 14 days of hormonal ovarian stimulation, followed by oocyte retrieval via a transvaginal approach.4,5,6,7,8,9,10,11,12,13,14,15 Hence, patients must be clinically stable enough to delay cancer-directed therapy. The decision about whether to cryopreserve oocytes versus embryos is a personal one for the patient. While embryo cryopreservation is associated with slightly higher live birth rates, this procedure requires a male partner (or willingness to use donor sperm) and no ethical objections to the freezing and storing of embryonic tissue. Hence, for some women, oocyte preservation may be preferable.

CASE 1: This patient is anticipated to receive high-dose alkylator-based therapy, which carries a high risk of permanent infertility and is certainly associated with prematurity ovarian insufficiency (POI), a condition defined as cessation of menstrual periods due to ovarian failure at a young age, typically under the age of 40.15–18 She is otherwise clinically well. There is little to no harm in delaying her chemotherapy for approximately two weeks. She should be referred urgently to gynecology/reproductive endocrinology for consideration of oocyte (or embryo) cryopreservation. In this circumstance, facilitated referrals are essential for the best care of the patient. Hematologists should cultivate relationships with their colleagues in reproductive endocrinology through educational opportunities such as grand rounds, multidisciplinary conferences and other collaborations; dedicated referral lines and social workers or patient navigators can be indispensable in securing expedited appointments.19–21

CASE 2: This patient is critically ill with disseminated intravascular coagulation, leukostasis, and likely sepsis. It is not appropriate to delay her therapy; on the contrary, emergency interventions are required to manage her AML (Figure).22–24 She should be counseled about fertility risks with AML therapy, which is infrequently associated with permanent amenorrhea.1 However, in view of the young age of this woman, the concern of transmitting leukemic cells back to the concern of transmitting leukemic cells back to the newborn is a valid one.25–27 Although ovarian tissue cryopreservation may be done on an emergency basis, there are concerns about whether to cryopreserve oocytes versus embryos. She may consider cryopreservation of oocytes in the future if herchildbearing is delayed.

CASE 3: This patient is critically ill with disseminated intravascular coagulation, leukostasis, and likely sepsis. It is not appropriate to delay her therapy; on the contrary, emergency interventions are required to manage her AML (Figure).22–24 She should be counseled about fertility risks with AML therapy, which is infrequently associated with permanent amenorrhea.1 However, in view of the young age of this woman, the concern of transmitting leukemic cells back to the concern of transmitting leukemic cells back to the newborn is a valid one.25–27 Although ovarian tissue cryopreservation may be done on an emergency basis, there are concerns about whether to cryopreserve oocytes versus embryos. She may consider cryopreservation of oocytes in the future if herchildbearing is delayed.

Fertility preservation approach for women (postpuberty) with newly diagnosed hematologic malignancy.


The Hematologist: ASH News and Reports
Precision Medicine in Clonal Hematopoiesis: New Data on Risk for Transformation

JENNIFER O’SULLIVAN, MD; ADAM MEAD, MD, PhD

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Editor’s Note: The first mention of clonal hematopoiesis (CH) of indeterminate potential as a named entity appeared in PubMed in 2015. Since then, this condition has been the subject of numerous, critically important investigations. In this Mini Review, Drs. O’Sullivan and Mead highlight two recent publications that expand what we know about the relationship between CH and the development of outright hematologic malignancies.

CH is a well-recognized entity where a somatic mutation is acquired by a single hematopoietic stem cell, confers a fitness advantage, and leads to clonal expansion. Eventually, the hematopoietic stem cell-derived clone expands such that it contributes to a considerable proportion of mature blood cell production in apparently healthy individuals. CH is more prevalent with increasing age and carries an increased risk of later evolution to a myeloid neoplasm (MN).1,2 Most mutations often detected in CH mirror those seen in overt MNs; these include epigenetic modifying genes (DNMT3A, TET2, ASXL1, IDH1/2), splicing factors (SF3B1, SRSF2, U2AF1), and DNA damage response (DDR) pathways (TP53, CHEK2, and PPM1D). MN-associated signaling pathway mutations are less frequent in CH, except for the JAK2 V617F mutation. Numerous studies have helped to refine the risks of transformation in CH,1,2 and a crucial role for mutations affecting DDR pathways is emerging. Several important studies have described that TP53 or PPM1D mutation–associated CH is particularly common after previous cancer treatment.13 Furthermore, MNS arising after chemotherapy or radiotherapy (therapy-MNs [tMNs]) are also enriched for TP53 and PPM1D mutations, which are often present prior to cancer treatment.14

TP53, a tumor suppressor gene, is the most commonly mutated gene across all cancers and in MNS is associated with very poor outcomes and resistance to standard treatments.1 When present in patients with tMNS, TP53 mutation is associated with a similarly dismal prognosis.4 Therefore, new approaches for early detection and prevention of TP53-associated MN are badly needed. However, not all patients with TP53 mutation develop tMNS. So how do we identify patients who might be at particular risk of tMNS through cell-intrinsic or cell-extrinsic factors? Regarding cell intrinsic factors, a wide spectrum of different TP53 mutations can occur as mono- or biallelic mutations. Whether such TP53 mutant allele imbalance has important clinical implications remains unclear, as monallelic TP53 mutation can mimic TP53 loss of function by exerting a dominant negative effect.15

Hoping to shed light on this question, Dr. Elko Bernard and colleagues16 recently reported an analysis of TP53 mutations in myelodysplasia (MDS) patients (n=3,324), inclusive of a subgroup with therapy-related MDS (n=229). They studied the TP53 allelic state using a combination of conventional G-banding analyses and a next-generation sequencing (NGS) panel covering TP53 and genomewide copy number probes. One-third of TP53 mutation cases of MDS were monallelic and two-thirds had multiple hits, consistent with biallelic mutation. Variant allele frequency (VAF) measurements for most cases correlated with mononuclear or biallelic states. However, some patients with VAF below 50 percent (n=19,378 patients) had copy neutral loss of heterozygosity at the TP53 locus and would have been misclassified as mononuclear if based on VAF alone. Therefore, VAF should not be used as the sole method of assigning allelic state.

Biallelic TP53 mutations were associated with poorer survival and an increased risk of acute myeloid leukemia (AML) transformation in contrast to persons with mononuclear TP53 mutation where the outcome was comparable to that of TP53 wild-type patients. Serial sample analysis in patients with AML transformation detected a higher proportion of biallelic TP53 alteration at the time of transformation, underlining the key role for loss of wild-type TP53 as the event that drives disease progression. Previous studies have also reported better survival in MDS patients with TP53 mutation with VAF below 20 percent (mononuclear for the majority).11 However, analyses in AML found TP53 mutations to be associated with adverse prognosis irrespective of VAF.11

Patients with tMNS in this study4 had a higher frequency of multiple mutations of TP53 as compared with de novo cases (84% vs. 65%; OR, 2.8; p=0.002). The same observations for clinical outcomes were made in this tMN subgroup; those with biallelic mutations had poorer outcomes compared to those with mononuclear TP53 mutations who had a lower risk of death, though this did not achieve statistical significance.

The relationship between CH associated with DDR mutations, chemotherapy treatment, and risk of IMN was studied in greater depth by Dr. Kelly Bolton and colleagues.13 Their group set out to determine novel approaches that might be used for early intervention and targeted prevention for patients with cancer at high risk of developing tMN. They analyzed targeted, deep coverage NGS data (MSK-IMPACT) to detect CH in a very large cohort of patients with cancer (n=24,439). Strikingly, the observations correlated targeted, deep coverage NGS data (MSK-IMPACT) to detect CH in a very large cohort of patients with cancer (n=24,439). Strikingly, the authors reported a prevalence of CH mutations in almost one-third of patients. More than half of the mutations detected were classified as putative driver mutations of cancer, and virtually all affected genes recurrently mutated in MNS. Reflecting prior studies, the most

Correlation between CH and clinical characteristics was performed for 10,207 patients; 61 percent had exposure to cancer therapy prior to CH analysis, and 39 percent were treatment naïve. The authors found that CH was associated with increasing age (OR, 1.8; p≤10^-4) and cell extrinsic factors such as smoking exposure (OR, 1.1; p=4.1 × 10^-4) and previous exposure to cancer therapy (OR, 1.2; p=4.2 × 10^-4). Although splicing factor mutations were less commonly detected, they were more strongly correlated with older age. TP53 (OR, 2.7; q=9.0 × 10^-4), PPM1D (OR, 3.6; q=1.2 × 10^-4), and CHEK2 (OR=4.6, q=10^-4) mutations were significantly associated with previous exposure to cancer therapy, an association not seen for mutations in epigenetic modifiers or splicing factors. CH was most strongly associated with topoisoenzyme II inhibitors (OR, 1.3; p=0.01) and platinum drugs (OR, 1.2; p=0.01) — carboplatin specifically (OR, 1.3; p=0.002) — for the latter class. Consistent with these observations, carboplatin is particularly associated with risk of MN.17 A dose-response relationship was observed between cancer therapy and the prevalence of CH; a higher cumulative exposure to external beam radiation and platinum chemotherapies was positively associated with the presence of CH. Specifically for TP53 mutations, significant associations were found in patients with exposure to platinum, taxanes, and radiation therapy.

Serial analysis in 325 patients allowed interrogation of the clonal dynamics of CH after cancer therapy. Sixty-two percent of persons with a CH mutation at both time points had a stable allele frequency, whereas in 28 percent of cases, mutations increased in VAF, and 10 percent showed a decrease in clone size. In patients receiving radiation or cytotoxic therapy, there was a selective increase in DDR mutations; moreover, increasing exposure to either

(Cont. on page 14)
As many hematology/oncology trainees will learn during their fellowship, certain germline mutations give rise to hematologic disorders. Some of these relationships are seemingly simple—a single base pair alteration leads to a change in the globin gene, for example. But others are more complex, including the germline mutations that confer leukemia risk or diseases of the megakaryocytes or platelets. There is a growing understanding that to ensure that genetic diagnoses are accurate and patient-centered, the scientific and clinical communities require a solid interpretative infrastructure to safeguard the many stakeholders of these data: What are the true genetic and phenotypic associations? Are all variants equally important? Are tests performed in a valid manner? Are the findings actionable?

This is the mission of the National Institutes of Health (NIH) Clinical Genome Resource (ClinGen), a federally funded project aimed at building shared knowledge repositories for genes and their variants. In early 2018, ASH partnered with the University of North Carolina (UNC) at Chapel Hill, an NIH ClinGen grantee, to develop a broad and accessible collection of genomic data aimed at improving the diagnosis of myeloid malignancies and hereditary platelet disorders. The two expert review panels have been working for nearly two years on this and have begun publishing their findings.1 Drs. Lucy Godley (Section of Hematology/Oncology and Center for Clinical Cancer Genetics, The University of Chicago, Chicago, IL) and Brett Godfrey (Section of Hematology/Oncology and Center for Clinical Cancer Genetics, The University of Chicago, Chicago, IL) co-lead the panels developing curation rules for variants in genes that confer risk for hereditary myeloid malignancies, and Drs. Jorge Di Paola (Department of Pediatric Hematology Oncology, Department of Pediatrics, Washington University, St. Louis, MO) and Wolfgang Bergmeier (Department of Biochemistry and Biophysics; UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC) co-lead the team developing similar rules for genes that cause inherited platelet disorders.

Editor-in-Chief Dr. Laura Michaels recently interviewed Drs. Godley and Di Paola about the panels’ work.

Laura Michaels: Let’s start by talking briefly about why ASH got involved with ClinGen. What need was being addressed by the collaboration?

Lucy Godley: Several years ago, the ASH Task Force on Precision Medicine recognized that germline mutations are responsible for many hematologic conditions. Also, increasingly commonly, we are performing molecular profils of tumors. In performing these studies, however, we are inadvertently finding germ-line variants. If you look at the ways these are categorized by clinical laboratories in the United States and internationally, there is no consistency. ASH sought to bring consistency to the way those are categorized by clinical laboratories. In the absence of that, we’ve got to catalog this in a systematic way, and that’s what we are doing.

We started this a year-and-a-half ago, and I think, looking back, we’ve made a lot of progress. These two pilot programs for ClinGen (myeloid malignancies and platelet disorders) were chosen because there had been a lot of discoveries over the last two decades in those particular areas and because there is, again, a proliferation of genetic panels that are available to order in the clinical setting.

Michaelis: It seems like the impact of variants might be different if one were focusing on different issues. I take it that your two panels are, for now, specifically interested in the bone marrow or megakaryocyte lineage?

Godley: Exactly right. Yes, we are classifying variants that are conferring risk to the bone marrow to develop myeloid malignancies. We started with RUNX1 because it had the largest number of variants in publicly available ClinVar, which is where the variant data are first deposited. And we also thought it would be helpful, since RUNX1 mutation carriers also have thrombocytopenia and the other ASH-sponsored ClinGen committee is working on germ-line predisposition to thrombocytopenia. So we thought that would be a nice starting place. It took us about a year-and-a-half to generate these rules for RUNX1. It’s a quite rigorous process I have to say, and it’s been really interesting and eye-opening.

Di Paola: Our group opted to start with probably the most well-known platelet disorder on earth—Glanzmann thrombasthenia, first described by the Swiss pediatrician Eduard Glanzmann in 1918, where there is an absence or deficiency in the platelet fibrinogen receptor (GPIIIa). This receptor is encoded by two different genes: ITGA2B and ITGB3. The reason we started with that is that this disease has been genetically well characterized all over the world. We said, “we have a lot of biological information, we have a lot of publications, we have a lot of genetic data that have already been published, and we have databases on these genes.”

Michaelis: Initial publications from your panels are out or pending, correct?

Godley: Yes, a paper discussing our detailed curation rules is now published in Blood Advances.1 For the most part, I think the end users of our Blood Advances paper are the clinical laboratories that are generating the variant interpretation. So now when a clinical laboratory sees a RUNX1 variant, it will be able to use these rules to say whether this variant is known pathogenic, likely pathogenic, variant of unknown significance, likely benign, or known benign—the five different levels of functional annotation. The variants that are in ClinVar are already being classified by our committee using those rules, so that if a clinical laboratory sees a variant, they’ll first go to the ClinGen/ClinVar website and see whether this variant has been classified already by the committee.

Any variant that’s already deposited has essentially been classified by the rules, and that makes their job very easy. If they have a new variant that’s never been described, then they’ll use these rules to classify it. If you see the

Strengthening the Potential of Genetic Diagnoses: A Conversation With Drs. Lucy Godley and Jorge Di Paola

Michaelis: Which genes or disorders will you tackle next?

Godley: We started with RUNX1, and we’re working on curation rules for GATA2 right now. After that, we probably need to do DDX41, CEBPA, [and] ETV6. Although ETV6 could be shared with the thrombocytopenia committee.

Di Paola: We’re going to curate the most traditional platelet diseases first, such as Bernard-Soulier syndrome caused by defects on the von Willebrand factor receptor, the GPIb-IIIb-V complex on the platelet surface. After that, we’re going to start to go deeper into other disorders, and probably after we finish what we know about platelet function disorders, we’re going to go into other thrombocytopenias such as those caused by MYPH mutations.

Michaelis: What does your output mean to patients and, not necessarily geneticists, but the working hematologist?

Godley: The first thing I would say is that we are identifying more and more families and individuals based on molecular profiling of leukemia cells. Because the prognostic significance of the leukemia is so dependent on when the molecular testing was performed, and the biological data included in those panels, we are very often finding these individuals and families at the time of diagnosis of the first leukemia in the family. Recognition that molecular profiling of a tumor cell yields both germline and somatic data is extremely important. Labeling these tests as “somatic panels” is extremely misleading. It’s critical to recognize that when you see a TP53, a RUNXI, a DDX41, a CEBPA, or a GATA2 mutation in a myeloid leukemia, you have to question: Is this a germline variant? And once you determine that it’s germline, you now have rules that are quite rigorous in terms of the functionality of that variant.

Di Paola: Most of us dream of platforms that are comprehensive enough that when you get your genetic tests back, your doctor or hematologist would be able to check on a website and confirm with other sources and eventually tell you, “This is everything that we know about this variant.”

There is a lot of misinformation out there and a lot of fear. I think that overall if we do our job well, in several years, we’re going to be able to truly diagnose these disorders in a way that the patient will understand if they do have or do not have the disease, and the physical and genetic counselors will be absolutely needed to interpret with patients and families the implications of these findings. The fact that ASH is supporting this is fantastic because it has to happen.
The MEDALIST Trial: Are We on the Podium for Lower-Risk MDS?


KRISTEN O’Dwyer, MD

The myelodysplastic syndromes (MDS) are a diverse group of malignant hematopoietic stem cell disorders characterized by ineffective hematopoiesis, blood cytopenias, and an increased risk of transformation into acute myeloid leukemia (AML). The revised International Prognostic Scoring System (IPSS-R) is a commonly used clinical prognostic tool that utilizes variables from the blood and marrow to place patients into one of five risk groups, based on risk of mortality and transformation to AML. For patients with “lower-risk” MDS, which comprises the IPSS-R very-low, low, and intermediate-risk groups, the disease is characterized by a low risk of transformation into AML, a relatively prolonged survival, but a high prevalence of anemia. Over time, approximately 40 percent of patients will need frequent red blood cell (RBC) transfusions.

The first-line treatment for transfusion-dependent patients with lower-risk MDS (those without the chromosome abnormality of deletion 5q) are the erythropoiesis-stimulating agents (ESA). For patients who do not respond to or are ineligible to receive ESAs, there are few treatment options for chronic anemia. Accordingly, the identification and translation of novel therapies capable of improving the marrow function in patients with MDS has the potential to significantly improve quality of life and overall outcomes for patients with MDS.

Luspatercept is a novel recombinant fusion protein that consists of a modified extracellular domain of the human activin receptor type IIB (ActRIBB) linked to the human immunoglobulin G1 Fc domain. It binds to select transforming growth factor-β superfamily ligands and neutralizes them, and consequentially inhibits erythropoiesis in the mature erythroid compartment, thereby reducing erythropoiesis differentiation. Importantly, the mechanism of action seems to be independent of erythropoietin regulation. In November 2019, the U.S. Food and Drug Administration (FDA) approved luspatercept for the treatment of anemia in adult patients with β-thalassemia who require regular RBC transfusions, and luspatercept is being studied for treatment of MDS-associated anemia. The safety and efficacy of luspatercept was tested in a single-arm phase II study of 58 patients with lower-risk MDS with anemia. Sixty-three percent of patients demonstrated an erythroid response, and 38 percent were transfusion independent for 8 weeks or longer. Patients with the ring sideroblast phenotype had the optimal responses, however, with 69 percent achieving an erythroid response and 42 percent becoming transfusion independent for 8 weeks or longer. Based on these promising phase II results, a randomized phase III trial (NCT02831070) was initiated to test luspatercept for the treatment of anemia in patients with lower-risk MDS with ringed sideroblasts who were transfusion dependent.

In the current article, Dr. Pierre Fenaux and colleagues report on the prospective, international, multicenter, double-blind, placebo-controlled, phase III MEDALIST trial. The trial enrolled 229 patients in 65 trial sites in 11 countries. Eligible patients had lower-risk MDS and were RBC transfusion dependent, defined as receiving at least two or more red blood cells per eight weeks. In addition, each patient’s anemia had to be classified as refractory to or unresponsive to ESAs, or they had to have discontinued ESAs due to a prior adverse event. Patients were randomly assigned in a 2:1 ratio to receive luspatercept or placebo, at a dose of 1 mg/kg administered subcutaneously every three weeks for 24 weeks. The dose of luspatercept could be escalated during the treatment to 1.75 mg/kg. The MDS disease assessment was performed at 24 weeks after the start of study treatment and then every 6 months thereafter. The primary endpoint was transfusion independence for 8 weeks or longer during weeks 1 through 24. Important secondary endpoints were transfusion independence greater than 12 weeks during the first 24 weeks and also within the first 48 weeks. Patients were observed for three years following the last dose of study treatment for AML progression and overall survival.

Thirty-eight percent of patients in the luspatercept group achieved the study’s primary endpoint of at least eight weeks without the need for transfusion compared to 13.2 percent in the placebo group (p<0.001). The median duration of transfusion independence was 30.6 weeks in the luspatercept group and 13.6 weeks in the placebo group. For the key secondary endpoint, 28 percent of patients in the luspatercept group achieved red cell transfusion independence for 12 weeks compared to 8 percent in the placebo group. Also, during weeks 1 through 24, an erythroid response was observed in 53 percent of patients in the luspatercept group compared to 12 percent in the placebo group. The adverse events most frequently reported in the luspatercept group were fatigue, diarrhea, asthenia, nausea, diarreha, and back pain. The risk of progression to higher risk MDS was low (1 patient in each treatment group), as was the development of AML (2 percent in the luspatercept group compared to 8 percent in the placebo group). Also, during weeks 1 through 24, an erythroid response was achieved in 53 percent of patients in the luspatercept group compared to 12 percent in the placebo group. The adverse events most frequently reported in the luspatercept group were fatigue, diarrhea, asthenia, nausea, diarreha, and back pain. The risk of progression to higher risk MDS was low (1 patient in each treatment group), as was the development of AML (2 percent in the luspatercept group and 1 percent in the placebo group) in the three-year study period. Long-term follow-up of the patient cohorts is ongoing, however.

Overall, luspatercept treatment lead to transfusion independence for eight weeks or longer, improved the erythroid response, and was associated with low-grade adverse events in patients with lower-risk MDS. The FDA announcement for luspatercept is expected in April 2020. It is expected that the MEDALIST trial will earn its spot on the FDA’s podium with an approval for luspatercept. The phase III COMMANDS trial (NCT03882536) is evaluating the efficacy of luspatercept versus placebo in patients with lower-risk MDS with and without ringed sideroblasts who have not received prior ESA for MDS. This agent represents a promising treatment for chronic anemia in patients with myelodysplastic syndromes. N Engl J Med 2020;382:140-151.

The Hematologist: ASH News and Reports
There is a rapid pace, and crowded space, in CD19 CAR-T clinical development. The degree to which the incremental and hinting that the humanized scFv may have decreased immunogenicity impacting persistence. CD28Z were decreased both in vitro and in vivo, and were linked to hinge and transmembrane domain differences, CD28Z costimulatory domain, are intrinsically linked to high incidence of neurotoxicity. Levels of cytokine release in Hu19-CD828Z, the CAR their group previously developed, now marketed as axicabtagene ciloleucel, which contains a murine ScFv and have subsequent lower toxicity rates? The new CAR they tested, Hu19-CD828Z, contains a CD19-binding domain decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR T cells (gammaretrovirus vs. lentivirus) was not completely ruled out as a contributing factor. These data together indicate that decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR containing the CD8 hinge and transmembrane domain. The UCAR group previously developed, now marketed as axicabtagene ciloleucel, which contains a murine ScFv and have subsequent lower toxicity rates? The new CAR they tested, Hu19-CD828Z, contains a CD19-binding domain decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR T cells (gammaretrovirus vs. lentivirus) was not completely ruled out as a contributing factor. These data together indicate that decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR containing the CD8 hinge and transmembrane domain. The UCAR group previously developed, now marketed as axicabtagene ciloleucel, which contains a murine ScFv and have subsequent lower toxicity rates? The new CAR they tested, Hu19-CD828Z, contains a CD19-binding domain decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR T cells (gammaretrovirus vs. lentivirus) was not completely ruled out as a contributing factor. These data together indicate that decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR containing the CD8 hinge and transmembrane domain. The UCAR group previously developed, now marketed as axicabtagene ciloleucel, which contains a murine ScFv and have subsequent lower toxicity rates? The new CAR they tested, Hu19-CD828Z, contains a CD19-binding domain decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR T cells (gammaretrovirus vs. lentivirus) was not completely ruled out as a contributing factor. These data together indicate that decreased secretion of cytokines may be responsible for the lower neurotoxicity seen with the humanized CAR containing the CD8 hinge and transmembrane domain.
Identification of Malignant Cells in Populations Years Before Development of Treatment-related Leukemia in Patients With Myeloma


Amy E. DeZern, MD, MHS

Treatment-related myeloid neoplasms (t-MN), including t-lymphomysodysplastic syndromes (t-MDS) and t-acute myeloid leukemia (t-AML), are a rare but feared complication in patients who previously received chemotherapy and/or radiation for the treatment of a primary cancer; the risk varies by primary disease as well as the therapies used.1 Multiple myeloma is a chronic hematologic malignancy for which patients, throughout their lifetime, can receive multiple treatments along with high-dose therapy and autologous hematopoietic stem cell transplantation (aHSCT). As such, MDS-associated cytogenetic abnormalities as well as clinical presentations of t-MDS and t-AML have relatively high rates reported in patients with myeloma.1 As these secondary malignancies are most often associated with an unfavorable prognosis, it is valuable to identify risk factors and predispositions that can lead to their development. Information about clonal hematopoiesis of indeterminate potential (CHIP; defined by the presence of somatic mutations in the blood in the absence of cytopenia or overt hematologic malignancy3), which may already be present at the time of primary therapy, informs our thoughts about future risk in these patients.4 Additionally, it is important to note where the cell populations that may harbor these mutations may fail in the hematopoietic lineage, as subclones, or in stem or progenitor cells.5

In the current study, Dr. Ashwin Sridharan and colleagues sought to identify whether stem and progenitor cell reservoirs were the targets of the myeloid mutations that were ultimately seen in the t-MN developing in patients. Six myeloma patients who developed t-MN after aHSCT from a single institution had stem cells, identified by CD34 positivity, available from transplant harvest. The mean time to t-MN in this small group was five years (range, 3 months to 7 years). The group used established and rigorous cell sorting methodology to separately phenotype normal and abnormal stem cells. Sorted cell populations were used for DNA isolation for targeted sequencing of known genes in MN. All six patients had the identical driver mutation (TP53 or RUNX1) observed in the t-MN samples. These mutations were detected in stem or progenitor cell populations at the time of myeloma treatment. The resultant data suggest that aberrant phenotypic leukemic stem cells can be detected years before the clinical onset of t-MN. Unsurprisingly, the stem and progenitor cells likely act as reservoirs of these mutant subclones, harboring the CHIP that ultimately may become the t-MN. Additionally, the authors observed phenotypic aberrant stem cells that were a subpopulation of leukemia stem cell markers including CD123 positivity. Previous reports have also demonstrated that CD123+ populations are associated with higher risk of MDS and AML.6

Currently in the field of hematology/ oncology, there is an explosion of data based on our ability to do sophisticated sorting of cell populations as well as next-generation sequencing/molecular testing of peripheral blood and bone marrow aspirates. In this article, these tests have the ability to detect mutations in individuals without morphologic or cytogenetic evidence of MN. Certainly, t-MN represent a unique clinical scenario in which chemotherapy or radiation may select for a mutant hematopoietic stem cell clone, increasing the risk that this clone will acquire additional mutations and progress to malignancy.7 As shown here in myeloma, similar reports have also shown that individuals with CHIP, who are treated for solid tumors have an elevated risk of MN and an overall mortality 8,9 and that mutations in TP53 gain a selective advantage in response to radiation or chemotherapy.10 The ability to track genetic evolution of the mutant population to those cells with the leukemia stem cell phenotypes. Some could make the rational argument that this additional genetic information just “incites panic” in patients and providers alike, without the currently available tools to change therapy for the present disease or a future potential diagnosis. However, much of what we do in hematology/oncology (and medicine globally) is about managing expectations. Increasingly, we are going to have the capacity to sort cells and monitor certain populations to ensure we can make t-MN diagnoses earlier to enable therapeutic intervention. Future studies, based on the biological description here, may also test aberrant CD123 expression on stem cells and whether this presence could be a potential biomarker for future development of t-MN. Therapeutic avenues that target this population may ultimately be feasible.

In summary, the capacity for understanding the biology of t-MN is ever increasing. Our patients will benefit from mortality and management advancements for t-MN for longitudinal follow-up after initial therapies and further studies will refine these, based on scientific advances. Thereafter, we can look forward to additional therapeutics for our patients.1-3

Menin Inhibitors: A New Hope in MLL-Rearranged Leukemia?


Raymond (patient name had been changed for privacy) died on New Year’s Eve, only a few months after his initial diagnosis. He had achieved complete remission with standard chemotherapy, but the cytogenetics revealed an MLL-rearrangement and foretold of the adverse prognosis that patients and clinicians fear. He stoically endured three cycles of consolidation chemotherapy during workup for allogeneic bone marrow transplantation, but unfortunately relapsed two weeks after completion of chemotherapy and was referred to the Haematology Service salivary glands. Ray lived long enough to see his young son win his football grand final and to spend Christmas with his family, but by all other measures, nowhere near long enough.

Relapsed acute myeloid leukemia (AML) rapidly evolves into a chemotherapy-resistant disease and is incurable with standard approaches. The genetic factors driving the leukemia also govern chemotherapy resistance and prognosis after treatment. MLL-rearranged (MLL, KMT2A) leukemias are an aggressive clinical subgroup of AML and acute lymphoblastic leukemia (ALL) with poor clinical outcomes, particularly in the context of therapy-associated AML and infant ALL. In the past 10 to 15 years, the molecular pathways governing MLL-rearranged oncogenic transformation have been carefully elucidated, demonstrating the importance of MLL-fusion interactions with chromatin-associated protein complexes. This work has led to the identification of novel drug targets, including DOT1 inhibitors,1,2 bromodomain inhibitors,3,4 and drugs that interfere with the Menin-MLL interaction.5 Menin binds to the N-terminus of MLL-fusion proteins and is required for MLL-fusion proteins to regulate aberrant gene expression pathways via the action of DOT1L.

In an exciting recent publication, Dr. Andre Krivtsov and colleagues used an iterative structure-based design approach to identify a highly active inhibitor of the Menin-MLL protein-protein interaction, VTP50469, with a favorable pharmacokinetic profile. The crystal structure confirmed binding within the Menin-MLL binding pocket, and inhibition studies showed that this compound had antiproliferative activity evidenced by reduced cell growth, differentiation, and increased apoptosis. VTP50469 rapidly suppressed the gene expression program in MLL-rearranged cell lines and showed activity at low mM concentrations in AML and ALL. Interestingly, the kinetics underpinning the suppression of gene expression were more rapid than that seen with some other epigenetic therapies such as DOT1L inhibitors. Despite the remarkable efficacy, the effect of VTP50469 seemed to be limited to a discrete subset of MLL-fusion target genes including MEIS1, MEPFC2, and JUNDTC, but not the HOXA cluster. This specificity may have been related to genes with the highest occupancy of Menin and DOT1L binding, though it remains unclear why some specific AR activity had been achieved in the past 50 years, with survival rates exceeding 90 percent.1

AALL0331 was a Children’s Oncology Group (COG) cooperative trial that enrolled National Cancer Institute standard risk (SR; see Table 1 [online only] for definitions) patients between 2005 and 2010.2 All patients received a three-drug induction with daunorubicin, vincristine, and pegaspargase-asparaginase (PEG-ASP). Following induction, patients were risk stratified into three categories: SR-low, SR-average, or SR-high. This article describes the outcome for those with SR-average and SR-high disease.2

Risk stratification was based on central nervous system status, cytogenetics, and response to therapy (Table 1; online only). Patients were evaluated for response to therapy via bone marrow aspirate on day 8. If blasts were over 5 percent, the patients had a repeat bone marrow aspirate on day 15. Bone marrow response by morphology and minimal residual disease (MRD) were also assessed after induction (day 28). Patients were classified as rapid early responders or slow early responders based on marrow response (Table 1; online only). Patients with MRD greater than 1 percent or an MRD at day 29 of greater than 0.01 percent are referred to as slow early responders if they had MRD less than 1 percent at day 43. Those with M3 marrow at day 29 or who had not achieved a remission (M1) by day 43 were removed from protocol therapy.

Patients with SR-average disease were randomly assigned in a 2 × 2 factorial design to receive four treatment combinations (Table 2; online only). In addition to the standard consolidation (SC) of vincristine, mitoxantrone, and intrathecal methotrexate, intensified consolidation (IC) included 1 mg/m2 of cyclophosphamide, an increased number of doses of vincristine, cytarabine, two doses of PEG-ASP, and a lower dose interrupted schedule of mitoxantrone. Initially, patients in the standard interim maintenance arms received oral methotrexate. Augmented interim maintenance included Cytarabine metothrexate and a combination of two additional doses of PEG-ASP, with intensified consolidation included more doses of vincristine and a second dose of PEG-ASP. Following an amendment in 2008 based on the results of CCG-1991, all patients in the SR-average arm received mild myelosuppression and the augmented intensified induction, reducing the randomization to between SC and IC only. Patients with SR-high disease were nonrandomly assigned to receive intensified consolidation and two cycles of delayed intensive chemotherapy.

The study enrolled 5,377 patients and included a two-stage consent. Patients initially consented to induction therapy. A second consent was used for postinduction therapy and randomization. A total of 3,992 patients continued on to postinduction therapy; 86 percent of the 1,055 patients who received induction therapy had complete remission (CR) or OS between these groups. More recently, COG has redefined SR-average as day 8 peripheral blood MRD less than 1 percent and day 29 MRD less than 0.01 percent. The authors analyzed the difference between the SC and IC arms in the subgroup of patients who would meet this more stringent definition of SR-average and found no benefit of IC over SC. In those patients with day 29 MRD between 0.01 and 0.1 percent, patients had a worse event-free survival and overall survival outcomes between SC and IC. In all subgroups, IC was associated with significantly higher toxicity, especially infectious toxicities, with an infectious rate of 23 percent in the IC groups and 4.7 percent in the SC group (p<0.0001).

The study enrolled 635 patients on the SR-high arm, and these patients also had excellent outcomes, with OS greater than 90 percent. COG and OS rates in patients on the SR-high arm who were treated nonrandomly with intensified therapy were superior to those patients with low-level MRD who were treated with IC or SC and standard postconsolidation therapy. The 6-year CR rate in the SR-average low-level MRD group overall was 77.46 percent, versus 85.55 percent in the SR-high group.

Historically, intensification of therapy has led to improved outcomes in many patients with ALL. The results of the SR-average arm on AALL0331 demonstrate that for many patients with ALL, further intensification of therapy adds only toxicity without benefit. In contrast, the SR-high patients seemed to benefit from nonrandom intensification of therapy post-consolidation. Thus, there may be some groups of patients that would continue to benefit from intensified therapy if this is becoming increasingly expensive, and it can be difficult to identify whom those patients are. The current generation of COG trials (NCT03914625, NCT03959085, NCT03876769) have changed the paradigm and are testing whether the incorporation of immunotherapies into cytotoxic chemotherapy backbones will improve survival without additional toxicity. Time will tell if this approach will lead to superior outcomes.

Does Renal Function Deteriorate in Individuals With Sickle Cell Trait and Sickle Cell Disease? Now We Know


ELNA SAAH, MD, AND IFYEWI OSUNKWO, MD, MPH

The carrier frequency of the sickle gene in African Americans is 8 to 9 percent but can be as high as 20 to 40 percent in some African countries such as Niger.1 This relatively high carrier rate has deemed it imperative that we continue to probe its impact on all-cause mortality and morbidity related to end organ function in aging populations and those with chronic illnesses such as hypertension and diabetes, both in the United States and globally.2 Furthermore, as the population with sickle cell disease (SCD) increasingly survives into adulthood in the United States, chronic kidney disease (CKD) is evolving as a significant contributor to morbidity and mortality.3

To define the trajectory and the rate of decline of estimated glomerular filtration rate (eGFR) in patients with sickle cell trait (SCT) and SCD compared to the general noncarrier (AA) status among black patients, a group of investigators examined changes in eGFR over a minimum of three time points in 21,800 patients from the Research Patient Data Registry housed in Partners HealthCare (Boston, MA) throughout a 13 1/2 year period. Among the 10,210 patients eligible for analysis after excluding for missing genotype data and conflicts in coding, the researchers identified 2,310 with SCT, 210 with SCD, and 8,729 who had neither carrier status nor disease as the reference or control group.

In this study, Dr. Kabir Olaniran and colleagues simultaneously evaluated and compared the decline in eGFR among patients with both SCT and SCD, and for the first time, could elucidate “a dose response relationship” between sickle hemoglobin S (HbS) quantitation and eGFR. In comparing the SCT group to controls, they found that SCT was associated with a faster rate of eGFR decline (particularly in male patients) and with associated hyperfiltration at baseline. Their findings suggested that black Americans with SCT lose nearly half an eGFR unit (mL/min/1.73 m²) more of kidney function every year compared to black Americans with AA status. The findings in SCD of accelerated eGFR were both intuitive and confirmatory.4,5

Other elucidated factors associated with eGFR decline in SCT and SCD were hypertension, diabetes, cardiovascular disease, angiotensin converting enzyme inhibitors (ACEis) or angiotensin receptor blockers (ARBs), aspirin, statins, and high leucocyte counts. The study as a retrospective cohort study was not designed to tease out the various confounding variables but elicits the question of what changes in factors may affect the rate of eGFR decline. Hence, while SCT is not itself a “malady,” it is far from benign as it is a strong predictor of CKD in the general population. The potential effects of coinheritance of SCT and other conditions such as diabetes or hypertension should also be recognized.

In SCD, the authors found an inverse correlation between HbS quantitation and eGFR decline; with a lower HbS being associated with a faster eGFR decline implying that higher hemoglobin A quantitation (of > 70%) strongly associated with a lower HbS being associated with a faster eGFR decline. Their study also suggested that elevated hemoglobin A2 and hemoglobin F may be protective in SCT.

Counterintuitively, in SCT, the authors found an inverse correlation between HbS quantitation and eGFR decline; with a lower HbS being associated with a faster eGFR decline implying that higher hemoglobin A quantitation (of > 70%) strongly associated with a faster eGFR decline. Their study also suggested that elevated hemoglobin A2 and hemoglobin F may be protective in SCT.

This study adds to others19 confirming that the effect of SCT on decline in renal function in black patients is both “insidious and significant.” In SCD, hypertension was not associated with a faster rate of eGFR decline; could this be because patients with SCT may have lower resting blood pressure? Perhaps a more sensitive indicator of the role of blood pressure in this cohort would be the degree of change from baseline blood pressure rather than the absolute measure.

Hence, while SCT is not itself a “malady,” it is far from benign. To determine all the confounders of both increased risk for developing CKD as well as what characteristics are renoprotective, these factors must be incorporated in the design of our next generation of prospective longitudinal cohort studies. This study sheds additional light on the role of HbS on renal function decline in blacks. Further research is needed to frame the response to our findings.

References


Dr. Saah and Dr. Osunkwo indicated no relevant conflicts of interest.

FLT3-ITDs and NPM1 Insertions

(Cont. from page 1)

be expected during nontemplated polymerization by TdT. Finally, fitter sequence correlated with frequency of TdT expression across AML subtypes and was significantly overrepresented in FLT3-ITDs derived from ALL compared to AML.

The authors went on to show how TdT activity could also provide a basis for generation of ITDs in the absence of germline microhomology. In short, TdT-added nucleotide runs that, by chance, have homology with nearby 5’ sequence (occult microhomology, Figure, part C) could allow for polymerase repositioning and subsequent duplication. These runs would not be apparent as fitter sequence, but they should display the level of G/C bias associated with TdT activity. In a companion study, Dr. Borrow and colleagues extended their sequence analysis approach to 2,430 previously published NPM1 mutations in AML (114 unique mutations), showing that of 111 unique mutations, more than 90 percent of NPM1 mutations, contained fitter sequences that were consistent with TdT activity in terms of G/C and dinucleotide bias as well as length distribution (Figure, part D).

In summary, Dr. Borrow and colleagues demonstrated sequence-level evidence that TdT plays a causal role in the generation of FLT3-ITDs and NPM1 mutations in AML, perhaps explaining their common co-occurrence. However, the clinical context and prognostic value of fitter and microhomology status in FLT3-ITDs and NPM1 mutations remain to be determined. Most AMLs lack expression of TdT by immunohistochemical stains or flow cytometry. The authors have suggested that TdT must be expressed early in the leukemic stem cell, while the block in maturation is expressed slightly later, after down-regulation of the enzyme. One would assume that similarly, NPM1 mutations should also occur with greater frequency in cases with earlier block in maturation, although experientially, NPM1 is more common in AMLs with some degree of differentiation (especially monocytic). The prognostic implications are even more intriguing. While allelic fraction is perhaps the most widely recognized stratification for survival within FLT3-ITD AML, a recent study demonstrated that FLT3-ITDs containing fitter sequences are associated with poor response to both cytotoxic and tyrosine kinase inhibitor therapy, suggesting a potential role for these structural characteristics as predictive biomarkers.

Dr. Saah and Dr. Osunkwo indicated no relevant conflicts of interest.

Examples of replication showing (A) normal DNA replication, (B) microhomology resulting in mispairing and formation of an internal tandem duplication (ITD), (C) nontemplated addition of nucleotides by TdT and the use of the terminal nucleotide to create an occult single base mispair conversion to form an ITD with filler sequence, and (D) a similar occult mispriming event leading to an NPM1 four base pair insertion. (From Vasiliou GS. The curious incident of TdT-mediated mutations in AML. Blood 2019;134:2229-2231.)


Dr. LaMacchia and Dr. Kim indicated no relevant conflicts of interest.

The Hematologist: ASH News and Reports
Act Fast! Making a Difference After Traumatic Brain Injury With Tranexamic Acid


Patients with traumatic brain injury (TBI) and associated intracranial hemorrhage have high morbidity and mortality. Hematoma expansion after TBI occurs in part due to the release of tissue plasminogen activator (t-PA) and other fibrinolytic mechanisms that promote the activation of plasminogen to plasmin and subsequent fibrin degradation. These include the endothelial release of tissue plasminogen activator and activation of protein C.1

Tranexamic acid (TXA) is an inhibitor of fibrinolysis, a synthetic lysine analogue that blocks the activation of plasminogen to plasmin. Intravenous TXA has been shown in two randomized trials (>40,000 patients) to reduce death due to bleeding in scenarios driven by hyperfibrinolysis: postpartum hemorrhage (WOMAN)2 and extracranial bleeding after trauma (CRASH-2).3 A patient-level meta-analysis of these trials demonstrated that the relative survival benefit of TXA decreased by 10 percent for each 15 minutes of treatment delay, with no benefit after three hours from injury or bleeding onset.4 There was no increase in the risk of seizures or thrombosis with TXA in either study. The CRASH-3 study investigators examined the efficacy and safety of TXA in TBI. In this large multicenter randomized controlled trial, patients with TBI and reduced Glasgow Coma Scale (GCS) of ≤12 or intracranial hemorrhage on computed tomography scan were randomized to receive TXA (1 g iv over 10 hours) or placebo. Patients were initially enrolled within eight hours of injury. However, when the above data on early TXA administration were made available, the protocol was amended to enroll patients within three hours of injury such that 69% were enrolled within the 12.7±2.9 hours in the final analysis. The primary outcome was head injury-related death within 28 days of injury in patients randomized within three hours. Safety outcomes included seizures and thrombotic events.

Among patients treated within three hours of TBI, the primary outcome occurred in 18.5 percent of the TXA group versus 19.8 percent with placebo (RR, 0.94; 95% CI, 0.86-1.02), which did not reach statistical significance. In a prespecified sensitivity analysis excluding patients with GCS lower than 3 or bilateral unreactive pupils, the results were 12.5 percent with TXA versus 14.0 percent with placebo (RR, 0.89; 95% CI, 0.81-0.98). However, the effects of TXA differed depending on the severity of head injury—the severity of head injury was characterized as moderate to severe TBI (GCS, ≥9-15), the primary outcome occurred significantly less frequently with TXA (5.8%) compared with placebo (7.9%; RR, 0.78; 95% CI, 0.64-0.95); there was no significant difference in severe TBI (GCS, ≤3-8).

In a regression analysis including all 12,737 participants, early (≤3 hours) versus late (>3 hours) TXA administration did not significantly impact on survival at 28 days. However, early treatment with TXA was more effective than later treatment for reducing the primary outcome in patients with mild to moderate TBI (p=0.005), but not in severe TBI (p=0.79). There was no difference in the risk of adverse events including seizures and thrombotic complications.

Strengths of this trial included its large and international sample, prespecified sensitivity analysis, and relatively complete follow-up. Limitations included its wide confidence intervals despite large sample size, potential underestimate of thrombotic complications with a lack of screening diagnostic studies, and protocol changes affecting enrolment.

Although the benefit of TXA was not shown in the overall study population, there are important lessons to be taken from this trial. First, TXA reduces bleeding-related death in patients with mild to moderate TBI. Meanwhile, those with severe TBI have a poorer overall prognosis such that TXA is unlikely to improve outcomes. Second, it is important to act fast as the benefits of TXA in mild to moderate TBI are most pronounced if given within three hours, mirroring what was seen in postpartum hemorrhage and trauma-related extracranial bleeding. Finally, TXA used at low-moderate doses does not appreciably increase the risk of thrombosis or seizures. This is consistent with a substantial body of randomized evidence reflecting the safety of TXA and suggests it should be used as frontline therapy in addition to other hematostatic measures.

I look forward to future studies examining the use of laboratory measures of fibrinolysis to appropriately target antifibrinolytic therapies. Currently available viscoelastic techniques (such as TEG and ROTEM) appear to lack sufficient sensitivity for non-severe fibrinolytic thrombosis or seizures. This is consistent with a substantial body of randomized evidence reflecting the safety of TXA and suggests it should be used as frontline therapy in addition to other hematostatic measures.


Update on the ASH Research Collaborative Data Hub

The Data Hub represents an ideal vehicle to meet objectives outlined in the 21st Century Cures Act and elsewhere — to generate real world evidence that informs and accelerates development and research in SCD. These types of platforms are important ways to understand priorities, incentives, and barriers to participation in research for patients. We envision continued engagement throughout future CTN and Data Hub activities. We are also developing plans for a patient-friendly electronic informed consent process for ASH RC. We are also exploring the development of a web-based patient portal for longitudinal engagement; community newsletters; and opportunities for participants to engage in other projects or advisory opportunities as they arise. We are also developing ways to collect data that directly reflect the patient voice. Future versions of the portal will include the ability to capture patient-reported outcomes (PROs) in areas such as symptoms and quality of life, disease-related symptoms, symptomatic toxicities, and physical function.

Engaging the Research Community

The Data Hub potentially represents a valuable resource to help the research community, and we are continuing to develop the Data Hub with the needs of the research community in mind. We are actively refining policy for analyses and publications and will make these available to the hematology community soon. We are also working with experts in MM and SCD to identify potential early analyses as “test cases” to evaluate the fitness of our current procedures. We plan to use these early analyses to inform ASH RC models and data-mining strategies to facilitate subsequent projects and widespread use of the Data Hub within the hematology research community.

Engaging the Practice Community

We also envision that the Data Hub will serve as a catalyst for practice transformation, to improve outcomes in real time for patients affected by hematologic diseases. In 2020, we are working to develop reports for sites contributing data to the Data Hub and will collaborate with sites to ensure that these reports are constructed in a way that best meets their needs. These reports will include data on consecutive patients from each site and will support quality improvement and other operational needs. We are also developing basic analytic data cuts for sites and other stakeholders so that specific subpopulations of interest can be identified and tracked. In the future, we envision alignment of ASH RC with other ASH activities designed to support and improve practice, such as development and integration of metrics associated with ASH guidelines in SCD and other conditions.

Expanding the Scope

The long-term goal of the ASH RC is to address the entire spectrum of malignant and nonmalignant hematologic diseases. This means we will need to expand our scope beyond our current focus on MM and SCD once these initial efforts have reached maturity. We anticipate a general call to the hematology community for submission of future CTN and Data Hub activities. We are also developing plans for a patient-friendly electronic informed consent process for ASH RC. We are also exploring the development of a web-based patient portal for longitudinal engagement; community newsletters; and opportunities for participants to engage in other projects or advisory opportunities as they arise. We are also developing ways to collect data that directly reflect the patient voice. Future versions of the portal will include the ability to capture patient-reported outcomes (PROs) in areas such as symptoms and quality of life, disease-related symptoms, symptomatic toxicities, and physical function.

Dr. Wood indicated no relevant conflicts of interest.
**Clinical Trials Corner**

**Bispecific Antibodies in Multiple Myeloma: Do These T cell–Recruiting Antibodies Make a Difference?**

**STUDY TITLE:** Study of ISB 1342, a CD38/CD3 Bispecific Antibody, in Subjects With Previously Treated Multiple Myeloma

**ISRCTN NUMBER:** NCT03399799

**SPONSOR:** Ichnos Sciences SA

**ACCRUAL GOAL:** 125 patients

**PARTICIPATING CENTERS:** Nine centers around the United States

**STUDY DESIGN:** This is a phase I open-label, two part dose-escalation and cohort expansion study of ISB 1342, a bispecific antibody directed at CD38 and CD3, for patients with progressed or relapsed multiple myeloma (MM) refractory to proteasome inhibitors, immunomodulators, and daratumumab. The primary outcome measures are maximum tolerated dose defined by the number of dose-limiting toxicities, objective response according to the International Myeloma Working Group (IMWG) response criteria, and the frequency and severity of adverse events. Secondary outcomes will measure maximum serum concentration, immunogenicity by antidrug antibody formation, and disease control rate. ISB 1342 will be administered by intravenous infusion on day 1 and day 15 of each 28-day treatment cycle at escalating dose levels.

**RATIONALE:** Despite advances in the treatment of MM, current therapies fail to cure most patients with MM due to intrinsic resistance, acquired resistance, and/or persistent minimal residual disease leading to subsequent relapse. Thus, new therapies with a more potent mechanism of action are needed. Bispecific antibody therapy has proven to be clinically relevant in other hematologic malignancies through an alternative mechanism of action. In acute lymphoblastic leukemia, for instance, blinatumumab has moved into common usage before stem cell transplantation for patients with either refractory disease or with evidence of measurable residual disease. Bispecific antibodies induce redirected T-cell lysis of tumor cells by simultaneous engagement of endogenous T cells, via binding to CD3, and the tumor cell, via any extracellular tumor-associated antigen. CD3 is a tumor-associated antigen with near-universal expression in MM and has been validated as a target in MM with the human monoclonal antibody daratumumab. While daratumumab monotherapy is associated with an approximately 35 percent overall response rate (ORR) when combined with other treatment modalities, not all patients respond, and many eventually develop progressive disease. The efficacy of daratumumab is limited by the inability to stimulate T-cell killing of myeloma cells. In preclinical studies, ISB 1342 was able to overcome this limitation by redirecting the cytotoxic potential of T cells to human myeloma cell lines in vitro and in mouse xenograft models. The aim of this study is to evaluate the safety, tolerability, and efficacy of monotherapy with ISB 1342 in relapsed/refractory patients.

**COMMENT:** The development of numerous treatment strategies aimed at overcoming the progressive immune dysfunction in myeloma malignogenesis has been promising. Bispecific antibodies in particular represent a powerful tool for the treatment of hematologic malignancies by redirecting the activity of the T cell response against cancer. The first bispecific antibody construct with available clinical data in MM is the B-cell maturation antigen–targeting molecule AMG 420 showing a favorable adverse event profile and a 70 percent RR (7.0) at the recommended study dose of 400 µg. Data presented at the 2019 ASH Annual Meeting showed promising results for another B-cell maturation antigen bispecific antibody ORB-1889 percent and a 44 percent complete response rate at the highest dose of 10 mg. Like AMG 420 and CC-93269, ISB 1342 is being tested in a relapsed/refractory setting. However, the efficacy of other bispecific antibody combinations have been shown to be higher in patients with less tumor burden, suggesting that earlier timing is necessary for optimal effect. Since CD3 is highly expressed in the early stages of plasma cell clonal evolution, it will be interesting to evaluate whether an anti-CD38 bispecific antibody could prevent or delay progression to symptomatic MM. However, the broad expression of CD38 on other cells, such as lymphocytes, natural killer cells, dendritic cells, and bone marrow progenitor cells, raises the question of off-target toxicity. Next to timing and toxicity, resistance mechanisms due to shedding/downregulation of CD38 need to be studied further. This also raises the question of the necessity of CD38 expression upon start of treatment. Additionally, studying biomarkers for identifying early signs and best drug combinations will be necessary to see if an anti-CD3 bispecific antibody could be clinically successful. A second trial of a bispecific antibody dual targeting CD3 and CD38 (AMG 424) is currently recruiting (NCT03445663). Additional studies are investigating other known MM-associated antigens such as SLAMF7 and GPRC5D (NCT03399799) bispecific antibodies. While many questions remain to be answered, awaiting current trials, bispecific antibodies seem to provide an off-the-shelf alternative in the arsenal of anti-myeloma immunotherapy.


**Clonal Hematopoiesis**

(Cont. from page 6)

radiation or cytotoxic therapy led to a further increase in allele burdens of DDR mutations. This highlights the relationship between cancer therapy and TP53, PPM1D, and CHEK2 mutations; exposure to specific cytotoxic treatments and radiotherapy confers a competitive growth advantage in patients with cancer harboring a DDR mutant clone. TP53 mutations were among the strongest risk factors associated with subsequent progression to tMM in an analysis of almost 10,000 patients exposed to cancer therapies. Indeed, a further analysis of tMM patients detected TP53 mutations in 40 percent (n=14/35 patients), which were present in a pretransformation paired sample in 10 of 14 patients (median interquartile time was 24 months for these patients).

There are numerous important ramifications of these large cohort analyses of cancer and MDS/tMM patients. Monitoring for CH may have a predictive role in risk stratifying patients with cancer to identify those at increased risk of tMM. This would have the potential to influence therapeutic decisions made during their cancer treatment. Dr. Bolton and colleagues investigated how CH may be incorporated into a risk stratification model for tMM, including other relevant parameters such as age and blood counts using an established methodology (ICARE). They modelled the 10-year risk of MDS/AML in women with breast cancer, estimating the risk for those receiving and not receiving adjuvant chemotherapy. In patients with the highest risk of tMM (top 1%), the estimated increase in risk with the addition of adjuvant chemotherapy was greater than 5 percent, which would exceed the predicted survival benefit of adjuvant chemotherapy in many women with breast cancer. Furthermore, the findings of Dr. Bernard and colleagues suggest that identification of haematocline TP53 mutations could be used to further refine this risk stratification. Ultimately, single-cell analysis might be required to definitively identify subclonal haematocline TP53 mutations. Such an approach will require prospective validation before it is incorporated into routine clinical practice. It will also be important to develop clear recommendations to guide oncologists in the appropriate use of CH screening to enhance optimal therapeutic decision making.


Dr. O’Sullivan and Dr. Mead indicated no relevant conflicts of interest.

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Dr. Tahri, Dr. Lomas, and Dr. Ghobrial indicated no relevant conflicts of interest.
The goal is to underscore the remarkable research that is published in Blood and to highlight the exciting progress that is being made in the field.

JANUARY 9, 2020

Dr. Christian A. Di Buduo and colleagues report that mutant CALR also shows loss of binding to calcium-regulatory proteins, leading to constitutive increase in intracellular calcium, further increasing proliferation of megakaryocytes, and contributing to their prominence as a clinical feature.


Steroids are conventional frontline therapy for acute graft-versus-host disease (GVHD) but induce substantial toxicity. This randomized multicenter comparison of sirolimus versus prednisone for treatment of standard-risk acute GVHD demonstrates similar response rates at 28 days. Sirolimus therapy was associated with reduced steroid use and improved quality of life.

JANUARY 16, 2020

This month’s CML article reports on the largest prospective observational study of long-term central venous catheter–related complications in children to date, in which this dogma is overturned. The research provides important information for decision-making about when to use central catheters and which type to choose.

JANUARY 23, 2020

In a Plenary Paper, Dr. Shruti Chaturvedi and colleagues provide seminal new insights into the role of complement activation in APS pathophysiology and report a high prevalence of complement-regulatory gene mutations in patients with CAPS.


Two papers examining the heritability of clonal hematopoiesis of indeterminate potential (CHIP) yield highly concordant results. In independent studies of elderly mononuclear and dicytotic twins totaling over 350 pairs, Dr. Jakob Werner Hansen and colleagues and Dr. Margarete A. Fabre and colleagues demonstrate that CHIP has a limited heritable basis, suggesting that environmental exposures are the leading contributors to clonal hematopoiesis.

JANUARY 30, 2020

Patients with Philadelphia-negative myeloproliferative neoplasms are prone to the development of second cancers. In an international nested case-control study, Dr. Valerio De Stefano and colleagues identified an association between a new event of arterial thrombosis and the subsequent diagnosis of a second cancer.

FEBRUARY 13, 2020

This prospective open-label study of dabigatran in children for secondary prophylaxis against venous thromboembolism (VTE) reveals low rates of recurrence and clinically significant bleeding over durations up to one year. The data suggest that dabigatran is safe for extended treatment of VTE in children and does not require routine laboratory monitoring.

FEBRUARY 20, 2020

In a population-based analysis including a large database restricted to patients over age 70, the authors demonstrate that the A91V polymorphism in the familial hemophagocytic lymphohistiocytosis-related gene is a nonpathological polymorphism that confers no increase in cancer, death, or immunopathology.

Featured content from Blood Advances, Volume 4, Issue 3

Mutation Accumulation in Cancer Genes Relates to Nonoptimal Outcome in Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm accounting for ~15 percent of all leukemia. Progress of the disease from an indolent chronic phase to the more aggressive accelerated phase or blast phase (BP) occurs in a minority of cases and is associated with an accumulation of somatic mutations. Researchers performed genetic profiling of 85 samples and transcriptome profiling of 12 samples from 59 CML patients. They identified recurrent somatic mutations in ABL1 (37%), ASXL1 (28%), RUNX1 (16%), and BCOR (16%) in the BP and observed that mutation signatures in the BP resembled those of acute myeloid leukemia (AML). The authors found that mutation load differed between the indolent and aggressive phases and that nonoptimal responders had more nonsilent mutations than did optimal responders at the time of diagnosis, as well as in follow-up. Using RNA sequencing, they identified other than BCR-ABL1 cancer-associated hybrid genes in six of the seven BP samples. Uncovered expression alterations were in turn associated with mechanisms and pathways that could be targeted in CML management and by which somatic alterations may emerge in CML. Last, they showed the value of genetic data in CML management in a personalized medicine setting.

Bite Cells Abound

LINDSEY SHANTZER, MD,1 AND KELLY DAVIDSON, MD2

1. Hematology/Oncology Fellow, University of Virginia, Charlottesville, VA
2. Assistant Professor, Department of Hematology, University of Virginia, Charlottesville, VA

A 61-year-old woman presented to clinic with fatigue, weakness, and dark urine. She underwent renal transplantation two months prior and was taking sirolimus, azathioprine, and prednisone 7.5 mg daily for immunosuppression, as well as dapsone 50 mg daily for Pneumocystis jirovecii pneumonia (PJP) prophylaxis. Glucose-6-phosphate dehydrogenase (G6PD) enzymatic activity was previously normal (15.3 U/g hemoglobin). Laboratory evaluation is shown in the Table. Ultrasound of the transplanted kidney was unremarkable. Peripheral blood smear (shown) revealed anisocytosis, frequent degmacytes (bite cells, arrows), occasional dacrococytes, and rare schistocytes (0-1/hpf).

<table>
<thead>
<tr>
<th>Test</th>
<th>Result at Baseline</th>
<th>Result at Presentation</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count</td>
<td>10.04 × 10^9/L</td>
<td>8.73 × 10^9/L</td>
<td>4.00-11.00 × 10^9/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>12.3 g/dL</td>
<td>7.0 g/dL</td>
<td>12.0-16.0 g/dL</td>
</tr>
<tr>
<td>MCV</td>
<td>93.4</td>
<td>103.9</td>
<td>83.0-95.0 fL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>357 × 10^9/L</td>
<td>210 × 10^9/L</td>
<td>150-450 × 10^9/L</td>
</tr>
<tr>
<td>LDH</td>
<td>419 U/L</td>
<td>125-250 U/L</td>
<td></td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt;8 mg/dL</td>
<td>30-200 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>2.37%</td>
<td>5.67%</td>
<td>0.70-2.50%</td>
</tr>
<tr>
<td>Direct antiglobulin (Coombs)</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>26 U/L</td>
<td>45 U/L</td>
<td>15-37 U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>40 U/L</td>
<td>39 U/L</td>
<td>13-61 U/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.0 mg/dL</td>
<td>0.7 mg/dL</td>
<td>0.6-1.3 mg/dL</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>2.7 mg/dL</td>
<td>7.0 mg/dL</td>
<td>0.2-1.0 mg/dL</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>0.4 mg/dL</td>
<td>0.0-0.5 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>17.8 mg/dL</td>
<td>7.0-31.1 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Cyto megalovirus viral load</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epstein Barr virus viral load</td>
<td>Negative</td>
<td></td>
<td></td>
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<tr>
<td>TPMT</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; MCV, mean cell volume; TPMT, thiopurine methyltransferase.

Figure. Peripheral blood smear.

What is the most likely cause of this patient’s anemia?
A. Immune-mediated hemolysis
B. Oxidative stress-induced hemolysis
C. Bone marrow suppression
D. Nutritional deficiency
E. Thrombotic microangiopathy

For the solution to the quiz, visit The Hematologist online, www.hematology.org/TheHematologist/Image-Challenge.

Dr. Shantzer and Dr. Davidson indicated no relevant conflicts of interest.

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